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Study of the pharmacological effects of the ethanol extract of trunk barks of *Zanthoxylum gilletii* (From Wild Waterman 1975) on the weight parameters of the reproductive organs of male rats

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Abstract

In Cote d'Ivoire the bark of the trunk of *Zanthoxylum gilletii* is used as an aphrodisiac. The objective of this work was to evaluate the impact of the ethanolic extract of *Zanthoxylum gilletii* trunk bark on the male sexual organs. To achieve this objective, the triphytochemical and acute oral toxicity were previously carried out according to OECD guideline 423. Then 18 males rats were chosen, divided into 3 batches of 6 rats for the study of the effect of the extract on the sexual organs of male rats. The rats received daily for 60 days 1ml of distilled water for the control batch and 1ml of extract with respective doses of 500 and 1000 mg/kg of body weigth for the treated batches. At the end of the treatment the rats were sacrificed, the reproductive organs were removed and weighed. Then, histological sections of the testes and epididymis were made. Phytochemical screening revealed the presence of polyphenols, flavonoids, alkaloids, sterols and polyterpenes. The acute toxicity study showed that the extract is not toxic; the lethal dose (LD50) is greater than 2000 mg/kg bw. The extract caused a significant increase ($P < 0.05$) of the fresh weight of the testes, epididymis, seminal vesicles, prostate and levator muscle. Histological sections also revealed growth in the size of the testicular and epididymal structures of the treated rats. *Zanthoxylum gilletii* extract may have androgenic properties, which would justify the use of this plant for improving male fertility.

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Introduction

Plants are increasingly used all over the world for their therapeutic properties (Fernando, 2012). As a result, studies are carried out to discover new active ingredients and formulate drugs accessible to populations (Katiyar *et al.*, 2012). In Côte d'Ivoire also, most populations resort to traditional medicine because of poverty and their safety (Logiel *et al.,* 2021). However, the excessive use of medicinal plants exposes populations to poisoning and various health risks, the outcome of which is often dramatic (Brima, 2017). Acute toxicity is the first step that must be carried out on a substance, when there is little information on toxicity. The determination of the LD50 in addition to the recording of the general behavior of the animals constitutes one of the critical parameters for the evaluation of the first signs of toxicity (Lima *et al.*, 2016). It makes it possible to measure and record the various adverse effects that appeared after administration (Leblanc, 2010) in order to approve and then guarantee phytotherapy and the consumption of plants (Bounihi, 2015). In Côte d'Ivoire, *Zanthoxylum gilletii* is commonly called African lemon tree and tchédjé in Baoulé. It is a plant of the Rutaceae family present in West and Central Africa. A tree that can reach 35 m high. The leaves, roots and bark of this plant are commonly used in traditional medicine in Côte d'Ivoire for their therapeutic virtues (Okagu *et al.,* 2021). Thus, according to Orsot *et al.* (2016), the decoction of the trunk bark of *Zanthoxylum gilletii* cures genitourinary and rheumatic disorders. The decocted bark of the tree is used against dental caries and the filtrate of the bark pounded or ground with the leaves of *Ricinodendron heudelotii* is used as an enema against female sterility. The bark of the trunk of *Zanthoxylum gilletii* in raffia wine is used in western Côte d'Ivoire as an aphrodisiac. Scientific data on the safety of this plant would allow it to be better exploited.

The objective of this work was to evaluate the impact of the ethanolic extract of trunk bark of *Zanthoxylum gilletii* on the reproductive organs of male rats.

The search for chemical compounds and acute toxicity were carried out to better evaluate the effect of the ethanolic extract of trunk bark of *Zanthoxylum gilletii* on the reproductive organs in male rats.

Materials and methods

Plant material

The vegetal material was composed of trunk bark of *Zanthoxylum gilletii* (Rutaceae) collected in March 2019 in the forest of Erimakoudjé 1, department of Agboville, in the commune of Anyama (located on the Anyama-Adzopé axis). A sample of this plant was authenticated at the National Floristic Center (CNF) of the Félix HOUPHOUËT BOIGNY University of Abidjan under the numbers UCJ016170.

