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Annona muricata Fruit Epicarp Improves Spermatogenesis in *Rattus norvegicus*

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Abstract

This study was part of the program to promote medicinal plants used in traditional medicine. Its objective was to demonstrate the effect of the aqueous extract of the epicarp of the fruit of *Annona muricata* on reproduction in mammals: the case of the rat *Rattus norvegicus* of the Wistar strain. The phytochemical study revealed the presence of polyphenols, quinones, sterols and terpenes, saponosides, alkaloids, leucoanthocyanins, flavonoids and cardiotonic glycosides; however, the absence of anthraquinones, phlobatannins, indoles, and antracenosides. Also, the antioxidant activity of the plant is 12.5 µg/mL. In addition, the acute toxicity study at a single dose of 2000 mg/kg BW orally revealed that the aqueous extract of the epicarp of the fruit of Annona muricata is not toxic and has an LD50 by oral route greater than or equal to 5000 mg/kg EP. Furthermore, the study of the androgenic effect of the aqueous extract of the fruit of Annona muricata was evaluated by observing the parameters of reproduction at different doses of the extract. An increase in concentration, mobility and a constant percentage of morphology was observed. This confirms that our plant has androgenic effects improving reproduction in mammals.

Keywords

Annona Muricata, Triphytochemical, Antioxidant Activity, Androgenic Effects

1. Introduction

Infertility is defined as the inability of a couple to conceive after at least one year

of regular unprotected intercourse [1]. Infertility can be primary or secondary [2]. In Africa, the prevalence of primary infertility is estimated at approximately 49.91%, with 41.57% in West Africa. Secondary infertility affects 49.79% of the population in Africa, with a prevalence of 57.64% in West Africa [3]. In most cases, women are the most stigmatized, making them victims of numerous forms of discrimination [4]. However, in 20% to 30% of infertility cases, the man is responsible, accounting for 50% of all cases [5]. Infertility in male couples can be caused by factors such as abnormalities in the reproductive organs and sperm parameters. In the latter case, the quality of male semen is therefore considered a biological indicator of male fertility [6].

In Côte d'Ivoire, infertility is a public health problem. Managing infertility is difficult due to the limited number of assisted reproductive technology (ART) centers, their concentration primarily in Abidjan to the detriment of rural areas, the small number of fertility specialists, and the high cost of ART techniques. Furthermore, existing treatment methods are not entirely effective. Indeed, although assisted reproductive technologies (ART) have been medically validated for several years and are commonly used, live birth rates per cycle are low, not exceeding 30% - 40% [7].

In fact, the low success rates of ART techniques often force patients to undergo further attempts. Given this high prevalence of infertility and the shortcomings observed in its management, research aimed at improving fertility and offering more accessible treatment alternatives is crucial. In this context, herbal medicine represents a significant alternative in the fight against infertility.

In Africa, people regularly use plants to treat infertility [8]. This practice is deeply ingrained in local customs, with rates ranging from 70% to 95% in developing countries [9]. The World Health Organization (WHO) recognizes the use of plants as a therapeutic method for treating various medical conditions. Consequently, several initiatives aimed at promoting and advancing traditional medicine on a global scale have been implemented since 2002. The new global strategy for the 2025-2034 period seeks to address global needs regarding traditional, complementary, and integrative medicine [10]. Indeed, plants have an unlimited capacity to synthesize aromatic secondary metabolites [11] [12]. These metabolites are biologically active and have several applications, including the development of pharmaceutical products [13] [14]. Consequently, the isolation and identification of these secondary metabolites, as well as their use as active ingredients, have attracted the attention of numerous researchers seeking to promote the appropriate use of herbal medicine [15] [16].

The Ivorian pharmacopoeia is rich in several plant species with numerous therapeutic applications. Among these species is Annona muricata, commonly known as soursop. According to ethnobotanical surveys, Annona muricata is widely used in traditional medicine to treat various ailments. Specifically, regarding fertility, an ethnobotanical study by Gbonsou *et al.* [17] showed that the leaves treat azoospermia. The fruit, and particularly the pulp, is widely consumed and highly val-

ued for its flavor. The fruit has a high content of nutrients and bioactive compounds, including phenolic compounds, alkaloids, flavonoids, and vitamins [18]-[20]. These compounds have recognized antioxidant and androgenic properties [21]. However, very few studies have identified the specific part of the fruit and the compounds it contains responsible for the androgenic effect. It is in this context that we focused on the epicarp of the unripe fruit. Alcoholic extracts of the fruit peel have been shown to have higher antioxidant activity than the whole fruit [22]. In contrast, very few studies address the fertilizing properties of the aqueous extract of the fruit epicarp. The overall objective of this work is to determine the effects of the aqueous extract of *Annona muricata* fruit epicarp in spermatogenesis. From this perspective, phytochemical sorting, toxicity, antioxidant activity, and sperm parameters were analyzed.

