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Evaluation of the Effects of Aqueous Leaves Extract of *Cnestis ferruginea* from Côte d'Ivoire on Male Rat Reproductive System

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Authors' contributions

This work was carried out in collaboration between all authors. Author ZNE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KKA and TA managed the analyses of the study. Author KK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Against growing male infertility in the world, investigations are undertaken to find new bioactive molecules. Thus, the aim of this study was to evaluate the pharmacological effects of aqueous extract of *Cnestis ferruginea* on the reproductive parameters of male rats. Indeed, 36 male rats were divided into 2 groups of 18 each and treated for 30 days (set I) and 60 days (set II). Each set was subdivided into three groups. Group 1 (control) received distilled water. Groups 2 and 3 were treated with 50 ($AECF_{50}$) and 100 ($AECF_{100}$) mg/kg body weight of the aqueous extract of *Cnestis ferruginea* respectively. The results showed that extract induces significant increase in the wet

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weight of testis, seminal vesicles, epididymis, prostate and levator ani muscle as well as the dry weight of the latter. On the sperm parameters, the extract produced a significant increase in the number of motile spermatozoa, number of spermatozoa and number of normal spermatozoa. The extract also increased serum levels of pituitary gonadotropins (FSH and LH) and testosterone. Histological study showed that, *Cnestis ferruginea* induced significant increase in the seminiferous tubules diameter. In conclusion, the extract of *Cnestis ferruginea* could contain androgen-like substances capable of improving the fertility of male rats.

Keywords: Cnestis ferruginea; testis; spermatozoa; gonadotrophins; testosterone.

1. INTRODUCTION

"Sterility" or infertility is a public health problem that has become increasingly recurrent in recent decades [1]. Indeed, about 45.8 million couples in the world could not have a child after five years of married life [2]. Glazener et al. [3] estimated this proportion to be one in six couples. It is very important to note that in Africa, infertility affects 12-21% of couples [4]. Previously Leke et al. [5], after studies in the regions sub-Saharan Africa estimated a prevalence rate of 30-40% infertility. It is accepted that 20% of this infertility is solely a male cause [1].

In addition, several studies show that the growing infertility in the world is caused by endocrine disruptors [6]. Thus, the deterioration of male reproductive health is at the center of the concerns of endocrine disruptor-human health relationships.

This deterioration is based on a triptych associating sperm quality degradation: hypospermia, oligospermia, azoospermia and asthenospermia [7,8]; increased incidence of testicular cancer and increased abnormalities of the genital tract [9,10]. To this must be added the erectile and ejaculatory dysfunction [11].

The aetiology of this affection requires several specific examinations for each organ of the genital tract including even hormonal homeostasis, immune system and body temperature [12]. These examinations can last for several months and sometimes prove unsuccessful.

Thus, poor couples resort to traditional medicine, whose essential components of recipes are plants [13,14]. Indeed, the extension of the medicinal plant as an alternative to public health care requires studies using modern scientific investigation methods. Thus, *Cnestis ferruginea*, a Connaraceae widespread in tropical Africa known for its many therapeutic virtues including the treatment of male infertility, interested us in this study. Hence, the aim of this work is to evaluate the pharmacological effects of the aqueous extract of this plant on the reproductive parameters of male rats.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *Cnestis ferruginea* were harvested in November in the Region of Nawa, Department of Soubré, precisely in the village named Trawlinkro (V8) (Côte d'Ivoire). A sample of this plant has been identified and authenticated by Professor Aké-Assi at the Laboratory of Botany and Plant Biology of Université Félix Houphouët-Boigny on the basis of taxonomic characters and by direct comparison with the herbarium specimens N°3974, 4327 and 15116 that available at the National Floristic Center (UJC).

2.2 Preparation of Extract

Harvested leaves have been rinsed with distilled water, dried in the shade (sheltered from the sun) at an ambient temperature (30±2°C). The dried leaves were crushed with a power mill (Retsch SM 100, Germany) to obtain a powder.

The powder obtained has been macerated by mixing 50 g and 1.5 L of distilled water and stirred for 24 hours by a magnetic stirrer (Janke & Kuntelika, Germany). After three times filtration on Whatman filter paper number 1, the filtrate was concentrated in an air circulating oven at 50°C until total dryness. The aqueous extract obtained (yield 11.51%) has been stored at 4°C in a refrigerator for the experimental studies.

2.3 Animal Material

Adult male rats, (*Rattus norvegicus*, Muridae), Wistar strain, weighing between 140-160 g and aged 55-65 days are from the animal facility of ENS (Ecole Normale Supérieure). These rats have been used for pharmacological studies of the aqueous extract of *C. ferruginea*. The animals were handled according to the guidelines of the Ethical Committee on the use and care of experimental animals of the Department of Biosciences, Université Félix Houphouët-Boigny.

2.4 Experimental Design

Thirty-six adult male rats were randomly distributed into 2 sets of 18 animals each and treated for 30 (set I) and 60 days (set II). Each set was then divided equally into three groups and treated as follows: Group 1 (control) was orally administered with distilled water once a day. Group 2 and group 3 were respectively treated with 50 ($AECF_{50}$) and 100 mg/kg of body weight ($AECF_{100}$) of aqueous extract of *C. ferruginea* orally once a day.

2.5 Body Weight

The body weight of each animal was recorded every two days during treatment. After 24 hours of last treatment, the final weight was recorded and the animals were sacrificed by cervical dislocation.

2.6 Reproductive Organ and Adrenal Gland Weight

Immediately after the sacrifice of animals, the testis, seminal vesicle, prostate, epididymis, cowper gland, adrenal gland, LAM (levator ani muscle) and penis of each rat were dissected out, weighed quickly using a sensitive balance (wet weight). These weighed organs, except seminal vesicle and prostate, were placed at the drying oven at 100°C during 24 hours and weighed again (dry weight).

2.7 Sperm Collection

The method is that described by Ngoula *et al.* [15]. Immediately after the sacrifice, the tail of the left epididymis of each rat was removed by opening the scrotum and then dilacerated in 10 ml of NaCl 9 ‰ previously incubated in water bath at 36°C. Thus the spermatozoa diffuse into the solution.