Animal material

Males and females Wistar strain rats (*Rattus norvegicus* Murideae) aged two to three months, weighing between 120 g and 170 g, were used for the experiments. The animals from the animal store of the Superior Normal School (SNS) were acclimatized after one week and fed.

Methodology

Preparation of 70% ethanolic extract of Zanthoxylum gilletii

The harvested *Zanthoxylum gilletii* trunk barks were transported in well-ventilated bags to the laboratory. Then the barks were sorted, rinsed with distilled water and dried for 20 days at room temperature (25°C). After drying, they were pulverized to obtain a yellowish powder which was used to prepare the hydroethanolic extract. Thus 100 g of *Zanthoxylum gilletii* powder were dissolved in one liter (1l) of 70% ethanol according to the method described by Zihiri *et al.* (2003). The ethanolic mixture was stirred for 24 hours using a magnetic stirrer and the homogenate filtered several times on a clean cloth, then three times on hydrophilic cotton and on Whatman No. 1 paper. The filtrate was dried at 50°C in an oven (Memmert) and the dry extract constituted the ethanolic extract of *Zanthoxylum gilletii* trunk bark coded EEZGTB**.**

Phytochemical screening of the ethanolic extract of Zanthoxylum gilletii

Phytochemical screening carried out according to the method described by Mangambu *et al.* (2014) and Bidié *et al.* (2011) was used for the detection of compound (Table 1).

Table 1. Differents phytochemical screening methodologys

Chimical groups	Reagents	Reactions
Alkaloïds	Dragendorff Bouchardat	Précipite or orange coloration Précipité riddish-brown
Polyphenols	Ferric chloride	Blackish_blue coloration
Flavonoïdes	Cyanidine	Précipitate orange-pink
Sterols and les polyterpenes	Liebermann	Green ring
Tannins	Stiasny	Precipite of flakes
Quinonic substances	Borntraegen	Red or violet coloration
Saponins	Agitation	Persistent foam

Acute toxicity

Acute toxicity was carried out on rats aged 8 to 9 weeks, with a body weight (bw) between 120 g and 170 g, using the ethanolic extract of *Zanthoxylum gilleti* trunk bark in order to determine an interval allowing an easier study of toxicity, preliminary tests were carried out. This toxicity was carried out according to guideline 423 of the Organization for Economic Cooperation and Development (OECD, 2001). The lethal dose (LD50) was determined from limit tests at 300 and 2000 mg/kg of body weigth. The animals were raised in the Vivarium of the Superior Normal School (SNS). The rats were placed in plastic cages with free access to food and water. The litter or wood shavings were changed every two days. The rats were acclimated to laboratory conditions for 15 days before the experiment. After acclimation, 3 batches of 3 rats were made. The rats were deprived of food 24 hours before the administration of the ethanolic extract of trunk bark of *Zanthoxylum gilletii* (EEZGTB) to avoid any digestive food interaction. After the fasting period, the animals were weighed and marked individually for recognition.

Each batch received orally a single dose at a rate of 1ml/g of body weight (bw), of which 2 batches of rats were treated with EEZGTB and the 3rd batch received distilled water. In this experiment a limit test was carried out. Thus, a single dose of 2000 mg/kg of body weight of EEZGTB is administered to each rat using a gastric tube. The animals were deprived of food for another 4 hours before having access to food. Then 48 hours after observing the absence of clinical signs in the first trial of 2000 mg/kg of body weigth (bw), the second group of rats received the same dose of 2000 mg/kg of body weigth of EEZGTB for confirmation of the LD50. A behavioral observation was carried out during the first 30 minutes after administration, then 1 hour, then for 24 hours with particular attention during the first 4 hours after administration of the extract. Then hydration and nutrition were carried out on a daily basis. The rats were monitored carefully once daily for 14 days. During this period, signs of toxicity include changes in coat, motility, tremors, mass, grooming, breathing, and sensitivity to noise after metal impact, appearance of stools, mobility as well as death were observed. The change in body weight of each batch was noted during the 14 days of experimentation.