2. Material and Methods

2.1. Animals

Guidelines for the use of laboratory animals were strictly followed in accordance with the DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes. The study was conducted with 26 rats (*Rattus norvegicus*), 20 males and 6 females of Wistar strain, aged of 12 weeks and whose weight was between 150 et 180 g. They were purchased and raised in the vivarium of Felix Houphouët-Boigny University. During the experimentation, rats were kept in cages in the vivarium with appropriate lighting ensuring a 12-hour cycle of light and darkness. Humidity was between 60 and 90 during experimental period. The animals have unlimited access to drinking water, distributed by water bottles installed on the cages. Their diet consists of pellets, bread, and a feed formulation composed of bread, corn, soy, fish.

2.2. Collection of Annona Muricata Fruit

Fruits of Annona muricata were purchased from a local market in Abidjan.

2.3. Extraction

The epicarp of *Annona muricata* fruit was isolated and then dried at 25˚C for three weeks. After grinding, an aqueous extraction was performed by macerating 100 g of the ground fruit in 750 mL of distilled water for 48 hours. This operation was repeated twice. The aqueous phases obtained after filtration were collected on cotton. The solvent was removed in an oven. The aqueous epicarp's extract (AEEAM) (1.2%) of *Annona muricata* fruit was thus obtained, and then stored at 25˚C in a tinted glass jar in a cupboard, away from moisture and sunlight.

2.4. Phytochemical Sorting

The major chemical groups were identified by color and precipitation tests in test tubes according to the methods of [23] and [24]. The presence of the following

secondary metabolites was investigated: sterols and terpenes, alkaloids, polyphenols, saponins, anthocyanins, tannins (catechin and gallic), phlobatannins, cardiotonic glycosides, flavonoids, leucoanthocyanins, anthraquinones, quinones, indoles, and anthracenosides.

2.5. Antioxidant Activity

Antioxidant activity was evaluated according to the method of Scherer and Godoy [25] based on the extract's ability to reduce DPPH, a free radical. Seven ranges of the extract with concentrations from 200 to 3.125 µg/mL (200-100-50-25-12.5-6.25-3.125), obtained by progressively diluting a 400 µg/mL stock solution, were prepared. The reaction medium changes from violet to complete decolorization when a reaction occurs, depending on its intensity. An equal-volume mixture of ethanol and DPPH was used as a control. Optical densities (OD) were read three times at a wavelength of 517 nm. Standards such as vitamin C and quercetin were used as references. The percentages of DPPH inhibition are determined by the formula: I: Inhibitory power (in%) = $[(A_0 - A_1)/(A_0)]*100$; where A_0 represents the blank and A_1 the absorbance of the DPPH solution in the presence of the extract. The IC_{50} represents the extract concentration required to scavenge 50% of the radicals. The IC_{50} values for the extract and the reference samples were determined using linear regression. Indeed, the IC_{50} corresponds to the concentration obtained by projecting the intersection between the inhibition curve and the parallel to the concentration axis passing through 50% of inhibition.

2.6. Acute Toxicity Study of the Aqueous Extract of Annona Muricata Epicarp

The acute oral toxicity study was conducted in accordance with OECD guideline 423, established by the acute toxicity class [26]. Rats were divided into two groups, n = 3 for each group. Rats in group 1 received 1 mL/kg of body weight of distilled water. For group 2, 2000 mg/kg of body weight was administered via a gastric tube. The animals were placed in individual cages for observation. Observations were performed after 30 minutes, 4 hours, 24 hours, one week, and two weeks. During the 14-day observation period, the animals were weighed every two days at the same time.

2.7. Animals Treatment

The extract (EAAM) was administered to rats according to OECD guideline 441 [27]. A total of 20 rats weighing between 150 and 180 g were divided into 4 groups (n = 5 for each group): the control group received distilled water (1 mL/kg of body weight), group 1 was treated with 125 mg/kg of body weight of AEEAM, group 2 received 250 mg/kg of body weight AEEAM, and group 3 was treated with 500 mg/kg of body weight of AEEAM. The different doses of the extract and distilled water were administered orally daily for 54 days. The animals were weighed every three days during the 54-day treatment period. Weight gain curves for each group

of animals during the treatment period were then established. After 54 days of daily administration, the rats in each batch are sacrificed after anesthesia with ether.