2.8 Study of Motility

Sperm motility was assessed by direct examination of the previous solution. Thus, a fine drop of this solution was placed between the slide and the coverglass (previously maintained at 36°C). The evaluation was done using the photonic microscope (Olympus CX31RBSF, Philippine) at 100× magnification. The mobile and immobile spermatozoa were rapidly counted on 5 random fields and the percentage of the mobile forms was determined from the formula:

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

2.9 Density of Spermatozoa

The density of the spermatozoa was determined using the Malassez cell. Thus, a drop of macerate from the epididymis was removed and deposited on the Malassez cell and then covered with a coverglass. Sperm counts were performed using a photonic microscope (Olympus CX31RBSF, Philippine) (×100). The number of spermatozoa per mm^3 was estimated using the formula [16]:

$$N = \frac{X \times df \times 10^6}{4}$$

X = Number of sperm count in 5 squares of 20 small squares of the Malassez cell
df = Dilution factor (20)
N = Number of spermatozoa per mm^3

2.10 Study of Spermatozoa Morphology

A drop of the preceding solution is deposited on a slide bearing object and spread by means of a slide. The smear thus produced is stained with an eosin solution. The smear was examined using a photonic microscope at ×400 magnification.

2.11 Reproductive Hormone Levels

During the sacrifice, blood was collected. Sera were separated by centrifugation 3000 r/min for 10 minutes and stored at -20°C until used for the assessment of FSH, LH, testosterone and prolactin levels by the ELFA technique (Enzyme Linked Fluorescent Assay) using specific kits (Bio Merieux, Lyon, France).

2.12 Histology Study

Testis and cauda epididymis were fixed in Formaldehyde 10% fixative and cut into pieces and processed through ethanol-toluene. It was then embedded in paraffin. Sections were cut at 5 µm thick and stained with Harris haematoxyline and eosin (H & E).

2.13 Statistical Analysis

The data and graphical representation of the data was performed using the Graph Pad Prism 5.01 software (Microsoft, USA). The experimental results were expressed as Mean ± SEM and data were assessed by the method of analysis of one-way ANOVA followed by Tukey test with least significant test. P value <0.05 was considered significant, P value <0.01 considered highly significant and P value <0.001 considered very highly significant.

3. RESULTS

3.1 Effects of *Cnestis ferruginea* on the Body Weight of Rats

The body weight of the rats treated with the extract as well as those of the controls increase gradually until the end of the treatment. This increase statistically showed no significant difference between treated and control rats ($p > 0.05$) (Fig. 1). At the end of the treatment, the mean rate of weight increase of the control rats was 71.28 ± 4.25% relative to their initial weight. The mean increase rates of the treated rats were 83.43 ± 5.64% and 82.27 ± 5.69%, respectively, for doses of 50 mg/kg PC ($AECF_{50}$) and 100 mg/kg of body weight ($AECF_{100}$).

3.2 Effects of *Cnestis ferruginea* on the Wet Weight of the Reproductive Organs and Adrenal Glands

The respective wet weights of the various organs taken from the rats after 30 days and 60 days of treatment with the extract of the leaves of *C. ferruginea* and the distilled water (control) are shown in Tables 1 and 2.

After 30 days of treatment, both doses $AECF_{50}$ and $AECF_{100}$ did not induce any change in testes and prostate wet weight ($p > 0.05$). On the other hand, after 60 days of treatment, the wet weight of the testes and prostate of the rats treated with $AECF_{100}$ increased significantly ($p < 0.05$) respectively by 8.02% and 38.38% compared to the control. While treatment with $AECF_{50}$ showed only a slight, insignificant increase of these organs.

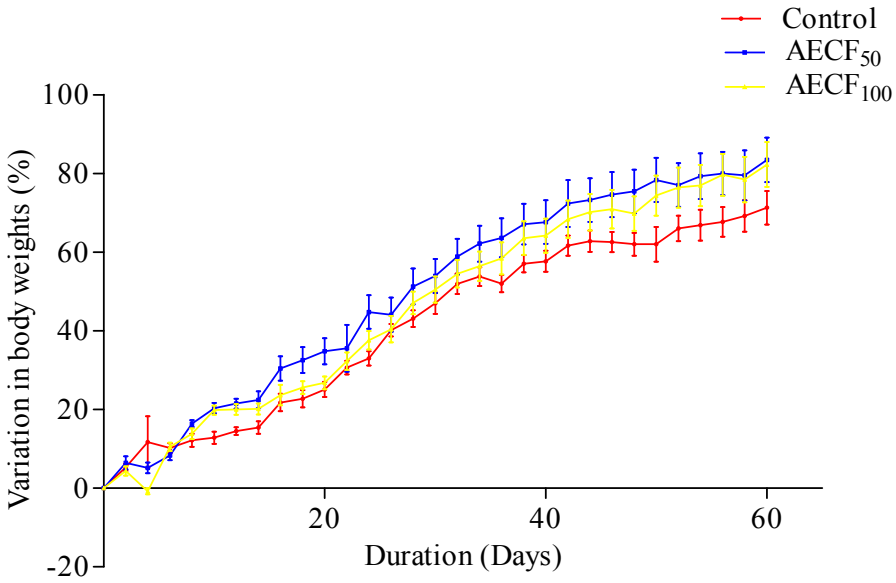

Fig. 1. Effect of *Cnestis ferruginea* on changes in body weight of rats

Table 1. Effect of aqueous extract of *Cnestis ferruginea* on wet weight of some reproductive organs and adrenal gland of rat after 30 days of treatment

Treatment	Wet weight (mg/100 g of body weight)							
	Testis	Seminal vesicle	Prostate	Epididymis	Adrenal gland	Cowper gland	LAM	Penis
Control	430.0±5.849	347.3±2.639	190.6±13.77	159.9±8.289	6.898±0.4361	9.591±0.7690	334.1±13.29	47.02±1.445
$AECF_{50}$	492.5±19.24	523.4±22.70***	197.0±10.99	196.4±4.881*	7.03±0.9426	10.45±1.095	412.5±15.68**	57.93±2.183
$AECF_{100}$	449.5±17.80	406.7±7.241*	223.6±7.164	201.8±7.859**	7.67±0.9418	10.23±0.6015	346.6±4.038	49.89±4.776