Preparation and distribution of animals

18 males rats are divided into 3 batches of 6 subjects each and treated as follows:

Lot 1: (control): 1 ml / 100g of body weigth of distilled water

Lot 2 (EEZGTB 500): 500 mg/kg of body weigth of EEZGTB

Lot 3 (EEZGTB1000): 1000 mg/kg of body weigth of EEZGTB

The volume of extract administered daily in a single dose was 1ml per 100g. Evaluation of the effects of the trunk bark extract of *Zanthoxylum gilletii* on the evolution of the weight of rats

Before their sacrifice, the animals were weighed using a balance (Mettler BW 400) with a capacity of 500 g. The rate of change in percentage of weight change was calculated according to the following formula:

$$\text{Weight variation}(\%) = \frac{\text{Final weight} - \text{initial weight}}{\text{Initial weight}} \times 100$$

Rats sacrifice

At the end of the treatment, the rats from the different groups were sacrificed the day after the last gavage (day 60) by decapitation, after anesthesia with ether.

Weight parameters of sexual organs

After the sacrifice of the animals, the testicles, the glans and the ancillary organs (seminal vesicles, ventral prostate, epididymis, elevator any muscle) were removed, freed of adipose tissue and then weighed. The relative weight of each organ was determined by the following formula:

$$\text{Relative weight} = \frac{\text{organ weight}}{\text{Body weight}} \times 100$$

Histological study of male reproductive organs

For the histopathology study, the right testicle and right epididymis of each animal were fixed in 10% formalin then subjected to a series of dehydrations in ethanol baths and embedding in paraffin. Sections of 5 µm were made with a microtome. These sections mounted on slides were stained with hematoxylin and eosin (H&E), then observed under a light microscope (Olympus CKX100, Germany).

Statistical analyzes

Data analyzes and graphical representations were carried out using GraphPad Prism 8.01 software (Microsoft, USA). The values are presented as mean ± SEM and the data were evaluated by the one-way ANOVA analysis method followed by Tukey's multiple comparison test at the 5% threshold. If $P < 0.05$ the difference between the values is considered significant, if $P < 0.01$ this difference is considered very significant, if $P < 0.001$ it is considered highly significant and if $P > 0.05$ this difference n is not significant.

Results

Secondary compound of the ethanolic extract of Zanthoxylum gilletii trunk bark

The ethanolic extract of *Zanthoxylum gilletii* trunk bark (EEZGTB) phytochemical screening revealed the presence of polyphenols, flavonoids, alkaloids, sterols and polyterpenes and an absence of quinones, saponosides and tannins (catechic and gallic) (Table 2).

Table 2. Secondary compound of the ethanolic extract of *Zanthoxyum* trunk bark *gilletii*

Groupes chimiques		Observation
Sterols and les polyterpenes		+
Polyphenols		+
Flavonoïdes		+
Tannins	Gallic	-
	Catéchic	-
Quinonic substances		-
Alkaloïds	Dragendorff	+
	Bouchardat	+
Saponins		-

+ : Presence of phytochimicals - : Absence of phytochimicals

Acute toxicity of the ethanolic extract of trunk bark of Zanthoxylum gilletii

Oral administration of the ethanolic extract of trunk bark of *Zanthoxylum gilletii* (EEZGTB) at a dose of 2000 mg/kg of body weigth did not cause any mortality in the treated rats. Observation of the animals for 14 days showed no signs of toxicity (salivation, drowsiness, morbidity, coma, etc.). The animals showed a sign of well-being, that is to say normal movement, correct food and water intake like the controls (Table 3).

At the end of the 2 weeks of treatment, no deaths were observed. This study revealed a non-significant increase in body weight gains between treated rats and control rats (Table 4).

Effect of Zanthoxylum gilletii bark extract on the weight of rats

Fig. 1 represents the weight gain of rats after 60 days of daily administration of the ethanolic extract of *Zanthoxylum gilletii* trunk bark (EEZGTB). The values obtained show that the administration of EEZGTB did not cause any significant variation ($P>0.05$) in the weight of rats treated at doses of 500 and 1000 mg/kg of body weigth compared to the controls. Effects of EEZGTB at doses of 500 and 1000 mg/kg bw on the fresh weight of the reproductive organs of male rats.

