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Spermatogenic and antiradical activities of the hydro-ethanolic extract of *Strychnos camptoneura* (longaniaceae) trunk barks in Wistar rats

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Abstract

The objective of the present study were to evaluate the spermatogenic and antiradical potentialities of the hydro-ethanolic extract of *Strychnos camptoneura* trunk bark in male wistar rats. For this purpose, 20 rats were divided into 4 lots of 5 animals and received respectively for lots 1 and 2 distilled water and testosterone enanthate. Lots 3 and 4 were treated with the hydro-ethanolic extract of *Strychnos camptoneura* (100 and 250 mg/kg). The results obtained show that, the administration of hydro-ethanolic extract of *Strychnos camptoneura (*100 and 250 mg /kg/po) orally in rats caused significant dose-dependent increases ($p < 0.05$; $p < 0.01$ and $p < 0 .001$) in sperm concentration and motility rates compared to rats of the control group treated with distilled water. On the other hand, the vitality rates of sperm and normal sperm were only significant ($p < 0.01$) at the respective doses of 100 and 250 mg /kg of *Strychnos camptoneura* compared to the negative controls. In addition, there was no variation in sperm pH ($p > 0.05$) at any dose level. Chemical analysis of the hydro-ethanol extract of *Strychnos camptoneura* revealed the existence of phenolic compounds especially polyphenols and total flavonoids endowed with antiradical and antioxidant properties. The improvement of the characteristics of the semen and the reduction of the DPPH radical observed in the present work, justify its biological potentialities and its use in traditional medicine in the treatment of male infertility.

Keywords: *Strychnos camptoneura*; Spermatogenic; Antiradical; Male infertility

1. Introduction

For a very long time, man has used plants as food, shelter and main materials for the maintenance and care of his health. Despite the progress made by modern medicine, herbal medicine remains a widely used means of care, even the only means for some indigenous people and communities living in some developing countries [1]. According to World Health Organization (WHO) data, infertility affects 9% of couples worldwide and in 50% of cases this infertility is of male origin [2].

Several recent studies have shown that male germ cells are very sensitive to environmental factors (pesticides, heavy metals and synthetic drugs) [3 - 5] having adverse effects on animal and human health with the consequent production of large quantities of free radicals causing oxidative stress. Work conducted by [6] and [3] showed that this imbalance between pro-oxidants and antioxidants in favor of pro-oxidants causes a significant decrease in reproductive parameters (oligospermia, asthenospermia, teratospermia), which can lead to infertility or male sterility [7,8].

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The use of plants in the treatment of infertility can therefore represent an alternative especially in developing countries where techniques such as stem cell transplantation, Sertoli cells are not accessible. The detailed analysis and exploration of the biological effects of plants, particularly to remedy infertility problems, is therefore of great interest. *Strychnos camptoneura* is a plant used in traditional Congolese medicine for the treatment of male infertility [9]. Indeed, some studies have shown the effectiveness of this plant in improving sexual performance [9] and its androgenic potential in Wistar rats [10].

However, to the best of our knowledge, no spermatogenic and antiradical study of the trunk barks of the hydro - ethanolic extract of *Strychnos camptoneura* has been conducted. Therefore, the present study was initiated with the main objective to evaluate the biological effects of the hydro-ethanolic extract of *Strychnos camptoneura* in wistar rats.

2. Material and methods

2.1. Plant material

The plant material consisted of the trunk bark of *Strychnos camptoneura* collected in M'voula (Itoumbi, Cuvette-ouest, 760 km from Brazzaville-Congo) and then dried in the shade at laboratory temperature (25 ± 1 °C) for 15 days. A sample of this plant was authenticated at the Institut National de Recherche en Sciences Exactes et Naturelles (I.R.S.E.N.) and registered under N° 2271

2.2. Animal Material

Wistar albino rats weighing between 160 and 190 g were used. They were raised at the animal house of the Faculty of Health Sciences of the Marien Ngouabi University and fed with a standard diet with free access to water and a nocturnal-diurnal (12/12) lighting rhythm.