*Values are means ± SEM (n=6); *=p<0.05; **=p<0.01; ***=p<0.001; For values whithout (*), p>0.05*
LAM: Levator ani muscle
Control: Distilled water,
$AECF_{50}$: Aqueous Extract of C. freruginea (50 mg/kg of body weight),
$AECF_{100}$: Aqueous Extract of C. ferruginea (100 mg/kg of body weight)

Table 2. Effect of aqueous extract of *Cnestis ferruginea* on wet weight of some reproductive organs and adrenal gland of rat after 60 days of treatment

Treatment	Wet weight (mg/100 g of body weight)							
	Testis	Seminal vesicle	Prostate	Epididymis	Adrenal gland	Cowper gland	LAM	Penis
Control	471.4±11.72	457.2±5.634	210.5±17.70	207.9±3.488	7.286±0.3849	26.47±1.985	472.6±22.51	50.00±1.837
$AECF_{50}$	491.4±6.645	553.0±6.594***	257.1±21.20	244.4±4.748*	7.519±0.5128	25.17±1.734	584.2±22.65*	58.68±2.927
$AECF_{100}$	509.2±8.717*	490.3±9.368**	291.3±15.07*	292.4±12.09**	6.588±0.2095	27.24±2,354	570.6±28.41*	54.07±4.786

*Values are means ± SEM (n=6); *=p<0.05; **=p<0.01; ***=p<0.001; For values whithout (*), p>0.05*
LAM: Levator ani muscle
Control: Distilled water,
$AECF_{50}$: Aqueous Extract of C. freruginea (50 mg/kg of body weight),
$AECF_{100}$: Aqueous Extract of C. ferruginea (100 mg/kg of body weight)

About the seminal vesicles, the AECF causes a very significant increase in the wet weight of this organ. In fact, the weight of the seminal vesicles increased by 50.70% ($p < 0.001$) and 17.10% ($p < 0.05$) respectively with $AECF_{50}$ and $AECF_{100}$ after 30 days of treatment. Similarly, treatment with AECF for 60 days increased the wet weight of this organ by 20.95% ($p < 0.001$) and by 7.22% ($p < 0.01$), respectively, with $AECF_{50}$ and $AECF_{100}$.

Concerning the epididymis, the treatment with $AECF_{50}$ resulted in a significant increase ($p < 0.05$) of 22.83% and 17.56% of the weight of this organ, respectively after 30 days and 60 days of treatment. $AECF_{100}$ induced a very significant ($p < 0.01$) increase in epididymis weight of 26.20 and 40.64% respectively for 30-day treatments and 60 days.

Adrenal gland, Cowper gland and penis experienced no change in wet weight regardless of dose and duration of treatment.

After 30 days of treatment, the wet weight of the levator ani muscle (LAM) of the treated animals increased significantly by 23.47% ($p < 0.01$) $AECF_{50}$. The dose of $AECF_{100}$ produced only a slight increase statistically non-significant. At the end of the 60 days of treatment, the wet weight of the organ underwent a significant increase of 23.47% ($p < 0.05$) and 20.74% ($p < 0.05$) respectively for $AECF_{50}$ and $AECF_{100}$ compared to the control.

3.3 Effects of *Cnestis ferruginea* on the Dry Weight of Some Organs

Tables 3 and 4 summarise the respective dry weights of testes, adrenal glands, Cowper's glands, levator ani muscle (LAM) and penis removed from animals after 30 days and 60 days of treatment with AECF.

The dry weight of testes, Cowper glands, adrenal gland and penis did not show any significant variation ($p > 0.05$) regardless of dose and duration of treatment with *C. ferruginea* leaf extract.

Treatment with AECF for 30 days resulted in a significant ($p < 0.05$) increase in LAM dry weight of 27.17% compared to control with $AECF_{50}$. After 60 days of treatment, alone $AECF_{50}$ again produced a significant increase ($p < 0.05$) of 20.36%. The slight increase induced by $AECF_{100}$ of the dry LAM weight of the treated rats is not significant ($p > 0.05$) compared to the control.

Table 3. Effect of aqueous extract of *Cnestis ferruginea* on dry weight of some reproductive organs and adrenal gland of rat after 30 days of treatment

Teatment	Dry weight (mg/100 g of body weight)				
	Testis	Adrenal gland	Cowper gland	LAM	Penis
Control	76.40±1.730	2.751±0.230	6.019±0.490	85.40±5.896	13.51±0.612
$AECF_{50}$	76.97±4.430	2.076±0.448	6.793±0.820	108.6±3.518*	15.08±0.640
$AECF_{100}$	72.80±3.181	3.553±0.312	7.705±0.431	87.41±4.280	14.20±1.810

*Values are means ± SEM (n=6); *=p<0.05; **=p<0.01; ***=p<0.001; For values whithout (*), p>0.05*
LAM: Levator ani muscle
Control: Distilled water,
$AECF_{50}$: Aqueous Extract of C. freruginea (50 mg/kg of body weight),
$AECF_{100}$: Aqueous Extract of C. ferruginea (100 mg/kg of body weight)

Table 4. Effect of aqueous extract of *Cnestis ferruginea* on dry weight of some reproductive organs and adrenal gland of rat after 60 days of treatment

Treatment	Dry weight (mg/100 g of body weight)				
	Testis	Adrenal gland	Cowper gland	LAM	Penis
Control	67.31±2.416	2.124±0.211	8.310±1.544	111.0±5.740	12.64±0.398
$AECF_{50}$	68.67±1.392	2.331±0.163	7.877±0.583	133.6±2.332*	15.08±0.837
$AECF_{100}$	69.62±3.919	2.441±0.110	9.459±0.861	123.6±3.951	15.35±0.630

*Values are means ± SEM (n=6); *=p<0.05; **=p<0.01; ***=p<0.001; For values whithout (*), p>0.05*
LAM: Levator ani muscle
Control: Distilled water,
$AECF_{50}$: Aqueous Extract of C. freruginea (50 mg/kg of body weight),
$AECF_{100}$: Aqueous Extract of C. ferruginea (100 mg/kg of body weight)

3.4 Effects of *Cnestis ferruginea* on Rat Spermatic Parameters

The histograms in Fig. 2 represent the spermatic parameters (A: motility, B: density and C: morphology) recorded in this study.

