

Effect of Different Parts of *Kigelia africana* Fruit Aqueous Extracts on Sperm Parameters and Testis

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Abstract

Kigelia africana is a plant of ivoirian pharmacopae commonly used in infertility treatment. The *Kigelia africana* whole fruit extracts are known to improve sperm parameters like density, morphology and mobility. However, data related to efficiency of different parts of the fruit are lack. The aim of this study was to know the effects of aqueous extracts of the whole *Kigelia africana* fruit (AP) and of the fruit without the peel (SP) on the sperm parameters and testicular tissue of mice. The phytochemical composition analysis of the SP and AP aqueous extracts of *Kigelia africana* fruit reveals several secondary metabolites in variable abundance. The sperm densities (**Figure 1**) of the SP ($\sim 78.10^6$ spz/ml ± 2.60) and AP ($\sim 96.15 \times 10^6$ spz/ml ± 2.44) groups were higher than that of the control ($\sim 63.95.10^6$ spz/ml ± 2.93) with a highly significant difference ($p < 0.001$). However, the treatment with the AP extract showed a higher value than that observed with the SP extract. The percentage of motile spermatozoa is higher in AP group ($\sim 34\% \pm 2.44\%$) than control group ($\sim 29\% \pm 1\%$) but the difference is not significant (**Figure 2**). However, the percentage of motile spermatozoa was much higher in SP group ($\sim 76\% \pm 5.48\%$), at least twice as high compared to the control and AP group with a highly significant difference ($p < 0.001$). AP and SP aqueous extracts preserve the gonad and enhance spermatogenesis. All together, our data revealed that AP and SP aqueous extracts of *Kigelia africana* stimulated spermatogenesis, sperm mobility and didn't affect the gonads. There is however a difference in

the effects of the two extracts with better efficacy of AP extracts for increasing sperm number while SP more significantly improves sperm mobility and morphology.

Keywords

Kigelia, Spermatogenesis, Density, Mobility, Testis

1. Introduction

Couple infertility is defined as the inability to conceive a child after a minimum of 12 months of sexual intercourse without contraception. Infertility affects approximately 70 million people worldwide. According to World Health Organization (WHO), infertility affects 9% of couples worldwide and in 50% of cases, couple infertility is attributable to the man [1] [2].

In developing countries, infertility is a public health problem, the highest prevalence in low-resource countries, particularly in sub-Saharan Africa [3]. Particularly in Côte d'Ivoire, infertility affects around 14.03% of couples [4]. The origins of infertility can be diverse, such as the pace of life, diet, some infections. In male infertility, abnormalities can affect the quantity and quality of spermatozoa as well as their excretion pathway [1]. Despite the available treatments, access to care remains problematic due to the inaccessibility of expensive therapies and the low income of the population. In this context, using remedies formulated from plants represents a great alternative for populations.

The Ivorian pharmacopoeia is full of numerous plants whose fertilizing properties in humans have been reported by ethnobotanical surveys [5]. *Kigelia africana* (Lam Benth) or "sausage tree" is one of its tropical plants of the Bignoniaceae family, widely distributed in Ivory Coast. The *Kigelia* plant is well known by rural communities, especially for its various medicinal properties. All parts of plant, including fruit, bark, roots and leaves, are used for medical purposes [6] [7]. *Kigelia africana* is used in traditional medicine like anticancer, antiulcer, anti-aging, antioxidant, and anti-malarial. It is also widely applied in the treatment of genital infections, gynecological disorders, renal ailments, fainting, epilepsy, rheumatism, sickle-cell anemia, psoriasis, eczema, central nervous system depression [8]. The fruit, edible by several mammalian species, contains flavonoids, terpenes, tannins, steroids, saponins and caffeic acid [9]-[14]. It is included in herbal prescriptions especially for treatment of infertility, poor libido, sexual asthenia and impotence [15]. In particular, the fruit is known to improve fertility in males. Studies in different species have shown that aqueous and ethanolic extract of the fruit has an androgenic effect and significantly increases sperm count, motility and morphology [16] [17] [18]. A relationship between the antioxidant and androgenic properties of the fruit and the improvement of sperm parameters as well as its protective role on the testis has also been demonstrated

[19] [20] [21]. However, there are still questions regarding the secondary metabolites of the fruit that improve fertility in males. Indeed, very little works has been done to specifically identify the phytochemical composition of aqueous extracts from different parts of *Kigelia* fruit (whole fruit with pericarp and fruit without pericarp). The properties of aqueous extracts of these different fruit's parts on the improvement of spermatogenesis, gonads integrity, as well as the molecules involved are poorly documented.