2.3. Methods

2.3.1. Preparation of the reference androgen solution

Testosterone Enanthate (Androtardyl)R 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 150 g rat. To facilitate administration, two successive dilutions were made to obtain a 2.5 mg/ml solution. We therefore administered 0.2 ml of this solution to each animal by intramuscular route in one go [10].

2.3.2. Preparation of the hydroethanolic extract of Strychnos camptoneura

The barks of the hydro-ethanolic extract of *Strychnos camptoneura* were cut into small pieces and dried on a clean laboratory bench at room temperature (28-30 °C) for 15 days. They were then crushed and ground in a mortar to obtain a homogeneous powder. The hydro-ethanolic extract of *Strychnos camptoneura* barks was prepared by maceration, 100 g of the barks were macerated in 1000 mL of a hydro-ethanolic solution (in equi-volumic proportion) during 72h.

After filtration, the obtained macerate was evaporated at 70 °C using a BUCHIR-II rotavapor. The dry extracts obtained were then stored in flasks protected from humidity and heat for possible pharmacological tests and phytochemical analyses.

2.3.3. Evaluation of the spermatogenic activity of the hydro-ethanolic extract of Strychnos camptoneura in rats

The study of the effects of the hydro-ethanolic extract of *Strychnos camptoneura* on the microscopic and macroscopic characteristics of the semen was carried out in order to evaluate the effects of the extract on the spermatic function (motility, vitality, concentration, morphology and pH of the spermatozoa). For this purpose, 20 animals weighing between 180 and 190 g and randomly divided into four (4) lots of five (5) animals each and received for 26 days:

- Lot1: 0.5 ml distilled water/100g;
- Lot2: 0.86 mg/kg /im of testosterone enanthate (reference molecule);
- Lot3: 100 mg/kg/po of *Strychnos camptoneura* hydro-ethanol extract;
- Lot4: 250 mg/kg/po of the hydroethanolic extract of *Strychnos camptoneura*.

2.3.4. Effects of the hydro-ethanolic extract of Strychnos camptoneura on some characteristics of the seed

The study of the effects of the hydro-ethanolic extract of *Strychnos camptoneura* on some microscopic characteristics of the semen was carried out in order to evaluate the effects of the extract on the spermatic function (mobility, vitality, concentration, morphology and PH of the spermatozoa). For this purpose, after the animals were sacrificed by ethyl ether overdose and the organs were 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, and then incubated in a water bath at 36 °C [8,11].

2.3.5. Effect of the extract on sperm motility

Mobility (or motility) is an important index of sperm vitality. In poor quality ejaculates, sperm move slowly and have non-straight movements [12]. The objective of our study was to evaluate the effect of the extract on sperm mobility. Indeed, a good quality sperm should have at least 60 to 70% motile spermatozoa with a degree of mobility or coefficient of 4 or 5[12]. Thus, sperm motility was assessed by direct examination of the solution. 20µl of this solution was placed between slide and coverslip at x40 magnification. Motile and immobile spermatozoa were counted on 12 randomly selected microscopic fields and the percentage of mobile forms was determined from the formula proposed by [11]:

$$\%\ of\ mobile\ spermatozoids\ = \frac{\text{Number of mobile spermatozoids}}{\text{Total number of spermatozoids}}\ \text{X}\ 100$$

2.3.6. Effect of the extract on sperm concentration

Sufficient sperm production in the ejaculate is also an important criterion to assess the quality of the semen as insufficient production could cause infertility in the subject [13,14]. Sperm count was done using Thoma's cell. For this purpose, semen was diluted 100 times with a formolated solution (35%) in order to fix the spermatozoa to make them immobile during counting. 10µl of this diluted semen was placed in the Thoma cell chamber (area = 0.2 mm^2 and depth = 0.1 mm), and covered by a cover slip. Counting was done under a microscope (Leica DM 750) at X40 magnification. Spermatozoa were counted in five large squares. The operation was repeated 2 times and the average thus calculated was used to determine the number of spermatozoa per epididymis tail, by the following formula Number of sperm / tail = number of sperm / mm3 x 1000 x 10. Where 10 is the volume of the solution in ml and 1000 is the conversion factor of mm3 to mL [8,11].