Table 3. Clinical signs observed during acute toxicity

Période	1h	2h	3h	4h	J2	J4	J6	J8	J10	J12	J14
Grooming	N	N	N	N	N	N	N	N	N	N	N
Pelage	N	N	N	N	N	N	N	N	N	N	N
Trembling	N	N	N	N	N	N	N	N	N	N	N
Motility	N	N	N	N	N	N	N	N	N	N	N
Reaction to noise	N	N	N	N	N	N	N	N	N	N	N
Stool appearance	N	N	N	N	N	N	N	N	N	N	N
Number of deaths	0	0	0	0	0	0	0	0	0	0	0

N= Normal ; Dose : 2000 mg/kg

Table 4. Variation in body weight of rats during the 14-day treatment

Treatment mg/kg bw	Mean of body weight ± SEM	
	before treatment	After le treatment
Control (distilled water)	139,8 ± 0,85	142,8 ± 0,39
Treaties (2000 mg)	135,7 ± 0,39	141,1 ± 0,94

Values are expressed as means followed by the standard error of the mean (M ± SEM); n = 3 rats; without significant value correspond to P>0.05 bw: body weight

Table 5. Weight of reproductive organs of rats after 60 days of treatment with EEZGTB

Parameters	Organs g/100g)						
Treatments	Testicular	Epididyms	Seminal vesicles	Prostate	Cooper glands	Levator muscle	Gland
Controls	1,16 ± 0,02	0,18 ± 0,02	0,47 ± 0,05	0,23 ± 0,04	0,15 ± 0,01	0,35 ± 0,02	0,14 ± 0,01
$EEZGTB_{500}$	1,31 ± 0,03*	0,31 ± 0,04	0,74 ± 0,08	0,24 ± 0,01	0,19 ± 0,04	0,51 ± 0,03*	0,15 ± 0,01
$EEZGTB_{1000}$	1,36± 0,04*	0,37 ± 0,03*	0,78 ± 0,06 *	0,35 ± 0,01 *	0,19± 0,01	0,57± 0,05*	0,17 ± 0,01

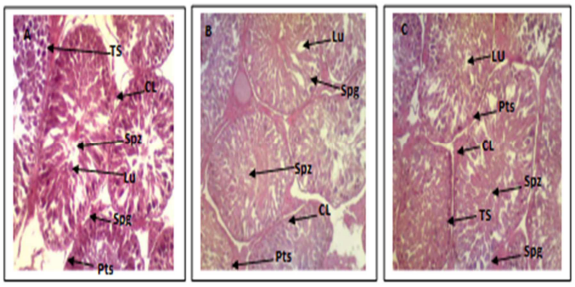
Values are expressed as means followed by the standard error of the mean (M ± SEM); n = 6 rats; *= p< 0.05 correspond to a significant difference compared to the control. EEZGTB: Ethanolic extract of Zanthoxylum gilletii trunk bark, body weight: body weight, EEZGTB 500: 500 mg/kg body weight and EEZGTB $_{1000}$: 1000 mg/kg body weight.

Table 5 presents the fresh weights of the reproductive organs of male rats after 60 days of treatment with EEZGTB at doses of 500 and 1000 mg/kg bw. These are the testicles, epididymis, seminal vesicles, prostate, Cooper glands, levator muscle and glans (penis). Concerning the weight of the testicles, a significant increase ($P < 0.05$) of 10.61% (1.31 ± 0.03) and 13.27% (1.36 ± 0.04) respectively was observed. for rats treated at doses of 500 and 1000 mg/kg bw compared to control rats (1.16 ± 0.02).At the level of the epididymis, significant increases ($P < 0.05$) of 21.05% (0.37 ± 0.03) of the epididymis were noted for rats treated at a dose of 1000 mg/kg of pc compared to control rats (0.18 ± 0.02). However, no significant difference was observed (P > 0.05) between rats receiving the 500 mg/kg dose compared to controls. However, for the seminal vesicles, the dose of 1000 mg/kg bw of EEZGTB caused a significant increase ($P < 0.05$) in the weight of the seminal vesicles of 65.95% (0.78 ± 0.06) per compared to controls (0.47 ± 0.05). Conversely, no significant difference ($P > 0.05$) was observed in those treated with 500 mg/kg bw compared to the controls. At the prostate level, a significant increase ($P < 0.05$) of 52.17% (0.35 ± 0.01) in prostate weight was revealed in rats treated at a dose of 1000 mg/kg. of bw compared to controls (0.23 ± 0.04). On the other hand, the statistical analysis showed that there is no significant difference ($P > 0.05$) between the weight of the prostate of the treated rats at a dose of 500 mg/kg bw and that of control rats. For Cooper's glands, analysis of the table revealed no significant difference ($P > 0.05$) between rats treated at doses of 500 and 1000 mg/kg bw and the controls.