In this work, we aimed to know the effects of aqueous extracts from different parts of *Kigelia africana* fruit (pulp and whole fruit) on sperm parameters and gonads of male wistar albino mice.

2. Material and Methods

2.1. Plant Matériel

Matured fruit of *Kigelia africana*, collected in the forests around Ayama was obtained from the local market of this city. The fruit was authenticated at the Floristic National Center of Felix HOUPHOUËT-BOIGNY University of Ivory Coast. The reference number is UCJ001955. After identification, the fruits were washed and then separated into 2 lots. In the first batch, the whole fruits (with peel; AP) were cut into small pieces and dried under air conditioning at 18°C. While in the second batch, the peel was carefully removed to retain only the pulp (without peel; SP). Pulp is then cut into small pieces and also dried under air conditioning at 18°C. In both cases, drying lasted 2 weeks followed by spraying. AP and SP powder were weighed and kept for further phyto-chemical analysis/extraction.

2.2. Extraction of Plant Material

From the powder obtained, we carried out an aqueous extraction according to the protocols of Zirihi *et al.* (2003) [22]. Indeed, one hundred grams (100 g) of fine powders were dissolved in one liter (1 L) of distilled water by grinding in a Blinder Mixer for 10 to 15 minutes. The homogenate obtained was drained in a square of cloth, then filtered successively twice through cotton wool and once through Whatman 3 mm paper. The filtrate obtained was dried by evaporation in a Venticell type oven at 50°C. The evaporate recovered as a powder constituted the total aqueous extract (Aq).

2.3. Phytochemical Screening

2.3.1. Identification of Alkaloids

To five ml of extract concentrated (evaporate to dryness). The residue obtained was dissolved in 3 mL of HCl diluted to 2%. Then a few drops of Mayer's reagent were added. The appearance of a precipitate or a white-cream color indicates the presence of alkaloids.

To 2 ml of plant extract, 5 drops of each reagent are added. Depending on the nature of the reagent, various colorations are obtained in the presence of alkaloids.

ids. Two of the three reagents were used to characterize the alkaloids. Those are:

- Dragendorff's reagent (yellow-orange precipitate);
- Bouchardat's reagent (brown-black, dull-brown or yellow-brown precipitate).

2.3.2. Demonstration of Flavonoids

The flavonoids were first identified by a general reaction to soda. In a tube containing 2 ml of plant extract solution, a few drops of a 1/10 soda solution were added. The yellow-orange color made it possible to characterize the presence of flavonoids.

2.3.3. Demonstration of Sterols and Polyterpenes

In a tube containing 2 ml of plant extract solution, a drop of concentrated sulfuric acid and a triperpene solution in chloroform were added. The appearance of a color that is first yellow, then dark red indicates the presence of sterols and polysterols.

2.3.4. Identification of Polyphenols

To 2 ml of each solution was added a drop of 2% alcoholic ferric chloride solution. Ferric chloride causes in the presence of polyphenolic derivatives. The appearance of a more or less dark blackish-blue or green color.

2.3.5. Highlighting Tannins

1 ml of 10% aqueous lead acetate solution was added to 3 ml of the extract. The formation of a blue, black-blue, whitish or brownish precipitate indicates the presence of tannins. To identify the classes of tannins, 1 ml of extract was added to 2 ml of distilled water and then 1 to 2 drops of 0.1% ferric chloride were added. The appearance of a blue, blue-black or black coloration indicates the presence of gallic tannins, the green or dark green (or greenish-brown) coloration indicates the presence of catechetal tannins.

2.3.6. Identification of Quinones

To 1 ml of extract was added 1 ml of sulfuric acid concentrate (H_2SO_4). The appearance of a red coloration indicates the presence of quinones.

2.3.7. Demonstration of Saponins

We put 5 ml of each of the aqueous extracts into a test tube. The tube was shaken vigorously for 15 seconds and then allowed to stand for 15 - 60 min. A height of persistent foam, greater than 1 cm, indicates the presence of saponins.

2.4. Animal Treatment

Fifteen 8 - 10 weeks old male wistar mice weighing 20 - 35 g were used for the experiments. The animals were obtained from the animal facility of Nangui Abrogoua University. They were kept in the Animal Room of the ENS under standard conditions of temperature ($27^{\circ}C$ - $30^{\circ}C$), with a 12-hour light: 12-hour dark cycle to acclimatize for 1 week prior to beginning of the experiments. Each

male was housed individually in wire mesh cage. All animals were allowed unrestricted access to water and feed with commercial granules and properly housed. All treatment was administered orally through a metal oropharyngeal cannula. Animals were divided into 3 groups (control group, AP group, SP group) of five mice per group. Control group received 1 ml normal saline. AP group animals received 100 mg/kg of AP aqueous extract while SP group animals received 100 mg/kg of SP aqueous extract. The treatment was carried out for 35 days.