2.3.7. Effect of the extract on pH and sperm vitality

The pH was measured not from the pure spermatozoa, but directly from the suspension of spermatozoa in 2 ml of physiological water and the pH reading was done using urine strips. Vitality was assessed using a particular methodology: slide preparations used to count live and dead spermatozoa; staining of spermatozoa, then light microscopy (objective 40) reading of 200 spermatozoa. On the smears, live sperm stained red with 2% eosin and dead sperm were not stained [5].

2.3.8. Effect of the extract on sperm morphology

Sperm morphology is an important parameter in the exploration of sperm from an infertile man (Saïdi et al., 2008). The eosin-nigrosin technique based on an eosin dye (physiological dye) and nigrosin (background substance) was used. The dye was prepared at the beginning of the study according to the protocol proposed by [15] and stored at 4 °C at a regularly controlled pH (6.8). Before use, the temperature was reduced to 37 °C in a water bath. One drop of seed is mixed with 4 drops of dye and after 1 minute, 10 µl of mixture is spread on a slide preheated to 37 °C, stored in an oven at 30 °C until analysis. The identification of abnormalities is done with a microscope (Leica DM 750) with a Gx 100 objective based on the models proposed by [16]. The types of sperm morphological abnormalities in males have been classified into two main categories [17]: - Primary abnormalities (macrocephalic, microcephalic, elongated and thinned head, irregular head sperm,) - and secondary abnormalities (sperm without tail, with angled or coiled flagellum, with short flagellum,..). Abnormal and normal spermatozoa were counted on a total of 200 cells and the percentages of abnormal and normal forms were determined from the formula proposed by [11].

$$\%\ of\ normal\ spermatozoids\ = \frac{\text{Number of normal spermatozoids}}{\text{Total number of spermatozoids counted}}\ \text{X}\ 100$$

$$\%\ of\ abnormal\ spermatozoids\ = \frac{\text{Number of abnormal spermatozoids}}{\text{Ntotal number of spermatozoids counted}}\ \text{X}\ 100$$

2.3.9. Anti-radical activities of the hydro-ethanolic extract of Strychnos camptoneura

This test was performed according to the DPPH radical reduction method as described by [18]. This method was also used by [19].

It consists in reducing the DPPH by the antioxidant substances contained in the hydro-ethanolic extract of *Strychnos camptoneura.* This method consists of mixing the solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) at 10 mg / 250mL in ethanol and 100 µl of extract at concentrations of 40; 20; 10; 5; 2.5 mg/ml with the hydro-ethanolic extract. The activity was then measured at 517 nm after 30 minutes of dark incubation using a UV-visible spectrophotometer compared to (Lamaison et al., 1988). The calculation of the percentages of inhibition was according to this relation:

$$\%\ \text{I} = \frac{[\text{D. O blank} - \text{D. OEI}]}{\text{D. O blank}}\ \text{X}\ 100$$

OD blanc = Optical density of the negative control; ODEI= Optical density of the extract/inhibitor

The concentration that inhibits 50% of DPPH (IC50), is determined graphically.

2.3.10. Anti-oxidant activity of the hydro-ethanolic extract of Strychnos camptoneura

This chromatographic technique was performed with the 50% (v/v) hydro-ethanolic extract.

An eluent system and a developer were used to highlight the polyphenols

- The ethyl acetate/formic acid/water (8/1/1) system followed by sputtering with Neu's reagent [20] , Plant Drug Analysis, a Thin Layer Chromatography Atlas, 2nd Ed, Springer, NY, USA), for polyphenols (λ = 366 nm) ;
- The Neu reagent is used for flavonoids because, it forms a complex with the hydroxyl-flavone moiety that is highly visible under UV light at 365 nm. Neu is obtained from a 1% (w/v) solution of 2-aminoethyldiphenylborate and 5% (w/v) PEG400 in methanol.