Indeed, spermatozoa collected from the caudal epididymis of rats treated for 30 days and 60 days showed a very significant ($p < 0.01$) increase in their motility in a dose-dependent manner (Fig. 2A). Thus, rats treated for 30 days showed 78.00 ± 2.781% ($p < 0.05$) and 85.33 ± 3.639% ($p < 0.01$) of motile spermatozoa respectively with doses of $AECF_{50}$ and $AECF_{100}$. Similarly, the 60-day treatment induced a significant increase in motile sperm count of 83.67 ± 2.390 ($p < 0.05$) and 88.67 ± 2.512% ($p < 0.01$) respectively with $AECF_{50}$ and $AECF_{100}$ compared to the control (73.67 ± 2.140%).

Fig. 2B shows the mean sperm density of the control and treated rats for 30 and 60 days. Thus, after 30 days of treatment, the increase in the sperm density of the groups treated with $AECF_{50}$ and $AECF_{100}$ is not statistically significant. On the other hand, after 60 days of treatment, the increase was highly significant ($p < 0.001$) with $AECF_{50}$ (593.300 ± 33.900 Million Spz/mL) and $AECF_{100}$ (610.000 ± 26.010 Million Spz/mL) compared to the control (379.000 ± 27.280 Million Spz / mL).

The analysis of the morphology of the spermatozoa taken after 30 and 60 days of treatment revealed a decrease in abnormal spermatozoa (double head, double flagella, short flagella, head abnormal, absence of intermediate piece, immature spermatozoa ...). Thus, the percentage of normal spermatozoa increased significantly by 76.70 ± 0.9015 ($p < 0.05$) and 83.47 ± 1.910 ($p < 0.01$), respectively, with $AECF_{50}$ and $AECF_{100}$ which is 70.95 ± 1.485 after 30 days of treatment. Similarly, after 60 days of treatment, the increase in the percentage of normal spermatozoa in the treated rats was significantly different from the controls. Thus, the sperm count after treatment for 60 days was 81.83 ± 1.306 ($p < 0.01$) and 83.47 ± 1.790 ($p < 0.001$) respectively with the $AECF_{50}$ and $AECF_{100}$ doses. While that of controls is 71.97 ± 2.044 (Fig. 2C).

3.5 Effects of *Cnestis ferruginea* Extract on Male Rat Sex Hormones

Oral administration of the aqueous extract of *Cnestis ferruginea* for 30 days and 60 days in adult male rats induces a significant increase in the serum level of FSH and LH. Indeed, $AECF_{50}$ results in an increase in the FSH rate of 31.46% ($p < 0.05$) compared to the controls. However, the increase in the level induced by $AECF_{100}$ compared to the control is not significant for this duration. After 60 days of treatment, the extract induced an increase of 43.17% ($p < 0.05$) and 56.53% ($p < 0.01$) respectively with $AECF_{50}$ and $AECF_{100}$ compared to the controls. Regarding the LH level, the 30-day treatment resulted in a slight, non-significant increase ($p > 0.05$). This hormone experienced a significant ($p < 0.05$) increase in rates after treatment for 60 days. Thus, the LH level increased by 27.02% and 23.80%, respectively, for the $AECF_{50}$ and $AECF_{100}$ compared to control. In the case of serum prolactin, the extract produced no significant variation ($p > 0.05$) compared to controls regardless of the duration and dose administered. Nevertheless the results showed an insignificant increase with 60-day treatments of this hormone (Table 5).

3.6 Effects of *Cnestis ferruginea* Extract on the Histological Structure of the Testis and Epididymis

Figs. 3 and 4 respectively show transverse sections of the testes and epididymis of control and treated rats for 30 and 60 days. They indicate changes in the morphology of the testes in the groups treated with plant extract. Indeed, an increase in the number of spermatozoa in the seminiferous tubules of the rats treated with the extract is observed in comparison with the controls. This increase is more important with the dose of $AECF_{100}$. However, the structure of the testes of the treated rats revealed a normal histological texture as did the controls. All stages of spermatogenesis are observed with the presence of spermatogonia, spermatocytes, spermatids and spermatozoa. Sertoli cells are observed between the germ cells. In the interstices of the seminiferous tubules, Leydig cells and the blood and lymphatic vessels are also observed.

In the epididymis, histological sections revealed a normal appearance of the structure in controls such as those receiving the plant extract.

The morphometric analysis performed on the structure of these sexual organs also revealed changes (Table 6). Indeed, in the testis, there was a growth in the diameter of the seminiferous tubes in the rats treated. Thus, for the 30-day treatment, the rate of increase was 12.39% and

15.93% compared to controls for $AECF_{50}$ and $AECF_{100}$, respectively. These differences are not statistically significant (p> 0.05). For the 60-day treatment, growth rates were 15% and 17.40% compared to controls for $AECF_{50}$ and $EACF_{100}$, respectively. For this duration of treatment, only the increase induced by the $AECF_{100}$ is significant (p <0.05).