2.5. Collection of Samples

At the thirty-seventh day, animals were sacrificed by decapitation after ether anesthesia. The testes were removed through the opening of the scrotum.

2.6. Semen Parameters Analysis

Spermatozoa were collected according to the method of Ngoula *et al.* (2007) [23]. After sacrifice, the caudal parts of the epididymis of each mouse is removed by opening the scrotum, then dilacerated in 10 mL of NaCl concentrated at 9% previously incubated in a water bath at 36°C.

Sperm motility was assessed by direct examination of the sperm collecting solution under a light microscope (Olympus CX31RBSF, Philippine) at 100X magnification. A fine drop of solution containing the spermatozoa is placed between slide and coverslip (previously maintained at 36°C). Motile and immobile sperm were quickly counted in 5 random fields and the percentage of motile forms was determined from the formula:

$$\% \text{ motile sperm} = (\text{number of motile sperm} / \text{total number of sperm}) \times 100.$$

The sperm density was determined using the Malassez cell. A drop of mace-rate from the epididymis was taken and placed on the Malassez cell then covered with a coverslip. The sperm count was performed under a light microscope (Olympus CX31RBSF, Philippine) (X 400). The number of spermatozoa per mm³ was estimated according to Sultan and collaborators (1982) [24].

Analysis of sperm morphology was performed using a sperm smear. Briefly, a drop of the previous solution is placed on a slide and spread using a coverslip. The smear is stained with eosin solution. The smear was examined for normal and abnormal sperm (abnormalities of the head and tail) under a light microscope at X400 magnification.

2.7. Histopathological Analysis

Testes were fixed in 10% formalin for 48 hours at room temperature. They were then dehydrated by successive passage through alcohol baths of increasing degree (80°, 90° and 100°) for varying times at room temperature. Thinning of the testes was done in three toluen baths for varying times. Impregnation was carried out in the oven (Mettmert, Germany) between 58°C and 60°C in 2 succes-

sives paraffin baths. Testes were then embedded in paraffin at room temperature in molds. 5 µm testicular sections were made with a microtome (Leica RM2125 RTS; Germany). The sections are mounted on slides and then dewaxed at 58°C to 60°C in an oven (Memmert, Germany) for 30 minutes to be dewaxed.

2.8. Statistical Analysis

Statistical analysis was performed using the R 4.02 software. For each parameters (sperm density, motility and morphology) Shapiro and wilk test was using to verify distribution's normality. We used Bartlett test to verify variance homogeneity. After these verifications, we performed an Anova test to compare control, SP and AP groups. Howether, for the motility of spermatozoon, we only used Kruskal and Wallys test to compare differents groups.

3. Results

3.1. Phytochemical Screening of *Kigelia* SP and AP Aqueous Extract

The study of the phytochemical composition of the SP and AP aqueous extracts of *kigelia africana* fruit (**Table 1**) reveals several secondary metabolites in variable abundance. In particular, the proportions of sterols and polyterpenes, polyphenols, catechic tannins, alkaloids and anthraquinones are equivalent between the SP and AP extracts. On the other hand, differences were observed in the detection of flavonoids and quinones. Indeed, flavonoids are more abundant in the AP extract. Quinones are more abundant in the SP extract. Gallic tannins, anthocyanins and saponosides were not detected in either extract.

3.2. Effect of Aqueous Extracts SP and AP Increased Sperm Density

The sperm densities (**Figure 1**) of the SP ($\sim 78.10^6$ spz/ml ± 2.60) and AP ($\sim 96.15 \times 10^6$ spz/ml ± 2.44) groups were higher than that of the control ($\sim 63.95 \times 10^6$ spz/ml ± 2.93) with a highly significant difference ($p < 0.001$). However, the treatment with the AP extract showed a higher value than that observed with the SP extract. The differences observed between these two extract are highly significant ($p < 0.001$). These data showed that the AP and SP aqueous extracts increase sperm density. Howether AP extract is more effective in increasing sperm density than the SP extract.