2.4. Determination of total polyphenols

The determination of total polyphenols was carried out using a spectrophotometer. We determined the optical densities of our extracts and then compared the result to that obtained by a standard of gallic acid of known concentration. The assay was as follows: to 0.1 ml of plant extract introduced into a test tube, were added 0.9 ml of distilled water; 0.9 ml of Folin-Ciocalteu reagent (1N); then immediately 0.2 ml of a $Na2CO_3$ solution (20%). The resulting mixture was incubated at room temperature for 40 minutes in the dark. The absorbance was then measured with a spectrophotometer at 725 nm against a methanol solution used as a blank. Note that a calibration line was previously performed before the analysis with gallic acid under the same conditions as the samples to be analyzed. The results obtained were expressed as mg gallic acid equivalent per 100 grams of dry matter (mgEGa/100 gMs) [21].

2.5. Determination of total flavonoids

Total flavonoids were also determined using a spectrophotometer as follows: 250 µl of the extract and 1 ml of distilled water were successively introduced into a test tube. At the initial time (0 minutes), 75 µl of a NaNO2 solution (5%) was added, followed by 75 µl of $AlCl_3$ (10%) 5 minutes 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 were expressed as mg rutin equivalent per 100 grams of dry matter (mgERu/100g Ms). A calibration curve was developed with Rutin standard solutions prepared at different concentrations [22].

2.6. Statistical analysis of the collected data

Statistical analysis of the collected data was performed using analysis of variance (ANOVA), Student's t-test and Mann-Whhitney test to compare the "test" groups. The results are expressed as mean ± standard error with $p < 0.05$ as the significance level.

3. Results

3.1. Effect of hydro ethanolic extract of *Strychnos camptoneura* on microscopic and macroscopic characteristics of semen

The effects of the hydro-ethanolic extract of *Strychnos camptoneura* trunk bark on the characteristics of spermatozoa are summarized in Table I. The results show that the administration of hydroethanolic extract of *Strychnos camptoneura* (100 and 250 mg /kg/po) orally in rats caused significant increases ($p < 0.05$; $p < 0.01$ and $p < 0$,001) in the concentration and mobility rates of spermatozoa compared to the rats of the control lot treated with distilled water. On the other hand, sperm vitality and normal sperm rates were only significant ($p < 0.01$) at 100 and 250 mg /kg of *Strychnos camptoneura*, respectively, compared to negative controls. In addition, sperm pH showed no variation ($p > 0.05$) regardless of the doses administered. These results show a significant increase ($p < 0.01$) in sperm concentration and vitality levels in testosterone enanthate treated rats compared to the control batch animals given distilled water.

Table 1 Effect of hydro-ethanolic extract of *Strychnos camptoneura* on microscopic and macroscopic characteristics of semen

Characteristics of spermatozoa	Treatment			
	Negative control 0.5 ml/100g	Enanthate of testosterone 3.6 mg/kg	Extract. H.ES.C 100 mg/kg	Extract H.ES.C 250 mg/kg
pH	6±0.28	6.5±0 .40ns	6±0.20ns	6±0.22ns
Number of spermatozoa / epididymis tail (x 10^6)	12±0.5	20±0.40**	20±0.81**	14±4.80 *
Mass mobility of spermatozoa (%)	17 ± 0.01	18 ± 0.02ns	72 ± 0.01***	79 ±0.00***
Sperm vitality (%)	60 ±0.04	77 ±4.08**	80 ±4.09**	63 ±4.4ns
Normal spermatozoa (%)	60 ± 0.04	65 ± 0.06ns	65 ± 0.05ns	80 ±0,00**
Abnormal spermatozoa (%)	40 ± 0.04	35 ± 0.10ns	35 ± 0.05ns	20 ± 0.00**