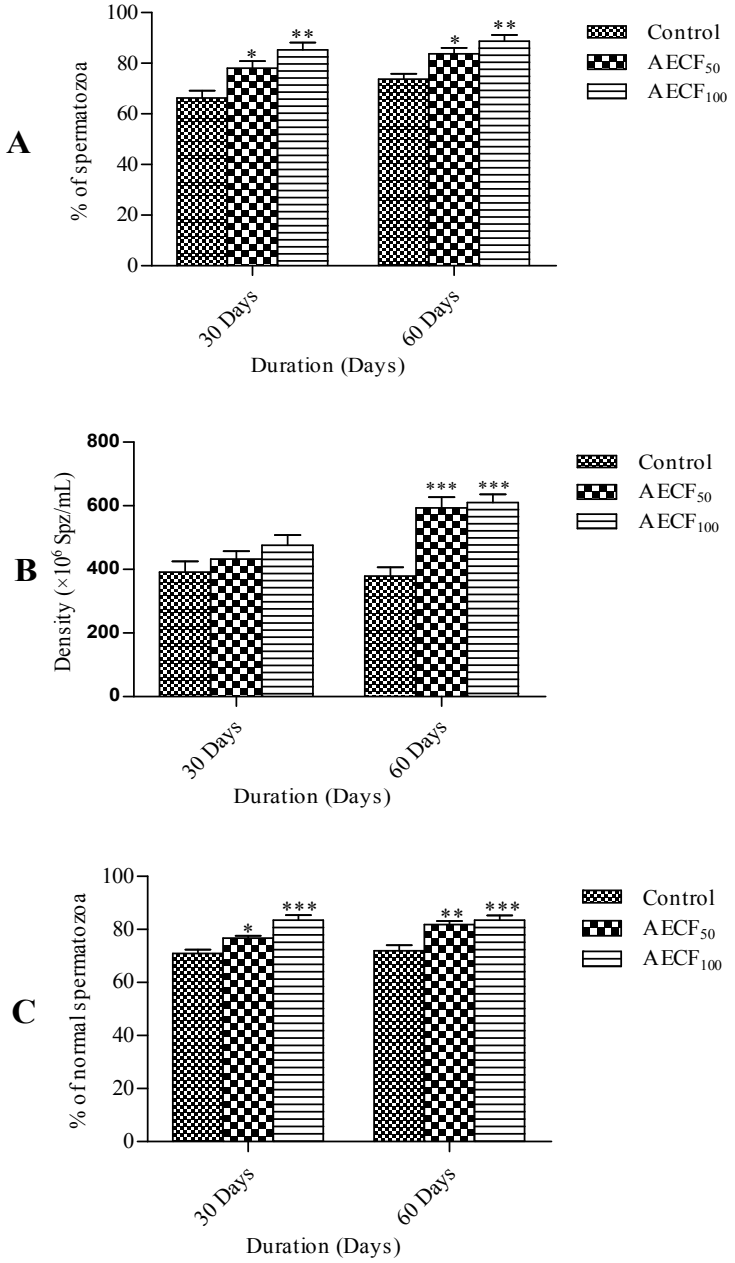

Fig. 2. Effects of the aqueous extract of *Cnestis ferruginea* on the sperm parameters of the male rat

A: Motility; B: Density; M: Morphology
*Values are means ± SEM (n=6); *=p<0.05; **=p<0.01; ***=p<0.001; For values whithout (ˆ), p>0.05*
Control: Distilled water,
$AECF^{50}$: Aqueous Extract of C. freruginea (50 mg/kg of body weight),
$AECF_{100}$: Aqueous Extract of C. ferruginea (100 mg/kg of body weight).
Spz: Spermatozoa

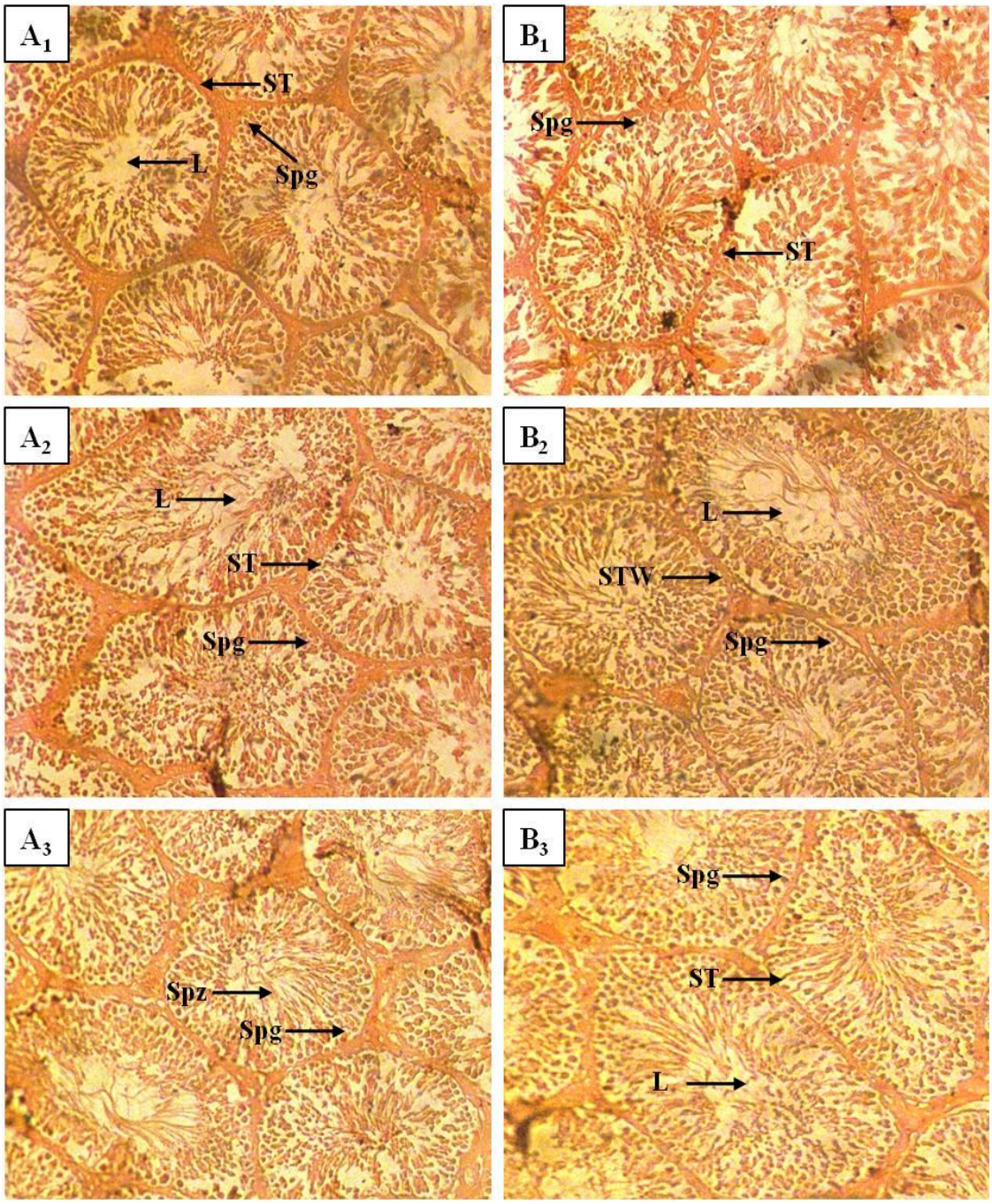

Fig. 3. Cross section of testis of control rats and rats treated with doses of *Cnestis ferruginea* after 30 and 60 days