Table 1. Phytochemical screening of Aq AP and AqSP *kigelia africana* fruit extract. Negative reaction (-), positive reaction (+), highly positive reaction (++)

Extracts	Sterol and polyterpene	Polyphenol	Flavonoid	Catechic Tanin	Gallic Tanin	Quinon	Alcaloïd	Anthocyan	Anthraquinon	Saponosid
AqAP	++	++	++	++	-	+	++	-	+	-
AqSP	++	++	+	++	-	++	++	-	+	-

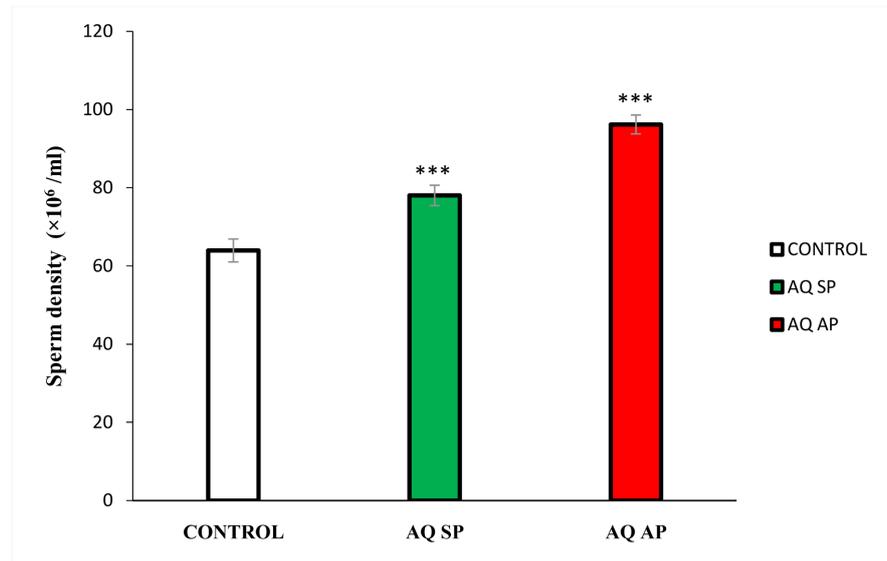


Figure 1. Effect of SP and AP aqueous extract of kigelia fruit on sperm density. Highly density observed for AP extract (96.15×10^6 spz/ml \pm 2.44). Difference is highly significant for $p < 0.001$ between control and SP groups, control and AP groups, SP and AP groups.

3.3. Effect of SP and AP Aqueous Extracts on Sperm Motility

The percentage of motile spermatozoa is higher in AP group ($\sim 34\% \pm 2.44\%$) than control group ($\sim 29\% \pm 1\%$) but the difference is not significant (**Figure 2**). However, the percentage of motile spermatozoa was much higher in SP group ($\sim 76\% \pm 5.48\%$), at least twice as high compared to the control and AP group with a highly significant difference ($p < 0.001$). These results suggest that SP aqueous extract more effectively improves sperm mobility than AP extract.

3.4. Effect of SP and AP Aqueous Extracts on Sperm Morphology

The percentage of normal spermatozoa (**Figure 3**) of AP ($\sim 90.1\% \pm 1.19\%$) and SP ($\sim 95.4\% \pm 1.19\%$) is higher than the control group ($\sim 82.4\% \pm 1.47\%$) with a highly significant difference ($p < 0.001$). Furthermore, the animals of the SP group exhibit a percentage of normal spermatozoa which is significantly much higher ($p < 0.001$) than control and AP groups. These data showed that SP aqueous extract improves sperm morphology more effectively than AP aqueous extract.

3.5. Histological Analysis of the Testes

3.5.1. Control Group

Control group (**Figure 4(a)**) section's revealed seminiferous tubules of various sizes and shapes homogeneously separated. Two types of tubes have been distinguished, those with a lumen (L) of variable diameter and visually more numerous and others without lumen at low amount (*). All the cells of the germ line representing the different stages of spermatogenesis and Sertoli cells have been identified (**Figure 4(a')**). From the basal to the lumen, we distinguished

spermatogonia, spermatocytes I and round spermatids. Elongated spermatids were observed in the seminiferous epithelium. Young immature spermatozoa were present in the seminiferous epithelium at the periphery of the lumen, from which their flagellum overflows. Numerous immobile mature sperm are also been observed in tube lumens (arrow). These observations suggested that spermatogenesis is normally active and fully complete in the majority of the seminiferous tubules of control animals.

3.5.2. AP Group Sections

Testicular sections of animals of AP group (Figure 4(b)) also reveal seminiferous tubes of variable sizes and shapes. A few cluster of Leydig cells are distinguishable in the interstitial spaces (Figure 4(b'); arrow). Almost all of the seminiferous