Values are means ± MSE, with n = 5. *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$significant difference from controls (distilled water), ns: $P > 0.05$non-significant difference from controls (distilled water) and H.E.S..C: *Strychnos camptoneura* Hydro-ethanolic

3.2. Antiradical activity of the hydro-ethanolic extract of *Strychnos camptoneura*

Figure 15 shows the evolution of the percentage of inhibition of free radicals as a function of concentration. It appears from this analysis that the highest percentage is 63.24% with an inhibitory concentration 50 of 23.64 mg/ml.

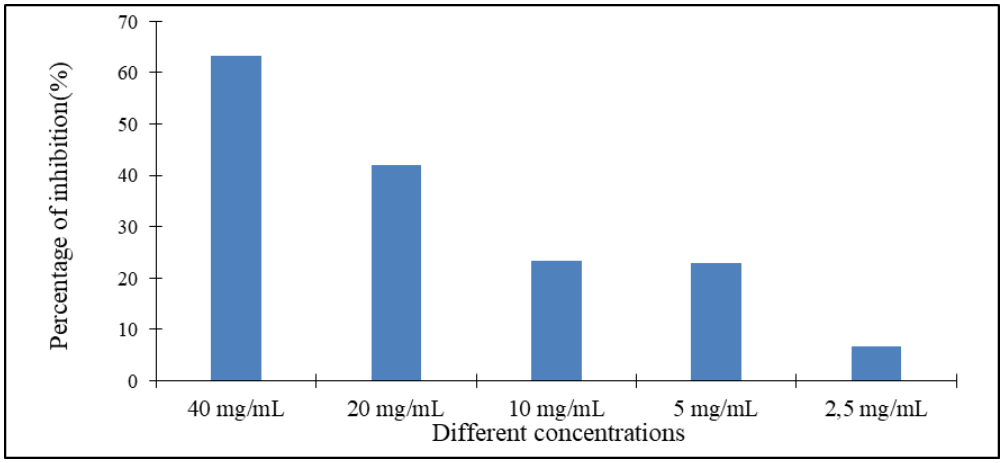

Figure 1 Percentage of DPPH inhibition by different concentrations of the hydro-ethanolic extract of *Strychnos camptoneura*

3.3. Anti-oxidant activity of the hydro-ethanolic extract of *Strychnos camptoneura*

Thin layer chromatography (TLC) of the hydro-ethanolic extract of the bark of the trunk of *Strychnos camptoneura*, followed by a revelation with Neu (figure B) and DDPH (figure C), shows that the tested extract is full of substances with anti-radical activities. It appears from these figures

- The figure A, marks the Co-migration of the different compounds to the visible light to a system of eluent formed by the acetate of ethyl, the formic acid and the water to the proportions 8/1/1, what allowed then to put in values its different compounds.
- The figure B, expresses a succession of spots of light blue fluorescence, light green with a yellow orange trail at the frontal retentions (0,4; 0,6 and 0,7) which will be allotted to the compounds of flavonoidic type and acid phenols.
- The figure C, materializes the highlighting of the anti radical activity by the revelation of the plate with DDPH. The tested extract shows the presence of yellow fluorescent compounds on violet light (figure C), which confirms that these different secondary metabolites are free radical scavengers (DPPH).