2.8. Body and Relative Organ Weigt

The reproductive organs (testis, prostate, seminal vesicles, Cowper's gland, levator ani muscle) were immediately removed and weighed after the animals were slaughtered. The relative weight of these organs was calculated using the following formula: Relative weight of the organ = (weight of the organ × 100)/weight of the animal.

2.9. Semen Parameters

Semen analysis was performed according to the method described by [28]. Before the animal was sacrificed, the right caudal epididymis was removed, incised, and diced in 10 ml of a 0.9% NaCl saline solution previously heated to 36˚C.

2.9.1. Sperm Concentration

A drop of the saline solution containing spermatozoa is placed between a slide and a Malassez coverslip and examined under an Olympus microscope at 400x magnification. Sperm counting is performed under an Olympus optical microscope at 100x magnification. The number of spermatozoa is determined from 5 randomly selected fields. Sperm concentration is estimated using the following formula: $N = X \times fd \times 10^6/4$. X = Number of sperm counted in 5 grids of 20 small squares of the Malassez cell; Fd = Dilution factor (10); N = Number of sperm per mm^3.

2.9.2. Sperm Motility

A drop of the saline solution containing spermatozoa is placed between a slide and coverslip and examined under a microscope (Olympus) at 400x magnification. Motile and non-motile spermatozoa are rapidly quantified in five fields chosen at random from a population of spermatozoa per field, and then the percentage of motile spermatozoa is determined using the following formula: Percentage of motile sperm = (number of motile sperm/total number of sperm) × 100.

2.9.3. Sperm Morphology

To assess sperm morphology (presence or absence of normal heads, mead piece, and flagella), a drop containing sperm and 0.9% saline solution was spread onto a slide. The slide was stained with methylene blue followed by eosin and allowed to air-dry. The stained slides were then examined under a microscope at 400x magnification. During examination, ten (10) spermatozoa were counted in each of ten (10) randomly selected fields per slide, resulting in a total count of 100 spermatozoa per rat. In each ten counted spermatozoa, we have distinguished the number of normal and the number of abnormal spermatozoa. The percentage of normal spermatozoa was expressed using the following formula: Percentage of normal sperm = Percentage of normal sperm = (number of normal sperm/total number of sperm) × 100.

2.10. Statistical Analyses

Statistical analyses of the experimental results were performed using GraphPad Prism 7 software (Microsoft, USA). Values are presented as mean ± standard deviation. The weight of vital organs, reproductive organs, and sperm parameters were evaluated using one-way ANOVA. Tukey's multiple comparison test at a significance level of 5% was applied to assess the significance of the observed differences. If $p < 0.05$, the difference between the values is considered significant; if $p < 0.001$, this difference is considered highly significant; if $p < 0.0001$, it is considered extremely significant; and if $p > 0.05$, the difference was not significant. Furthermore, the graphical representations were generated using the same software.

3. Results

3.1. Phytochemical Sorting

The phytochemical sorting carried out on the AEEAM allows identification of chemical constituents of varying abundance recorded in Table 1. This table shows in particular the presence of sterols and terpenes, alkaloids, polyphenols (anthocyanins, catechin and gallic tannins, flavonoids), saponosides, quinones, leucoanthocyanins, cardiotonic glycosides in this extract of the plant.

Table 1. Chemical groups characterized in AEEAM.

Secondary Metabolites			Tests	Results
Sterols and Terpenes			Liebermann-Burchard	+++
Alkaloids			Dragendorff	++
Antracenosides			Borntraeger	-
Saponosides			Foam formation	+++
Polyphenols	Total Polyphenols		Ferric chloride	+++
	Anthocyanins		-	+++
	Leucoanthocyanins		Cyanidin	++
	Flavonoids		Cyanidin	+
	Tannins	Catechic	-	+++
		Gallic	Stiasny	+++
		Phlobatannins	-	-
Indoles			Salkowski	-
Cardiac glycosides			Keller-Killiani	+
Anthraquinones			-	-
Quinones			Borntraeger	+++

Note: (+++) Strong presence; (++) Medium presence; (+) Weak presence; (-) Negative reaction.