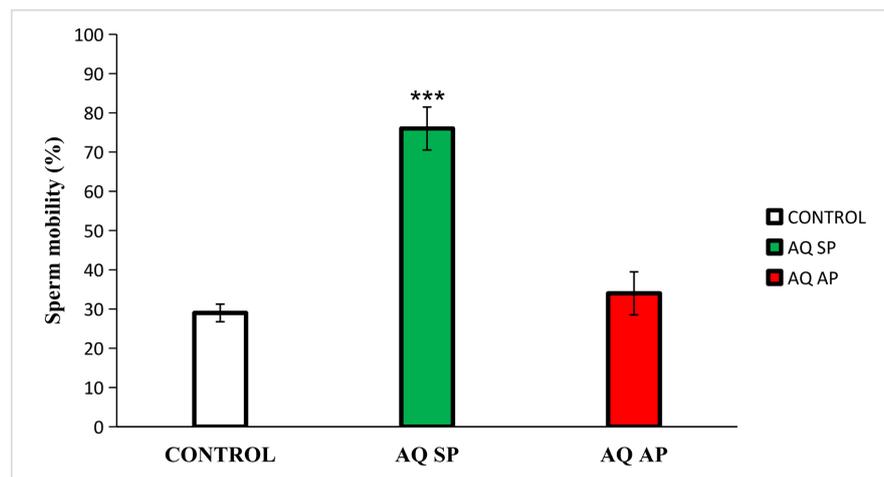


Figure 2. Effect of SP and AP aqueous extract of kigelia fruit on sperm density. Highly motility observed for SP extract (76% \pm 5.48%). Difference is highly significant for $p < 0.001$ between control and SP groups, SP and AP groups.

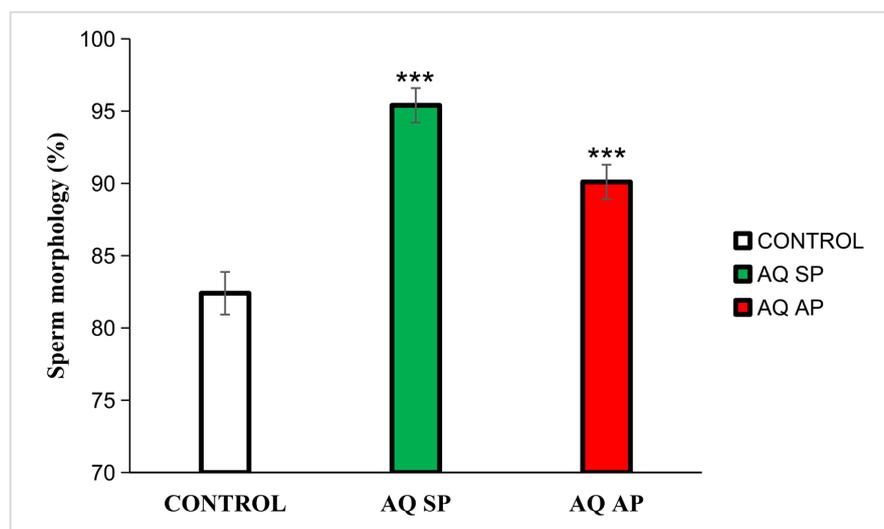


Figure 3. Effect of SP and AP aqueous extract of kigelia fruit on sperm morphology. Highly motility observed for SP extract (76% \pm 5.48%). Difference is highly significant for $p < 0.001$ between control and SP groups, control and AP groups, SP and AP groups.

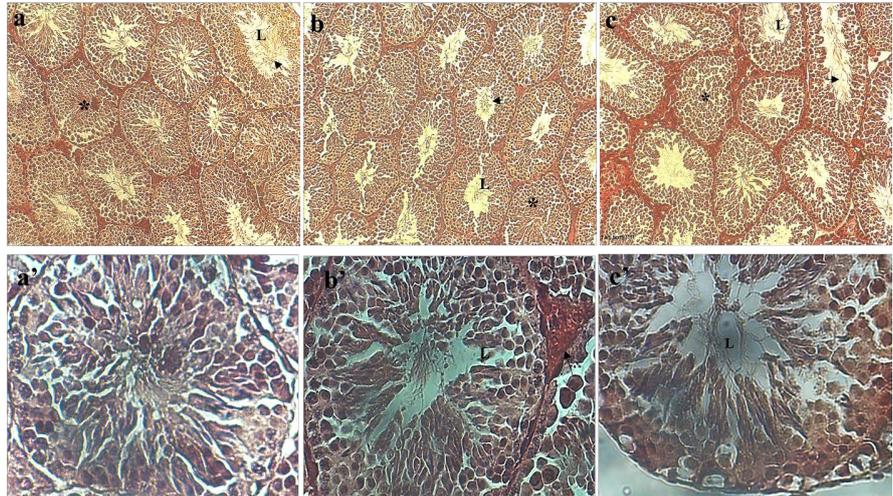


Figure 4. Histological sections of testis of control (a and a'), AP (b and b') and SP (c and c') groups. (a, b, c) $\times 100$ general observation of seminiferous tubules. (L) for lumen of tubes; (*) for tubes without lumen; Arrow show spermatozoon in lumen of a tube. (a', b', c') $\times 400$ seminiferous epithelium showing germinal cells, Sertoli cells and Leydig cells cluster (arrow). Hemalun-eosin coloration.