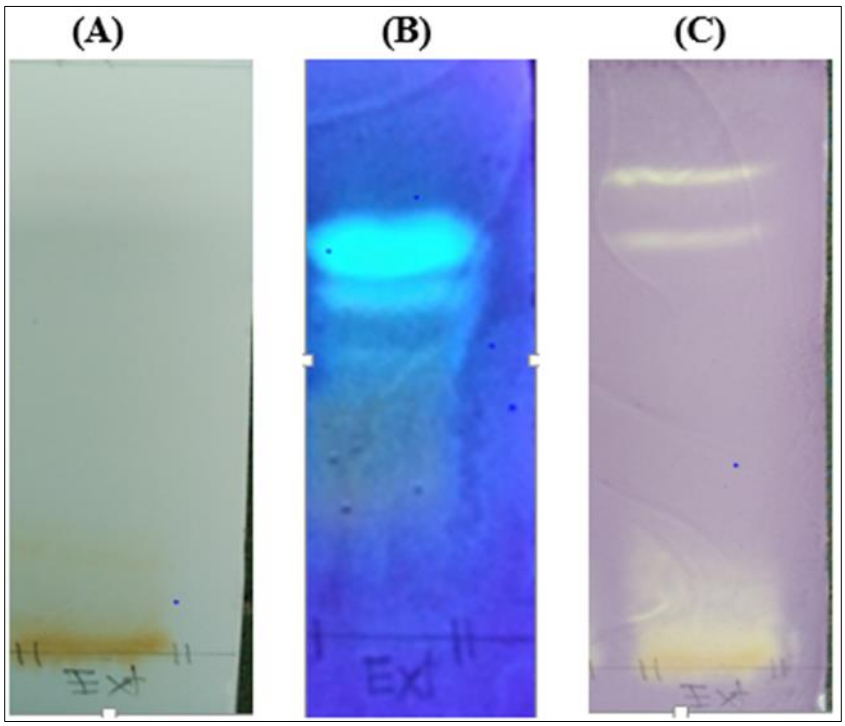

Figure 2 Detection of the different compounds on TLC of the hydroethanol extract of *Strychnos camptoneura* from the DDPH radical

Eluent: Ethyl acetate/formic acid/water (8/1/1)

Plate A: chromatogram under visible light

Plate B: UV-Visible chromatogram after development with NEU

Plate C: visible light chromatogram after DPPH revelation

3.4. Chemical analyses of the hydro-ethanolic extract of *Strychnos camptoneura* trunk bark

The results of the quantitative analyses by UV-visible spectrophotometer of the hydro-ethanolic extract of *Strychnos camptoneura* studied are represented by figure 3

This figure shows that the contents of total polyphenols are quantitatively higher than those of total flavonoids with respective values of 693 mg EAG /gMS and 222 mg EO/g MS.