At the end of the experiment concerning the levator muscle, the doses of 500 and 1000 mg/kg bw of the ethanolic extract of the trunk bark of Zanthoxylum

gilletii did not cause a significant increase ($P < 0.05$) of the levator muscle respectively by 54.54% (0.51 ± 0.03) and 62.85% (0.57± 0.05) compared to controls (0.35 ± 0.02). At the glans level, no significant difference ($P > 0.05$) in weight was revealed in rats treated at doses of 500 and 1000 mg/kg bw compared to controls. Effects of EETBZG at doses of 500 and 1000 mg/kg bw on the histological structure of the testis and epididymis.

Fig 1. Evolution of body weight of male rats during treatment



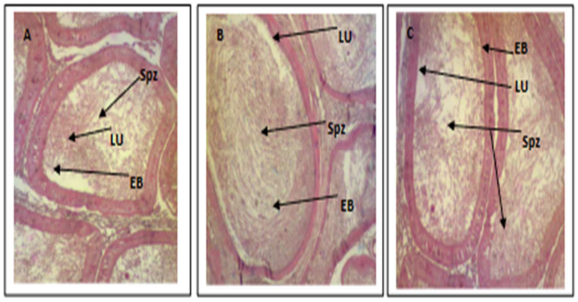
A: Section of testicle from a rat treated with distilled water, B: Section of testicle from a rat treated with 500 mg/kg bw of *EEZGTB*, C: Section of testicle from a rat treated with 1000 mg/kg bw

LU: Lights; Pts: Wall of the seminiferous tubule; Spg: Spermatogonia; Spz: Sperm;

CL: Leydig cell; TS: Seminiferous tube; Magnification, Coloring: Hematoxylin-eosin

Fig 2. Cross section of testis of control rats and treated with ethanolic extract of Zanthoxylum gilletii trunk bark (*EEZGTB*) after 60 days

The histological study of the testes (Fig. 2) revealed that daily administration of EEZGTB for 60 days caused in all treated rats an increase in the thickness of the seminiferous tubules of the treated rats compared to the controls. On the other hand, normal development of spermatogenesis was observed in control rats and rats treated at doses 500 and 1000 mg/kg bw. All stages of spermatogenesis (spermatogonia, spermatocytes, spermatids and spermatozoa) were observed through the histological sections. Regarding the epididymis (Fig. 3), observation of the histological sections showed a normal appearance and the presence of spermatozoa. However, the increased presence of spermatozoa was noted in rats treated at doses of 500 and 1000 mg/kg bw than in controls.



A: Section of the epididymis of a rat treated with distilled water, B: Section of the epididymis of a rat treated with 500 mg/kg bw of EEZGTB, C: Section of the epididymis a rat treated with 1000 mg/kg bw

LU: Epididymal light; EB: Basal epithelium; Spz: Spermatozoa; Magnification: X100; Coloring: Hematoxylin-eosin

Fig 3. Cross section of the epididymis of control rats and treated with ethanolic extract of trunk bark of *Zanthoxylum gilletii* (EEZGTB) after 60 days

Discussion

Medicinal plants are used by most populations for their reputation in the management of pathologies. Research on plants has confirmed their therapeutic effectiveness on several diseases (Sinan *et al.*, 2019). These plants in fact contain molecules which are phytochemical compounds giving them curative properties (Yoo *et al.*, 2018). Thus, the phytochemical screening of the ethanolic extract of trunk bark of *Zanthoxylum gilletii* (EETBZG) revealed the presence of sterols and polyterpenes, polyphenols, flavonoids, alkaloids and the absence of tannins (catechic and