3.2. Antioxidant Activity

The antioxidant activity curves for vitamin C, quercetin, and AEEAM are shown in Figure 1. Antioxidant activity is expressed as IC_{50}. The values obtained for vitamin C, quercetin, and AEEAM are 3.75 µg/mL, 1.75 µg/mL, and 12.5 µg/mL, respectively. Although the extract exhibits antioxidant activity, it is less intense than that of quercetin and vitamin C, which are reference compounds.

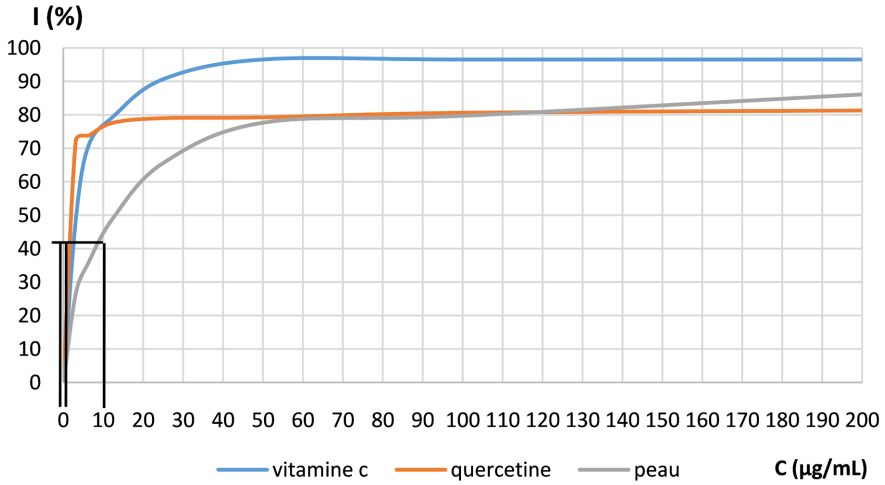

Figure 1. Antioxidant activity of vitamin C, quercetin and aqueous extract of the AEEAM.

3.3. Evaluation of Acute Oral Toxicity

Rats treated with AEEAM at dose of 2000 mg/kg of body weight showed no apparent signs of toxicity as shown in Table 2.

Table 2. General condition of the animals after administration of the aqueous extract of the epicarp of the fruit of *Annona muricata.*

Settings	Treatments	
	Control	2000 mg/kg of bw
Number of animals	03	03
Mobility	N	N
Aggressiveness	N	N
State of Stools	N	N
Sensitivity to pain	N	A
Tail condition	N	N
Vigilance	+	+
Number of deaths	0	0
Drowsiness	+	+
Salvation	--	--
Coma	A	A

A: Absent; N: Normal; +: Yes; --: No reaction.

The weight of the treated rats changed gradually during treatment, with a significant variation compared to the control rats. Furthermore, no mortality was observed in these rats in the minutes, hours, and days following gavage (Figure 2).

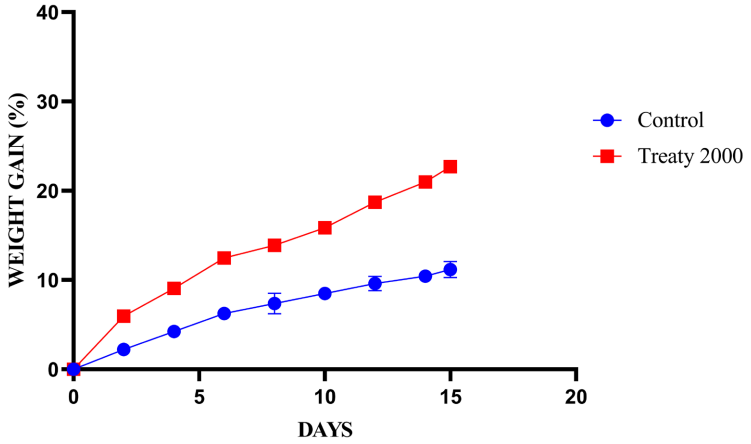

Figure 2. Acute toxicity curve of AEEAM in rat.

3.4. Effects of the AEEAM on Reproductive Parameters

3.4.1. Evolution of Body Weight

The body weight of the rats in the different groups (Figure 3) increased progressively over the 54 days. This increase was observed as early as day 7. Between days 7 and 28, the weight gain was greater in the treated groups than in the control group. However, from day 28 onward, the weight of the control group increased dramatically until day 54. Although the weights of the treated groups continued to increase, they nevertheless remained lower than those of the control group.