A_1: Control (30 days); A_2: Treated 50 mg/kg of BW (30 days); A_3: Treated 100 mg/kg of BW (30 days)
B_1: Control (60 days); B_2: Treated 50 mg/kg of BW (60 days); B_3: Treated 100 mg/kg of BW (60 days)
L: Lumen; STW: Seminiferous tubule wall; ST: Seminiferous tubule; Spg: Spermatogonia;
Spz: Spermatozoa
Magnification: × 100
Staining: Hematoxylin eosin

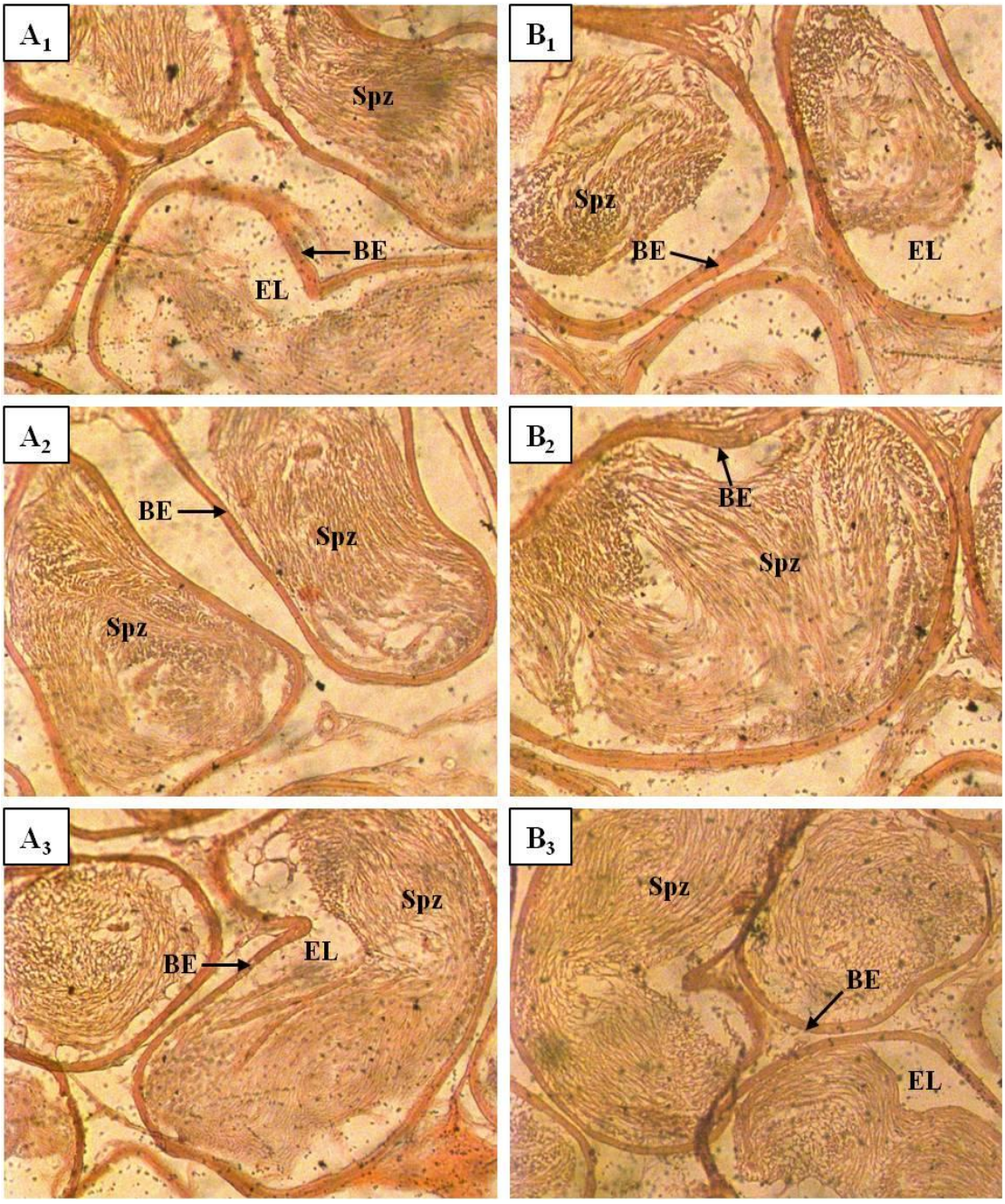

Fig. 4. Cross section of the epididymis of control rats and rats treated with doses of *Cnestis ferruginea* after 30 and 60 days

A_1: Control (30 days); A_2: Treated 50 mg/kg of BW (30 days); A_3: Treated 100 mg/kg of BW (30 days)
B_1: Control (60 days); B_2: Treated 50 mg/kg of BW (60 days); B_3: Treated 100 mg/kg of BW (60 days)
BE: Basal epithelium; EL: Epididymal lumen; Spz: Spermatozoa.
Magnification: × 100
Staining: Hematoxylin eosin

Regarding the epididymis, the morphometric analysis revealed no significant modification whatever the duration of treatment and the dose administered. Moreover, the increases induced by the different doses are not statistically significant.