gallic), quinones and saponosides. This richness in chemical elements would explain the therapeutic use of the different parts of *Zanthoxylum gilletii*. These results are similar to those of Zirihi *et al.* (2007), these authors indicated that *Zanthoxylum gilletii* exerts its antimalarial activity thanks to an alkaloid, dihydronitidine. Other metabolites such as flavonoids, sterols and terpenes have also been isolated from plants of this genus (Waterman and Grundon, 1983). Different classes of low nitrogen compound alkaloids molecular weight have already been isolated from different parts of the plant belonging to the genus Zanthoxylum. Polyphenols and flavonoids are in fact known for their anti-inflammatory (Basli *et al.*, 2012), antioxidant (Ghazghazi *et al.,* 2013), antidiabetic (Bayle, 2017), hepatoprotective (Jehangir *et al.*, 2010) activities. . The sterols and polyterpenes present give EEZGTB hypocholesterolemic (Bougherara, 2015), anti-atherogenic (Mamyrbekova-Bekro *et al.* 2013), anti-inflammatory (Kouadio *et al.*, 2021) and antioxidant properties according to Mbaïhougadobe *et al.* (2017). The alkaloids attribute hypotensive and vasodilatory properties to the ethanolic extract of *Zanthoxylum gilletii* trunk bark (Koné *et al.*, 2009). The purpose of acute toxicity is to evaluate the LD50 of a given substance. It was done by recording the various adverse effects which occurred after administration of the single dose of 2000 mg/kg body weight of EEZGTB. This method makes it possible to determine in which dose range the substance should be considered. Indeed, the study of the acute toxicity of the ethanolic extract of trunk bark of *Zanthoxylum gilletii* administered orally to Wistar rats revealed no mortality or morbidity at the dose 2000 mg/kg bw. At this dose of 2000 mg/kg bw of EEZGTB, no change in behavior or signs of intoxication were recorded after 14 days. This result made it possible to classify according to OECD (2001) the ethanolic extract of *Zanthoxylum gilletii* trunk bark in category 5 or unclassified of the globally harmonized classification system (GHCS) of chemical substances. This category identifies substances with low oral toxicity according to the Hodge toxicity Hodge *and* Sterner (1943) the lateral dose 50 (LD50) of EEZGTB would be greater than 2000 mg/kg bw. The ethanolic extract of Zanthoxylum gilletii trunk bark belongs to the category of weakly toxic extracts (OECD, 2001). The evolution of body weight on the 14th day indicated that the ethanolic extract of *Zanthoxylum gilletii* trunk bark (EEZGTB) did not induce any significant change in the body weight of rats compared to controls. Changes in body weights serve as an indication of the general health of animals (El Hilaly *et al.,* 2018). This change in weight is practically the same in the treated animals and in the controls. It could be assumed that the *Zanthoxylum gilletii* trunk bark extract did not interfere with the normal metabolism of these rats. Indeed, the body weight of animals is an important factor in evaluating the toxicity of a given substance. The reduction in body weight is a simple and sensitive index of toxicity after exposure of animals to a toxic substance (Raza *et al.*, 2002). EEZGTB would therefore not be toxic after a single oral intake of 2000 mg/kg bw in rats. The present study also revealed a significant increase in the weight of some reproductive organs due to the administration of EEZGTB for 60 days. Indeed, the relative weights of the testicles, epididymis, prostate, levator muscle and seminal vesicles compared to controls increased significantly ($P < 0.05$). This increase in the testes of treated rats would be due to stimulation of steroid synthesis by EEZGTB. The production of steroids that interact with androgens is one of the main causes of enlarged reproductive sex organs (Thakur *and* Dixit, 2007). Indeed, the increase in the weight of the reproductive organs would be an indicator of an improvement in fertility by stimulating androgen synthesis (Woode *et al.,* 2011). The androgenic effect observed is attributed to testosterone (Amini *and* Kamkar, 2005). The ethanolic extract of trunk bark of *Zanthoxylum gilletii* would stimulate the secretion of testosterone, hence the increase in the weight of the testes through the mitotic proliferation of gonadal cells. EEZGTB would cause the growth of seminiferous tubules in treated rats. According to Bordbar *et al.* (2013) increased testosterone production would increase gonad weight through growth of seminiferous tubules and active spermatogenesis.