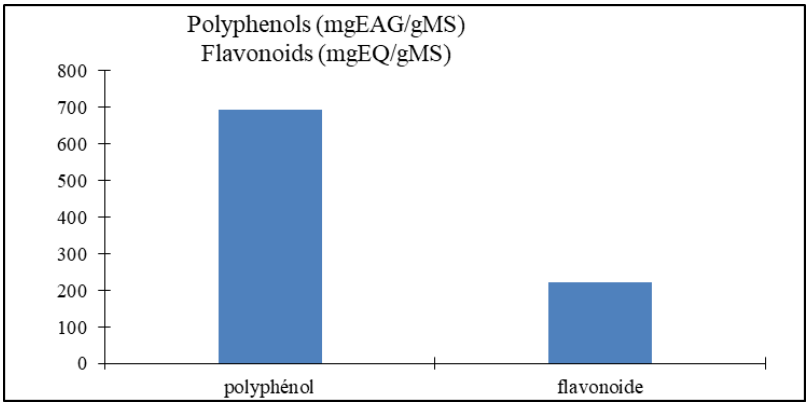

Figure 3 Determination of total polyphenols and flavonoids

4. Discussion

The main objective of this study was to evaluate the spermatogenic and antiradical potentialities of the hydro-ethanol extract of *Strychnos camptoneura*. It should be noted that sufficient sperm production in the ejaculate is an important criterion to assess the quality of the semen because insufficient production could be a cause of infertility in the subject [13,14]. Similarly, motility is an important index of sperm vitality. In poor quality ejaculates, sperm move slowly and exhibit non-straight movements [12]. The results obtained show that oral administration of the hydroethanolic extract of *Strychnos camptoneura* in rats at the doses studied caused a significant dose-dependent increase in the concentration per epididymis tail and in the rate of sperm mass motility compared to the control rats treated with distilled water. Our results are in agreement with those of [23], [8] and [24] who observed a significant increase in sperm concentration and motility in rats treated with ethanolic and aqueous extracts of *Pausinystalia yohimbe* and aqueous extract of *Kigelia africana* fruits respectively. Similarly, a significant dose dependent increase in sperm motility and concentration was observed by [25], [26] and [27] in rats treated with aqueous extract of *Zanthoxylum macrophylla* and ethanolic extracts of *Chlorophytum borivilianum* and *Tribulus Terrestris* with *Anacyclus Pyrethrum* respectively. This study also showed a significant increase in the percentage of vital and normal spermatozoa in rats treated with the hydro-ethanolic extract of *Strychnos camptoneura* at the respective doses of 100 and 250mg/kg in contrast to rats that received distilled water. Our results contradict those found by [5] who observed no variation in sperm concentration, vitality and morphology in rats treated with Haloperidol and Clomipramine. This difference would be attributed on the one hand to the types of drugs administered and on the other hand to the chemical composition of these products. It has been proven in the literature that some drugs (xenobiotics) would have harmful effects on the quality of spermatozoa at the origin of male infertility [4,5]. The pH of rats treated with the extract showed no variation compared to the control batch. This result is close to that noted by [5] 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 [5].

The improvement of the characteristics of the quality of the semen observed during this study suggests that the hydro-ethanolic extract of *Strychnos camptoneura* would contain a certain number of phenolic compounds (flavonoids, polyphenols ...) revealed during the chemical analyses which would confer anti-oxidant potentialities to it and would act as scavengers of free radicals [8] thus limiting the deleterious effects of these last ones on the spermatozoids. Indeed, the membrane of spermatozoa is particularly rich in polyunsaturated fatty acids, which makes them especially sensitive to reactive oxygen species (ROS) derived from oxygen metabolism. In addition to 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 acrosomal reaction, loss of motility, possibly leading to infertility [8]. In the light of the results, we can deduce that the hydro-ethanolic extract of *Strychnos camptoneura* trunk bark has a positive effect in the reduction of free radicals which would be attributed to its high flavonoid content.

5. Conclusion

The improvement of the characteristics of the semen and the reduction of the DPPH radical observed in the present work, justify its biological potentialities and its use in traditional medicine in the treatment of male infertility.

Compliance with ethical standards

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Disclosure of conflict of interest

There are no known conflicts of interest associated with this publication.

Statement of ethical approval

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

References

[1] Fleurentin j, Balansard g, (2002). Ethnopharmacological interest in the fields of medicinal plants, Tropical medicine ; (62) : 23-28

[2] Fainberg Jonathan, Kashanian, James A, (2019). Recent advances in understanding and managing male infertility. F1000 Research: 1-8.

[3] Ngoula F, Guemdjo TM, Kenfack A, Tadondjou CD, Nouboudem S, (2017). Effects of heat stress on some reproductive parameters of male cavie (Cavia porcellus) and mitigation strategies using guava (Psidium guajava) leaves. Journal of Thermal Biology; 64:67-72.

[4] 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.

[5] Landry Martial Miguel, Jean Alfred Mbongo, Jean De Dieu Bimangou Bia Nzambi,Etienne Mokondjimobé, Léon Hervé Iloki and Ange Antoine Abena,(2019). Latrogenic male infertility with psychotropic drugs in rats: case of haloperidol and clomipramine. International journal of scientific research, (8): 1-3

[6] Abarikwu S. O, Adesiyan A. C, Oyeloja T. O, Oyeyemi M. O, and Farombi E. O, (2010). Changes in sperm characteristics and induction of oxidative stress in the testis and epididymis of experimental rats by a herbicide, atrazine. Arch Environ Contam Toxicol.; 58: 874-882.