tubes in the AP group showed a lumen (L) and seems to be more numerous. Some tubes didn't have lumen and seems to be in low quantity (*). Seminiferous epithelium showed the spermatogonia and spermatocytes which appear to be more numerous (**Figure 4(b')**). Unlike control, round spermatids were rarely observed in the tubes. In contrast, elongated spermatids and young immature spermatozoa were numerous in the seminiferous epithelium and at the border of the lumen respectively. Mature immobile sperm were most abundantly observed in the lumens of the tubes (arrow). Thus unlike control, spermatogenesis was very intensive in AP group and fully complete in all seminiferous tubes with a more accelerated spermiogenesis.

3.5.3. SP Group Sections

Organization of testis and seminiferous tubes of SP animals was similar to that observed in AP sections (**Figure 4(c)**). We found that tubes with lumens were the most abundant compared to tube tubes without lumens. Like AP sections, we also found that seminiferous epithelium has spermatogonia and spermatocytes which appear to be more numerous. Round spermatids were still rarely observed while elongated spermatids and young immature spermatozoa were more numerous in the seminiferous epithelium and at the border of the lumen respectively unlike control (**Figure 4(c')**). Mature immobile sperm were most abundantly present in the lumens of the tubes (**Figure 4(c)**; arrow). However, a difference have been detected concerning the type of seminiferous tubes compared to AP and control sections. Even if seminiferous tubes with lumen were abundant compared to control group, their amount seems lower than in AP section. In addition, very few tubes without lumen were still present but at very low abundance compared to control group. These data suggest that spermatogenesis

is also very intensive and fully complete with accelerated spermiogenesis but not in all seminiferous tubes of SP group animals. The spermatogenic activity level seems lower than AP group.

These observations suggested that AP and SP extracts increased spermatogenic activity and seems stimulated spermiogenesis compared to control group.

4. Discussion

In this study, we aim to investigate the effects of different aqueous extracts of *Kigelia africana* fruit (pulp and whole fruit) on sperm parameters and testis of male wistar albino mice. In this context, mice were treated for 35 days with 100 mg/kg of body weight of aqueous extracts of the whole fruit (AP group) and aqueous extracts of the pulp only (without peel; SP group). We chosen this concentration basing us on the study of by Farah and collaborators (2018) [25]. In a toxicological study of *kigelia africana* fruit extract, these authors shown that all the concentration tested (50, 500 and 2000 mg/kg of body weight) was safe for the animal. In addition, it has been show that the fruit extract is more effective at 100 mg/kg of body weight in the case of ethanolic extract [19].

The study of phytochemical composition of aqueous extracts SP and AP of kigelia fruit revealed many classes of secondary metabolite. Our detection of sterols and polyterpenes, polyphenols, catechic tannins, alkaloids and flavonoids in the aqueous extract AP corresponding to whole fruit remains consistent with the work of other authors [26] [27] [28] on the aqueous extract of the whole fruit. The aqueous extract SP corresponding to the pulp (fruit without peel) has never been studied until now to our knowledge. Our results show that this SP extract has a similar composition to the whole fruit, in particular with regard to sterols and polyterpenes, polyphenols, catechic tannins, alkaloids, favonoids and quinon at difference abundance. The detection of compounds that were present in the two extract at the same abundance allowed us to suppose equivalent biological activities involving these molecules. It could justify the use of the pulp also in naturotherapy for the treatment of diseases requiring these metabolite of fruit. Abundance of these compounds detected in the SP and AP extracts is however different from that of these studies [26] [27] [28]. This could be attributed to the difference in protocols used for detection. The region (type of soil, temperature) of fruit collection and the season could also cause variations in phytochemical analysis. Our saponoside test was negative for the aqueous AP and SP extracts. This result is not consistent with those of Saini *et al.* (2013) and Abdulkadir *et al.* (2015) [26] [27]. It is nevertheless consistent with that of Fagbohun *et al.* (2020) [28]. The inability to detect saponins in SP and AP extracts could be related to the sensitivity of the detection technique employed and the environmental parameters of the experiment.