Table 5. Effects of aqueous extract of *Cnestis ferruginea* on reproductive hormones

Hormones	30 Days			60 Days		
	Control	$AECF_{50}$	$AECF_{100}$	Control	$AECF_{50}$	$AECF_{100}$
FSH (mUI/mL)	2.860±0.189	3.760±0.194*	3.500±0.176	3.143±0.295	4.500±0.253*	4.920±0.375**
LH (mUI/mL)	5.667±0.345	6.033±0.2305	6.317±0.162	5,133±0,217	6.520±0.359*	6.355±0.310*
Testosterone (ng/mL)	4.798±0.228	6.660±0.416**	6.875±0.232**	4.682±0.250	6.170±0.384*	6.463±0.362**
Prolactin (mUI/mL)	7.375±0.325	6.313±0.426	6.838±0.346	6.500±0.620	7.180±1.305	7.960±1.199

*Values are means ± SEM (n=6); *=p<0.05; **=p<0.01; ***=p<0.001; For values whithout (*), p>0.05*
Control: Distilled water,
$AECF_{50}$: Aqueous Extract of C. freruginea (50 mg/kg of body weight),
$AECF_{100}$: Aqueous Extract of C. ferruginea (100 mg/kg of body weight)

Table 6. Effect of the aqueous extract of Cnestis ferruginea on the diameter of seminiferous tubules and epididymal tubes of rats treated for 30 and 60 days

Treatments duration	Treatments	Measured parameters (µm)	
		Seminiferous tubule diameter	Epididymis tubule diameter
30 Days	Control	274.400±9.252	620.300±57.880
	$AECF_{50}$	308.400±8.451	731.200±59.560
	$AECF_{100}$	318.100±16.702	748.700±57.340
60 Days	Control	282.700±12.491	650.300±50.900
	$AECF_{50}$	325.1±13.260	809.500±63.530
	$AECF_{100}$	331.900±13.502*	775.400±45.410

*Values are means ± SEM (n=6); *=p<0.05; For values whithout (*), p>0.05*
Control: Distilled water,
$AECF_{50}$: Aqueous Extract of C. freruginea (50 mg/kg of body weight),
$AECF_{100}$: Aqueous Extract of C. ferruginea (100 mg/kg of body weight)

4. DISCUSSION

4.1 Effects of *Cnestis ferruginea* on the Wet and Dry Weight of Some Organs

Cnestis ferruginea extract was tested on the reproductive organs and the adrenal gland of the rats. Thus, after 30 days of treatment, the results showed a significant increase in the wet weight of the seminal vesicle, the epididymis and the levator ani muscle (LAM). After 60 days of treatment, there was a significant increase in the wet weight of the testes, seminal vesicles, prostate and levator ani muscle.

Moreover, the weight, size and secreting function of the testis, epididymis, seminal vesicle and prostate are closely regulated by androgens. Similarly, the development of the levator ani muscle and penis is also dependent on androgens [17].

Indeed, steroidogenesis is one of the causes of the increase in the weight of the sexual organs. Growth of these parameters could be considered as a biological indicator of the plant's effectiveness in stimulating steroidogenesis [18]. Since the androgenic effect is due to the level of testosterone in the blood [19], it is likely that the extract of *C. ferruginea* may have a role in the secretion of testosterone allowing a better availability of hormone to the gonads.

In the testis, this observed weight gain, in addition to steroidogenesis, could be attributed to stimulation of spermatogenesis. The extract behaves as a testosterone agonist by binding to its receptors to mimic its biological activity. The extract could also act via a central action on the hypothalamic-pituitary complex by the secretion of LH and FSH and which would play an important role in the establishment of spermatogenesis. A similar result was obtained by Woode et al. [20]. These authors administered the ethanolic extract of *Xylopia aethiopica* (Annonaceae) at 30, 100 and 300 mg/kg of body weight to male Sprague Dawley rats for 60 days and obtained an increase in testes weight. On the other hand, these results are contrary to the administration of ethanol extracts of *Cynoglossum zeylanicum* (Borraginaceae) [21] and *Dactyloctenium aegyptium* (Poaceae) [22], anti-fertility plants and bisphenol A [23] which induce a decrease in the weight of the testes.

The increase in the weight of the seminal vesicles and the prostate observed in this study is due to an intense stimulation of the seminal and prostatic fluid secretion. This abundance of fluid could be at the origin of the weight growth of these organs. As mentioned above, the secretion in the seminal vesicles and the prostate is due to androgenic action on these organs. Thus, the extract of *C. ferruginea* would have acted directly on these organs by an androgen-like action or via the hypothalamic-hypophyso-testicular complex. This could also be explained by the presence in the extract of flavonoids and saponosides [24,25]. These compounds have the ability to boost the level of androgen and hence the blood testosterone. This hypothesis is supported by the fact that the study of the dry weight of these organs revealed no significant difference that could explain protein synthesis in these organs. Similar results were noted by Zade et al. [26] on rats treated with the aqueous extract of *Moringa oleifera* (Moringaceae) known for its fertility properties. Conversely, Gupta et al. [27] working on the methanolic extract of *Strychnos potatorum* (Loganiaceae) an anti-fertility plant recorded a significant reduction in the weight of the seminal vesicles and the prostate.

As for the epididymis, in addition to secretions of fluids induced by androgens, the accumulation of spermatozoa in this organ could be at the origin of the increase in weight. Thus, extract of *C. ferruginea*, in addition to these androgenic actions incriminated above, would have a stimulatory activity on spermatogenesis. These results corroborate those obtained by Zade et al. [26], which after administration of 100 mg/kg of body weight of the aqueous extract of *M. oleifera* obtained a significant increase in the weight of the epididymis.

The increase in the weight of the levator ani muscle also reinforces the results that the extract of *C. ferruginea* contains androgen-like substances. Indeed, the extract of this plant mimic the endogenous androgens at the level of this organ. In addition, the increase in the dry weight of this organ of the treated animals would also be due to the capacity of the extract to stimulate protein synthesis.

4.2 Effects of *Cnestis ferruginea* on Rat Spermatic Parameters

The evaluation of the effects of AECF on sperm parameters showed a significant increase in the percentage of motile spermatozoa and the percentage of normal spermatozoa with both doses studied ($AECF_{50}$ and $AECF_{100}$) and both

treatment durations. The density of spermatozoa increased significantly after 60 days of treatment with both doses. Indeed, the number, motility and morphology of spermatozoa are recognised as fertility index in male [28,29]. Thus the extract of *C. ferruginea* could have positive effects on the fertility of males. This result confirms again the effects of this plant on the androgeno-dependent organs. Furthermore, spermatogenesis is under the regulatory influence of pituitary gonadotrophins and testosterone. Improvement in the quality and quantity of spermatozoa is dependent on the quality of spermatogenesis and its transit to the caudal epididymis.