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

[8] Akassa H, Makemba Nkounkou G.S, Nkoua Badzi C, Etou Ossibi A.W, Tamboura H.H, Abena A.A, (2019). Effects of the aqueous extract of the trunk barks of *Pausinystalia yohimbe* on the semen quality and reproduction performances in the male Wistar rat. Int. J. of Multidisciplinary and Current research; 7: 732-740.

[9] Morabandza C.J, Ondele R, Elion Itou R.G, Etou Ossibi A.W, Imbiella C, Mokondjimobe E, Ongoka P.R, Abena A.A, (2017). Aphrodisiac activity of aqueous and hydroethanolic extracts of the stem bark of *Strychnos camptoneura* (Longaniaceae) in wistar rat. Asian Journal of Science and Technology; 08 (10): 6055-6059.

[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] 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.

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

[13] Oyedeji K. O, Bolarinwa A. F, Azeez A. A. (2013a). Effect of methanolic extract of Vernonia amygdalina on reproductive parameters in male albino rats. Academic Sciences. Asian Journal of Pharmaceutical and Clinical Research ; 6(2): 234-236.

[14] Oyedeji K.O, Bolarinwa A.F, Akinbode A.A. (2013b). Effect of Corchorus olitorius extract on reproductive functions in male albino rats. Academic Sciences. International Journal of Pharmacy and Pharmaceutical Sciences ; 5(3): 427-431.

[15] Bjorndhal L, Barratt C. L, Kvist U, (2003).Evaluation of the one-step eosin-nigrosin staining tehcnique for human sperm vitality assessment. Human Reproduction ; 18: 813-816.

[16] Dumont P. Appreciation of the sexual function of the bull reproduccer , (1997). Le point vétérinaire ; 28 : 19-32.

[17] Abdel-Azim A, El-Kamash E. M, (2011). Evaluation of semen quality and its relation to mating system for some breeds of rabbits under environmental conditions in the middle of Egypte; Egypte poultry Science.; 31(2): 467-480.

[18] Brand-Williams W, Cuvelier M.E, and Berset C, (1995). Use of free radical method to evaluate antioxidant activity. Technology28: 25-30.

[19] Huang D.B, Prior R.L, (2005). The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry; 53: 1841-1856.

[20] Wagner H, Bladt S, (1996). Plant Drug Analysis, a Thin Layer Chromatography Atlas, 2nd Ed, Springer, NY, USA.

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

[22] Subhasree B, Baskar R, Keerthana R. L , Susan R. L, & Rajasekran P,(2009). Evaluation of antioxidant potential in selected green leafy vegetables. Food Chemistry; 115: 1213 -1220.

[23] Ogwo, E.U, Osim E.E, Nwankwo A.A, Ijioma S.N, (2016). Semen Quality in Male Albino Rats Fed with Various Concentrations of Pausinystalia Yohimbe Bark Powder (Burantashi). Journal of Medical and Dental Science Research; 3:16-24.

[24] Maïmouna Coura Koné, Blaise Wokapeu Kpan2, Ibrahima ,(2021). Effect of Different Parts of Kigelia africana Fruit Aqueous Extracts on Sperm Parameters and Testis Advances in Reproductive Sciences; 9: 171-188.

[25] 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.

[26] Syed Ammar Hussain , Ahsan Hameed, Furqan Nasir, Yang Wu, Hafiz Ansar Rasul Suleria and Yuanda Song,(2018). Evaluation of the Spermatogenic Activity of Polyherbal Formulation in Oligospermic Males.BioMed Research International :1- 10.

[27] Dariush Haghmorad,Mohammad Bagher Mahmoudi , Pardis Haghighi , Paria Alidadiani , Ensieh Shahvazian , Parsova Tavasolian , Mahmoud Hosseini , Mahmoud Mahmoudi, (2019). Improvement of fertility parameters with Tribulus Terrestris and Anacyclus Pyrethrum treatment in male rats. Int Braz J Urol; 45 (5): 1043-1054.