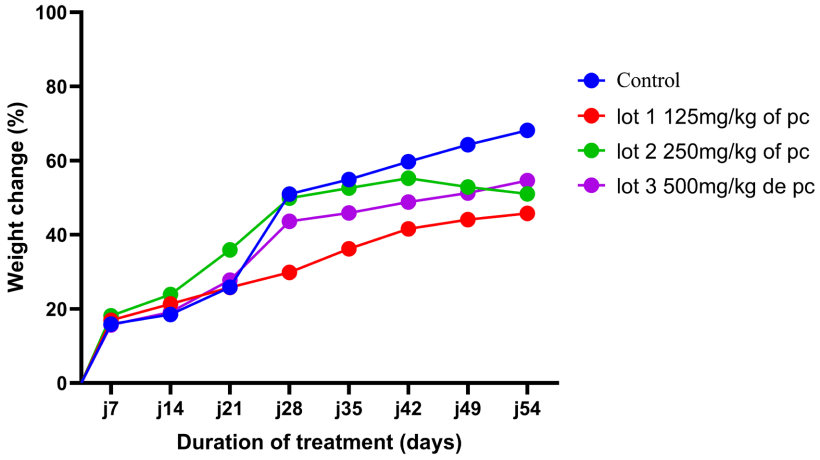

Figure 3. Effect of aqueous extract of AEEAM on body weight.

3.4.2. Reproductives Organs Relative Weight

AEEAM administered to rats at doses of 125, 250, and 500 mg/kg of body weight, resulted in a change in the relative weight of reproductive organs after 54 days of treatment (Table 3). This was particularly noticeable in the testes, where the fresh

weight increased in a dose-dependent manner. Compared to the control group, the fresh weight of the prostate gland increased at the 125 mg/kg of body weight dose. However, a decrease was observed at the 250 and 500 mg/kg of body weight doses. For the levator ani muscles and Cowper's glands, a slight dose-dependent increase was observed up to the 250 mg/kg of body weight dose. At 500 mg/kg of body weight, the fresh weight of these organs also increased compared to the control group, but the increase was lower than at the 250 mg/kg of body weight dose. Regarding the seminal vesicle, a decrease in relative weight was observed at doses of 125 and 250 mg/kg of body weight compared to the control group. Conversely, this weight increased at the dose of 500 mg/kg of body weight. Although variations were observed for all organs studied, the differences were not statistically significant.

Table 3. Evolution of the weight of reproductive organs.

	Control	Treated 125 mg/kg	Treated 250 mg/kg	Treated 500 mg/kg
Testis	0.612 ± 0.022	0.615 ± 0.020	0.621 ± 0.167	0.652 ± 0.024
Levator muscle	0.285 ± 0.019	0.297 ± 0.031	0.407 ± 0.093	0.301 ± 0.016
Prostate	0.231 ± 0.032	0.243 ± 0.037	0.194 ± 0.042	0.194 ± 0.020
Seminal vesicle	0.460 ± 0.061	0.390 ± 0.00	0.393 ± 0.070	0.465 ± 0.04
Cowper's glands	0.019 ± 0.002	0.021 ± 0.002	0.022 ± 0.003	0.015 ± 0.002

3.4.3. Sperm Parameters

1) Sperm Concentration

The AEEAM resulted in a dose-dependent and significant increase in sperm concentration at doses of 125 (34.95×10^6 spz/mL ± 2.175; $p < 0.05$), 250 (41.75×10^6 spz/mL ± 3.144; $p < 0.01$) and 500 mg/kg of body weight (65.05×10^6 spz/mL ± 3.286; $p < 0.001$) compared to the control group (23.70×10^6 spz/mL ± 1.348) after 54 days of treatment (Figure 4).

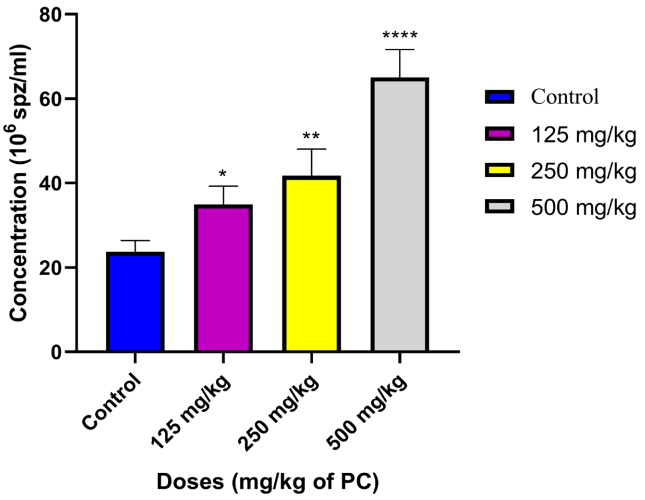

Figure 4. Effect of aqueous extract of the AEEAM on sperm concentration.