Thus, the increase in spermatozoa in the caudal epididymis found in this study could be explained by the ability of the extract to interfere with the spermatogenetic process in seminiferous tubules and epididymal function. It may also interfere with the activity of testosterone on hypothalamic release factors and anterior pituitary secretion of gonadotrophins. This interference may result in improved spermatogenesis in treated rats. Indeed, the presence of flavonoids in the extract of *C. ferruginea* revealed by Yakubu et al. [24] and Akharaiyi et al. [25], reinforces this hypothesis. It is established that flavonoids are capable of inducing antioxidant activities favorable to the improvement of testicular deficiencies related to stress-oxidants [30,31,32]. They also stimulate androgen synthesis and play an essential role in testicular differentiation, integrity and steroidogenesis function [33]. These results corroborate those of Zade et al. [26]. These authors administered the aqueous extract of *M. oleifera* at 100, 200 and 500 mg/kg body weight to rats and recorded an increase in sperm concentration. Conversely, administration of the alcoholic extract of *Citrus Limonum* (Rutaceae) induced a significant decrease in sperm concentration in the epididymis in rats [34]. Also in mice, Mimosine purified from *Leucaena leucocephala* (Fabaceae) induces a decrease in sperm concentration [35].

In this study, the observed increase in sperm motility may be due to a modification of the microenvironment in the caudal epididymis, which also had a synergistic effect on the spermatozoa of the treated rats. These effects could be attributed to the oral administration of the plant extract which has the ability to stimulate or boost the level of androgens through its phytochemical composition. At this level the extract could have a direct effect on the epididymis by making it conducive to the development of spermatozoa or by acting on the testicles to stimulate androgen secretion. These results are in agreement with those obtained by Mohan et al. [21]. Indeed, these authors administered to the rats the ethanolic extract of *Polycarpaea corymbosa* (Caryophyllaceae) at the dose of 500 mg/kg of body weight and observed a significant increase in the motility of the spermatozoa coming from the caudal epididymis.

In morphology, normal spermatozoa increased significantly in rats treated with *C. ferruginea* extract. This could be explained by the quality of spermatogenesis induced by plant extract in these rats. This result is consistent with the administration of *Polycarpaea corymbosa* (Caryophyllaceae) in rats [21]. Indeed, this plant induces a decrease of percentage of abnormal spermatozoa in the treated rats.

4.3 Effects of *Cnestis ferruginea* Extract on Male Rat Sex Hormones

The result of the determination of the reproductive hormones during this experiment revealed a significant increase in the serum concentration of pituitary gonadotrophins (FSH and LH). Indeed, gonadotrophins are central neurohormones that control gonadal functions and are alternately regulated by Gonadotropin-Releasing hormone (GnRH) [36]. The secretion of GnRH depends on activation of the GPR54 receptor, localised on the surface of the GnRH neurons, by the kisspeptin peptide. This increase in the serum concentration of FSH and the observed LH could be explained by a stimulation of the GnRH secretion through a mimetic action of the extract on the kisspeptin receptors (GPR54). The extract could also have a direct effect on gonadotropic cells of the anterior pituitary by behaving as a GnRH agonist. Furthermore, the results show that the concentration of LH increased only after 60 days of treatment compared to the control. It has been demonstrated by Ferris and Shupnik [37] that the low GnRH pulse frequency induces preferential FSH release probably due to the differential expression of the FSH receptor. This may explain a moderate effect of the extract of *C. ferruginea* on the secretion of GnRH during the first 30 days of treatment. After these first 30 days, the extract would have induced a strong impulse of the GnRH, hence the increase in the concentration of the serum LH. These results are consistent with those of Mohan et al. [21], which after administration of *Polycarpaea corymbosa*

(Caryophyllaceae) at a dose of 500 mg/kg of body weight obtained a significant increase in the concentration of FSH and LH.

The results of the testosterone assay confirm the previous results with respect to the weight of androgeno-dependent organs, sperm parameters and gonadotrophin hormones. Indeed, the serum testosterone level elevated in this test would probably be the cause of the increase in the weight of the organs measured. Similarly, the high level of testosterone may be a consequence of the increased serum level of pituitary gonadotrophins induced by the extract of *C. ferruginea* in the treated animals.

Indeed, gonadotropins stimulate the testes through Leydig cells to secrete testosterone [38]. Thus, the elevation of the LH level observed could be a factor triggering the high release of testosterone in the treated animals. The extract could possibly have a direct effect on the testes by acting as LH agonists on Leydig cells to induce testicular steroid synthesis.

4.4 Effects of *Cnestis ferruginea* Extract on the Histological Structure of the Testis and Epididymis

The histological and morphometric study carried out on the structure of the testis of treated subjects revealed an abundance of spermatozoa in the seminiferous tubules with a significant increase in the diameter of these. These results confirm the high concentration of spermatozoa found in the caudal epididymis of treated rats. Moreover, this could be related to the increase in serum levels of pituitary gonadotrophins induced by the extract. These results are consistent with those obtained by Woode et al. [20]. Indeed, these authors administered the ethanolic extract of *Xylopia aethiopica* (Annonaceae) to Sprague-Dawley rats at the dose of 30, 100 and 300 mg/kg for 60 days and observed an increase in the diameter of the seminiferous tubules and a Abundance of spermatozoa in these tubes. On the other hand, these results are contrary to the administration of the alcoholic extract of the seed of *Citrus limonum* (Rutaceae) and its fraction of ethyl acetate to albino rats. Indeed, the extract of this plant produces a decrease in the diameter of the seminiferous tubes and the concentration of spermatozoa in its lumen [34].

5. CONCLUSION

The aqueous extract of *Cnestis ferruginea* stimulates the growth of seminal vesicles, testis, prostate, epididymis and LAM. It also has an effect on the production and quality of spermatozoa. Histologically, the extract causes the diameter of the seminiferous tubes to increase. The results also show that the extract stimulates the synthesis and release of FSH, LH and testosterone. These effects could be related to the presence in our extract of androgen-like compounds.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The animals were handled according to the guidelines of the Ethical Committee on the use and care of experimental animals of the Department of Biosciences, Université Félix Houphouët-Boigny.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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