These results are similar to those of Mukhallad *et al.* (2009), the latter noted a significant increase in the weight of the reproductive organs and the number of spermatozoa after administration of black seed extract to male albino rats. The increase in weight of the seminal vesicles of rats treated with EEZGTB compared to control rats observed after 60 days would be due to intense stimulation of seminal fluid secretion (Marzouk, 2017).

The richness of EEZGTB in flavonoids, these secondary metabolites, would stimulate the hypothalamic-pituitary-testicular complex to act on adrogen-dependent organs by increasing the secretion of seminal fluid and their volume (Zougrou, 2017). Thus the weight of the testicles, epididymis and seminal vesicles increases. Indeed, flavonoids have the ability to boost the level of androgen and testosterone in the blood circulation. These same observations were made by Zade *et al.* (2013) after the administration of the aqueous extract of *Moringa oleifera* to albino male rats for 60 days during fertility tests. In this study the increase in the weight of the levator muscle of the treated rats could be attributed to the ability of EETBZG to stimulate protein synthesis according to Zougrou (2017) who in this work noted an increase in the mass of the levator muscles of anus of the treated rats. induced by aqueous extract of *Cnestis ferruginea* leaves in a study on male rat fertility. EEZGTB would therefore have an androgenic action on certain reproductive organs. Effect the sexual organs are androgen-dependent organs, their growth is closely regulated by androgen hormones (Agrarwal *et al.,* 1986). The analysis of the histological sections made it possible to make a difference at the level of the constituent cells of the testis and the epididymis. The length of the seminiferous tubules is linked to three structural parameters: testicular weight, diameter of the seminiferous tubes and tubular volume (Souza *et al.*, 2011). EEZGTB caused an increase in the size of the seminiferous epithelium in treated rats compared to controls. The seminiferous tubules correspond to the site of spermatogenesis, where diploid spermatogonia differentiate into mature haploid cells (Russell *et al.*, 1990). This process leads to an increase in the size of the epithelium of the seminiferous tubules thanks to hormonal control. EEZGTB would have stimulated the production of testosterone and therefore spermatogenesis. According to Stocco (2002) the increase in the size of the seminiferous tube and the weight of the tests is due to very active spermatogenesis, stimulated by the main male hormone testosterone secreted during steroidogenesis by Leydig cells. Identical results were obtained by Boudou *et al.* (2013) who showed that intraperitoneal administration of the essential oil of *Syzygium aromaticum* to rats improves the structure of testicular tissue. Thus, the increase in the weight of the testicles is therefore linked to that in the number of germinal and somatic cells of the testicles. The normal appearance of spermatogenesis with the presence of spermatozoa in the seminiferous tubules and in the epididymis in control and treated rats shows that EEZGTB would have a positive effect on spermatogenesis.

Conclusion

This study is part of the development of medicinal plants. The objective of this work was to evaluate the effect of the ethanolic extract of trunk bark of *Zanthxoylum gilletii* on the reproductive organs of male rats. The phytochemical study based on specific tests showed that the extract does not contain saponosides, quinones and tannins (catechic and gallic), but contains sterols and polyterpenes, polyphenols, flavonoids and alkaloids. The presence of these chemical elements would be at the origin of the therapeutic virtues of the trunk bark of *Zanthoxylum gilletii*. The study of the acute oral toxicity of the extract revealed that it is non-toxic, with a lethal dose greater than 2000 mg/kg bw and does not induce any change in body weight. Furthermore, doses of 500 and 1000 mg/kg body weight of the extract led to a significant increase in the weight of the reproductive organs in the treated rats. The ethanolic extract of trunk bark of *Zanthxoylum gilletii* would be a good stimulator of male fertility. The histological study of the sections carried out also revealed a normal-looking structure in the testicles and epididymis of the treated animals.

A high concentration of spermatozoa was observed in the seminiferous tubules and in the epididymis of rats receiving the extract. The ethanolic extract of trunk bark of *Zanthoxylum gilletii* therefore seems to have an androgenic effect modulating the fertility of male rats thanks to its richness in flavonoids. The results obtained are satisfactory, in order to complete this study, it would be of great interest to evaluate sperm parameters and direct research towards the production of improved drugs.

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