2) Sperm Motility

After 54 days of treatment, an increase in sperm motility was observed in all treated groups compared to the control group (Figure 5). For the 125 mg/kg of body weight dose, 78% of sperm were motile, a highly significant difference compared to the control (63%) ($p < 0.001$). The 250 mg/kg of body weight dose showed 73% motility. In this case, the difference compared to the control was not significant ($p > 0.05$).

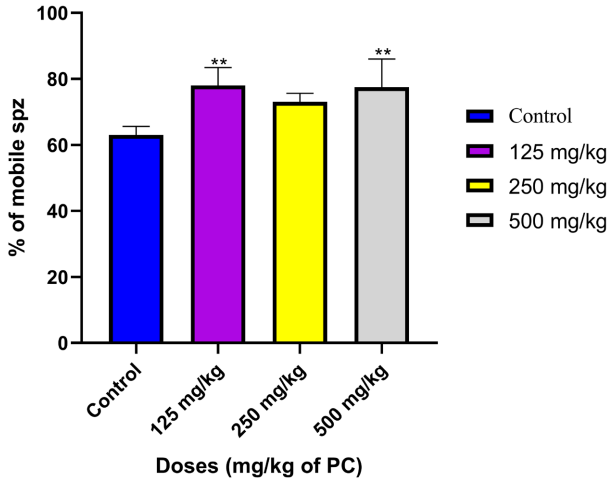

Figure 5. Effect of AEEAM on sperm motility.

3) Sperm Morphology

Observation of the morphology (head and flagellum) of sperm from rats treated with doses of 125, 250, and 500 mg/kg of body weight of AEEAM (Figure 6) revealed respective rates of 100.0% ± 00, 99.60% ± 0.54, and 100.0% ± 00 of normal sperm. These values, all approaching 100% normal sperm, show no significant difference ($p > 0.05$) compared to that of the control group, which was 99.60% ± 0.54.

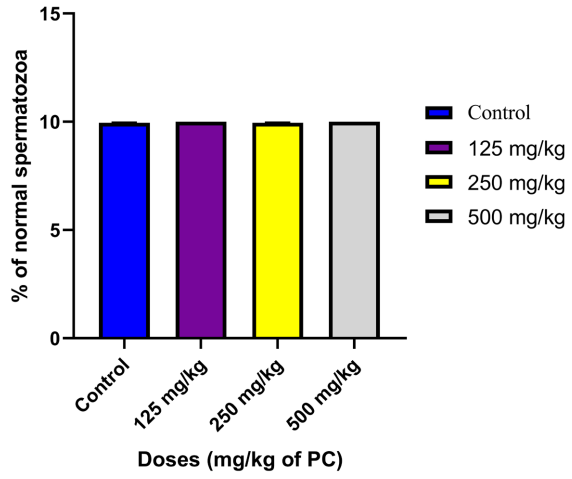

Figure 6. Effect of AEEAM on sperm morphology.

4. Discussion

The overall objective of this work was to determine the effects of the AEEAM on spermatogenesis. To this end, we determined its chemical composition, antioxidant activity, toxicity, and finally, its effect on sperm parameters.

The phytochemical study of the AEEAM revealed the presence of chemical groups such as sterols and terpenes, polyphenols, flavonoids, tanins (catechics and gallics), alkaloids, saponins, anthocyanins, and quinones. The phytochemical composition of the different organs (whole fruit, seeds, leaves, bark, roots) of *Annona muricata* is documented. However, very little data has determined the chemical composition of the aqueous extract of the fruit epicarp [29]. The detection of phenolic compounds and flavonoids was consistent with [30]. The majority of secondary metabolites that we identified have been found by [29]. Except tanins, anthocyanins, and steroids that have not been detected by these authors. The differences could be linked to the fact that fruits used in the two studies grown in geographical areas with differing environmental conditions. Indeed, it is known that soil characteristics influence the phytochemical composition of fruit [31]. The second point could be related to the differences in detection methods [32]. The classes of secondary metabolites detected in our extract were also found in the whole fruit (phenols, alkaloids, and flavonoids), the pulp (alkaloids, flavonoids, saponins, and terpenes), and the leaves (tannins and sterols) [33]. The observed similarities suggest two hypotheses: 1) the epicarp contains compounds identical in nature to those found in the other organs mentioned; 2) the compounds in the epicarp and other organs belong to the class of metabolites but are of a different nature. The richness of the epicarp in bioactive compounds enhances the value of the fruit epicarp extract and highlights the potentially interesting pharmacological properties it could possess, just like the other organs of the plant [34].