The aqueous extract of whole kigelia fruit has been shown to have antioxidant activity [26] [27] [28]. It contains antioxidant enzymes such as superoxide dismutase, catalase and ascorbate oxidase and non-enzymatic antioxidants (vitamin

C) which are active in the aqueous extract [26]. Flavonoids, polyphenols, quinones and anthraquinones also show antioxidant activity [29] [30] [31]. In addition, flavonoids are known for their aromatase inhibitory properties [32], preventing the conversion of androgens to estrogen. This helps increase the level of testosterone. In the case of quinones, they are also electron acceptors in the inner membrane of mitochondria, thus playing an important role in the metabolic pathways that take place there. Thus, our detection of these compounds in the aqueous extracts of SP and AP suggests effects against oxidative stress and in the increase in testosterone levels through aromatase inhibition. However, we show for the first time that SP and AP extracts contain flavonoids and quinones in varying abundance. Flavonoids are more abundant in the AP extract while quinones are more abundant in the SP extract. Based on this data, we can assume that these differences in abundance between extracts could lead to variations in the intensity of the effects attributed to these molecules.

The detection of all these classes of biologically active compounds in the different extracts of the kigelia fruit were consistent with its use in the treatment of various conditions [33] [34]. The particular presence of flavonoids, phenols, quinones, anthraquinones justified the use of the fruit in the treatment of male infertility because of its androgenic and antioxidant properties [15] [35] [36]. Our results of analyzes of sperm parameters and histological sections support this idea. Indeed, the comparison showed that the extracts SP (~ 78.106 spz/ ml ± 2.60) and AP (~ 96.106 spz/ ml ± 2.44) increased the sperm density with a highly significant difference ($p < 0.001$) compared to the control ($\sim 63.95.106$ spz/ml ± 2.93). These data show that the SP and AP extracts contain compounds that stimulate spermatogenesis with a more intensive effect of AP extract.

Seminiferous tube with lumen could be associated with a fully complete and active spermatogenesis as mature immobile spermatozoa were observed in the lumen. Tubes without lumen is associated with active but not fully complete spermatogenesis as mature immobile spermatozoa were not detected. In our observations of testicular sections of all groups, we saw these two types of seminiferous tubes but the frequency seems visually different between all the groups. Tubes without lumen have been observed in control group and at a very low frequency in AP and SP group compared to control. On the other hand, testicular sections of animals in AP and SP group saw mostly the presence of seminiferous tubes possessing a lumen with immobil spermatozoon and the frequency seems greater than control group. Testicular sections observations for all animal groups showed that spermatogenesis is normally active but not fully complete in some tubes. We could hypothesized that there could be a relationship between the number of seminiferous tubes with lumen and the increasing of spermatozoon showed by sperm density analysis for AP and SP group. In addition, round spermatid have been rarely observed in SP and AP group. Indeed, numerous elongated spermatid and young immature spermatozoon were present in these group. We could think that AP and SP could also stimulate spermiogenesis. No

alteration of the testicular tissue was observed, thus showing the histo-protective role of the aqueous extracts of SP and AP. Very few data describe the histo-protective function of the aqueous extracts SP and AP. However, our observations remain consistent with the work of Azu and colleague (2010b) [20] who worked on the ethanolic extract.

A highly significant ($p < 0.001$) increase in mobility was observed for the SP extracts ($\sim 76\% \pm 5.48\%$) compared to the control ($\sim 29\% \pm 2.24\%$). The AP extract ($\sim 34\% \pm 5.48\%$) also increased sperm mobility compared to the control, but the difference is not significant. SP and AP extracts also increased the percentage of normal sperm compared to the control group with a highly significant difference ($p < 0.001$). For mobility and morphology, the values obtained with the SP extract were nonetheless higher than the AP extract with a highly significant difference ($p < 0.001$). These results showed that the extracts contain active compounds improve spermiogenesis and sperm mobility, with a more intensive effect of SP extract.

To our knowledge, very little data describes the effects of aqueous extracts of *Kigelia africana* fruit (SP and AP) on sperm parameters in mice of the wistar albino strain. Although data on mice are poorly documented, our observations of the increase in these parameters nevertheless remain consistent with those of other authors [16] [17] [19] [21] using the concentration of 100 mg/kg body weight of alcoholic extracts of whole *Kigelia africana* fruit in rats. Thus, regardless of the type of extraction and the species, the fruit of *Kigelia africana* retains its effectiveness in improving the quality and quantity of sperm. These authors attribute this increase in sperm parameters on the one hand to the androgenic properties of the fruit and on the other hand to the antioxidant properties [17] [19] at a dose of 100 mg/kg of body weight. Indeed, it is well known that the flavonoids present in the fruit increase the level of testosterone and that this hormone stimulates spermatogenesis [37] [38] [39] [40]. Regarding antioxidant action, methanolic extract from kigelia fruit has been shown to cause increased catalase, glutathione and reduced malonaldehyde in rat testes and seminal fluid. These variations are known to be indicators of inhibition of oxidative stress and are closely associated with increased sperm quality and quantity [19] [20] [21] [41]. The flavonoids contained in the fruit are believed to be involved in increasing the intracellular level of glutathione [19] [30]. Several mechanisms can influence the number, mobility and morphology of sperm. We distinguish in particular, the osmolarity of the seminal fluid, the efflux of fluid caused by osmosis during the differentiation of spermatids into spermatozoa, aquaporins, phosphorylation, calcium, bicarbonate ions, the functioning of the mitochondria, the anti activity, oxidants [42] [43]. In our study, identifying the precise mechanisms involved in improving quality and quantity of sperm is difficult. However, the nature of the extracts as well as our detection of secondary metabolites whose antioxidant activities are known leads us to believe that this mechanism could be involve in the observed results. Indeed, morphology, number and mobility of spermatozoa are closely related to the production and activity of