The antioxidant activity evaluation revealed that the AEEAM fruit has an IC50 higher than that of vitamin C and quercetin. This result showed that the extract exhibits considerable antioxidant activity, even if it was low compared to that of vitamin C and quercetin, that have been used as reference molecules. These results were consistent with Sosa-Crespo *et al.* [35]. These authors showed that aqueous extracts of peels have the highest antioxidant capacity for DPPH and ABTS+ compared to other extracts that they study. It is known that reactive oxygen species are produced during numerous physiological processes, and their excessive accumulation can lead to oxidative stress, causing damage in the body. To prevent oxidative stress, the body uses antioxidants through various strategies. These antioxidants are either endogenous (antioxidant enzymes) or exogenous, provided by certain foods. Our findings about the antioxydant activity of AEEAM suggested a capacity for the elimination of free radicals or hydrogen donation, which were probably related to the higher deprotonation of the hydroxyl groups present in the aromatic rings of the compounds in the peel of soursop [35]. The antioxidant activity of the extract evaluated in this work could be explained by the particular richness of the extract in polyphenols, flavonoids, tannins, quinones and alkaloids,

[36]-[39]. It has showed that plant part or fruit part could have variable antioxydant activity according to the difference of phytochemical composition [40]. Also, the type and the total content of phenolic compounds is directly related to antioxydant power [41]. Here, antioxydant activity of AEEAM was lower than those of the reference molecules. Even if the total content of phenolic compound wasn't evaluated, we could assume that it would be weak.

The acute toxicity assessment of the AEEAM consisted of recording apparent signs of toxicity after oral administration of a single 2000 mg/kg of body mass dose of the extract to adult rats. At this dose, no changes in behavior, signs of intoxication, or mortality were observed. The LD_{50} of the extract was estimated to be greater than 5000 mg/kg of body mass orally. According to the Globally Harmonized System of Chemical Labelling [26] AEEAM is in category 5 or unclassified. Relative toxicity studies have shown that the epicarp of the fruit exhibits no toxicity in animals, just like the leaf [42]. According to OECD 423, the recommended doses would be 250, 500, and 1000 mg/kg of body mass. However, we administered doses of 125, 250, and 500 mg/kg of body mass due to the long duration of treatment. Our results show that ingesting of AEEAM is not harmful, although it is not edible like the pulp of ripe fruit.

During treatment, the weight of rats in the different groups increased progressively over the 54 days. Weight variations between the control and treated groups were observed primarily from day 7 onward. From day 7 to day 28, food intake was significantly stimulated, as the treated groups showed greater weight changes than the control group. Conversely, from day 28 to day 54, the control group showed greater weight changes than the treated groups. These data suggest that the epicarp extract has an orexigenic effect that manifests rapidly from the start of extract administration. However, the intensity of the extract's orexigenic effect is not constant over time, as food intake tends to regulate itself from day 28 onward. The chemical composition of the extract could be responsible for the observed orexigenic effect, particularly due to the presence of polyphenols [43]. Another mechanism could be considered, namely that involving flavonoids and saponins. Indeed, flavonoids are involved in the synthesis and metabolism of androgens [44]. It has been shown that flavonoids inhibit aromatase [45], thus preventing the conversion of testosterone to estrogen. This would contribute to increasing the endogenous concentration of testosterone. As for saponins, they would also contribute to increasing the endogenous level of testosterone by increasing the level of LH [46]. Furthermore, it is known that testosterone has an anabolic effect that increases muscle mass [47].

Daily administration of 125, 250, and 500 mg/kg of body mass of AEEAM for 54 days increased the fresh weight of some reproductive organs, particularly the testes, in a dose-dependent manner. However, the observed differences were not statistically significant. Furthermore, variations were also observed for other organs at the tested doses, but these differences were not statistically significant. The variation in the relative fresh weight of reproductive organs during administration

of AEEAM is poorly documented. The weight, size, and secretory function of the testes, epididymides, seminal vesicles, and prostate are tightly regulated by androgens. Indeed, steroidogenesis is one of the causes of increased weight in the sex organs [48]. The increase in the relative fresh weight of these organs could be considered a biological marker of the extract's effectiveness in stimulating steroidogenesis [49] following the action of flavonoids, saponins, and sterols that can stimulate endogenous testosterone synthesis [50] [51]. However, the numerous variations observed for other androgen-dependent organs such as the prostate, seminal vesicles, levator ani muscles and Cowper's glands raise questions about the mechanisms of action of the extract compounds on reproductive organs.