free radicals as well as antioxidant enzymes [44]. This is for example the case of catalase whose low activity is associated with low motility while high activity leads to increased motility [41]. Thus, the male reproductive system, the level of reactive oxygen species must not exceed that of anti-oxidants at the risk of disrupting spermatogenesis and the functioning of spermatozoa (an increase in lipoperoxidation of the lipids of their membrane, the mitochondrial activity; DNA defects and reduced motility [45] [46] [47] [48]). Oxidative stress is provided by anti-oxidants naturally present in male genital tract, including non-enzymatic anti-oxidants (vitamin E, C, glutathion) and anti-oxidant enzymes such as superoxide dismutase (SOD), catalase, glutathion peroxidases and reductase [49] [41] [43]. A good majority of these factors are detected in the aqueous extract of the kigelia fruit [26] [27] [28]. There would therefore be an additional supply of anti-oxidant which would strengthen the defense system against oxidative stress, thus making it possible to maintain the integrity of germ cells and gametes. In addition, the polyphenol, quinone, anthraquinone flavonoids that we detect also showed antioxidant activity [30] [50] [51] [52]. Based on these data, we believe that improvement in the quantity and the quality of the spermatozoa observed with the aqueous extracts SP and AP would be linked to the presence of flavonoids, polyphenols, quinones and anthraquinones. However, the precise mechanism of action of these molecules is not yet well known.

Our results also showed a variation in the intensity of the effects of AP and SP on sperm parameters. AP extract increased sperm count more efficiently, while SP extract significantly improved mobility and body shape. Some compounds present in each of the 2 extracts would have different mechanisms during spermatogenesis and the acquisition of motility. The suspected compounds are the flavonoids and quinones, with a greater amount of the flavonoids in the AP extract and a greater amount of the quinones in the SP extract. Thus, we could assume that these differences in the intensity of the observed effects with each of the 2 extracts would be the consequence of different mechanisms of action and the variable abundance of these 2 compounds. It is possible that the action of flavonoids manifests itself specifically during spermatogenesis and more precisely in all the stages preceding spermiogenesis. Quinones are thought to act more specifically during spermiogenesis and the acquisition of mobility. A second hypothesis relating to a synergistic action between flavonoids, quinones and other compounds that we were not able to reveal in this study would also be possible. However, the precise mechanisms of these secondary metabolites are not yet well understood. The idea that the effectiveness of antioxidants in a specific condition depends on its chemical structure, its redox potential, its solubility influenced by the site of action, its pH-related stability as well as the temperature is in agree with this hypothesis [53].

5. Conclusion

The aim of this study was to know the effects of aqueous extracts of the whole *Kigelia africana* fruit (AP) and of the fruit without the peel (SP) on the sperm

parameters and testicular tissue of mice at a concentration of 100 mg/ kg of body weight. The AP and SP extracts increase the quantity of spermatozoa and improve their mobility as well as their morphology. AP extract more effectively increases sperm density while SP extract is more efficient in improving motility and morphology. This supports the use of kigelia fruit for the treatment of male infertility as reported by naturotherapists. These extracts also preserve the integrity of the testicular tissue, thus highlighting a histo-protective role. These results provide complementary data on specific effects and efficiency of different parts of *Kigelia africana* fruit. These data open up many perspectives for the treatment of male infertility. In particular, the AP extract could be specifically recommended for the treatment of oligospermia and the SP extract for the specific treatment of asthenospermia and teratospermia. Further research in this direction would therefore be relevant. It would be also relevant to confirm the antioxidant activity of the aqueous extracts AP and SP. Then, it would be necessary to elucidate the precise mechanism, the secondary metabolites detected in these different extracts and to verify the fertilizing power of the spermatozoa produced during the treatments with the AP and SP extracts. More analysis of the different types of seminiferous tubules number could also be of great interest.

Conflicts of Interest

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

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