Another hypothesis regarding the increase in testicular weight concerns the increase in sperm count [52]. Indeed, the extract led to a significant and dose-dependent increase in sperm concentration. The optimal dose for this sperm parameter would therefore be 500 mg/kg body mass (65.05×10^6 spz/mL ± 3.286), which showed the greatest variation. The AEEAM also significantly increased the percentage of motile sperm. In contrast, the effect of the extract on the percentage of normal spermatozoa could not be assessed, as the values obtained with the various doses of the extract were very similar to those of the control group. These results showed that the AEEAM stimulates spermatogenesis and improves sperm motility. According to [53], sperm density, motility, and morphology are considered important parameters for evaluating the effect of chemicals on spermatogenesis. Very little data has described the effects of AEEAM on sperm quality and quantity. Nevertheless, our data regarding increased sperm density and improvement of motility are consistent with work carried out on *Annona muricata* leaves [54] [55]. The variations in sperm parameters following treatment with AEEAM are thought to be related to its richness in metabolites such as polyphenols, saponins, tannins, alkaloids, and quinones. These metabolites together implicate several mechanisms influencing sperm production and the acquisition of motility. First, an increase in endogenous testosterone levels, which would stimulate spermatogenesis following the action of flavonoids and saponins, is a plausible explanation. The influence of testosterone on spermatogenesis is well documented [50] [51]. Sterols could also play a role due to their similar action to that of endogenous steroids. They enhance fertility and are used in the treatment of erectile dysfunction. Secondly, the extract could contribute to improving these parameters through its antioxidant activity. The capacity of *Annona muricata* extracts against oxidative stress that impairs sperm quality has been demonstrated by many other authors [56]. Maintaining the balance between the production of free radicals and endogenous antioxidants significantly impacts sperm parameters [57], potentially altering the quantity and quality of gametes [58]. The result of other authors [59] and our finding about aqueous extract of *Annona muricata* fruit peel antioxydant activity support this hypothesis. Thus, the aqueous extract of the epicarp of *Annona muricata fruit*, due to its composition of polyphenols, alkaloids, tannins, and quinones with well-known antioxidant activity [60], would constitute a significant additional anti-

oxydants avoiding oxydative stress, improving spermatogenesis in the testes and the acquisition of motility in the epididymis. Third, the function of mitochondria located in the sperm flagellum, responsible for sperm motility, could be influenced by secondary metabolites such as flavonoids and quinones [61]. Given that sperm quality and male fertility are assessed based on parameters like sperm concentration, motility, and morphology [62], our results suggests that AEEAM could enhance male fertility.

The overall results relating to the increase in body weight, relative fresh weight of reproductive organs, and sperm count suggest that the aqueous extract of the epicarp of the *Annona muricata* fruit could have androgenic activity. But the specific mechanism of action remains difficult to identify.

5. Conclusion and Perspectives

The data presented in this work show that the aqueous extract of the epicarp of *Annona muricata* fruit, which is non-toxic when administered orally, contains numerous secondary metabolites that confer orexigenic, antioxidant, and probably androgenic properties. Thus, the epicarp of *Annona muricata* fruit, a long-neglected byproduct of the fruit, possesses pharmacological properties of interest that could be particularly useful in the treatment of male infertility. Its stimulating effect on spermatogenesis and the improvement of sperm motility could be especially helpful in resolving abnormalities in gamete quantity and quality. However, identifying the mechanisms involved remains problematic because our study only revealed the classes of secondary metabolites. In light of the above, in the short term, identifying the precise nature of the chemical compounds is essential to elucidate the mechanisms of action of the extract. Also, the androgenic effect will be confirmed by supplementary reproductive hormonal detection and testicular histology. Furthermore, validating the extract's effectiveness in resolving abnormalities in gamete number and motility will require additional testing.

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Conflicts of Interest

The authors declare in full transparency that they have no financial, personal or professional interest that could influence the conduct of this study, the analysis of its results or the writing of this manuscript.

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Abbreviations and Acronyms

AEEAM: Aqueous Extract of the Epicarp of Annona muricata fruit

IC_{50}: Median Inhibitory Concentration