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Effects of the aqueous extract of *Anthocleista liebrechtsiana* leaves (Longamiceae) on ethanol-induced spermatic disorders in rats

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ABSTRACT

Anthocleista liebrechtsiana is a plant used in traditional medicine in Cameroon to treat sexual asthenia and male infertility. In order to evaluate the effects of *Anthocleista. liebrechtsiana* hydroalcoholic extract leaves on ethanol-induced sperm disorders in rats, adult male rats were divided into 4 groups of five rats each. The experimental period lasted 75 days. It was divided into two phases: an oligospermia or azoospermia induction phase (21 days) during which all the animals were intoxicated with 40 ° ethanol at a dose of 6 g/kg except the normal control group, and the treatment phase which lasted 54 days. During the latter phase, the animals of the negative control group received ethanol and distilled water *per os*; those of test groups A and B received ethanol and hydroalcoholic extract at doses of 150 and 300 mg/kg, respectively. At the end of this period, the animals were anesthetized and then sacrificed. Reproductive organs and annex glands were removed and weighed; arteriovenous blood was collected in tubes for testosterone assay. Sperm count, motility, sperm vitality was determined, and histopathological analysis of the testis, epididymis and prostate was performed. The results of the study showed that treatment of rats with 40° ethanol has significantly reduced body weight, relative weight of reproductive organs and the annex glands, the number of spermatozoa, their mobility and vitality, and the level of testosterone compared to the normal group. The treatment with the hydroalcoholic extract of *Anthocleista liebrechtsiana* significantly corrected the weight of the reproductive organs and annexed glands, and the number, mobility and vitality of spermatozoa. These results indicate the ability of the hydroalcoholic extract of *Anthocleista. liebrechtsiana* to correct the deleterious effects of ethanol on sperm parameters. These results thereby justify the use of *Anthocleista. liebrechtsiana* leaves in the management of male infertility in the traditional pharmacopoeia.

Keywords: *Anthocleista liebrechtsiana*, Ethanol, Male rat, Spermatic disorder.

INTRODUCTION

Infertility is defined as the difficulty for a couple to have a child after a year of frequent unprotected sex. It is one of the major health problems affecting the socio-cultural life of many individuals. Infertility affects about 10 percent of couples worldwide, or 70 million people [1]. In Africa, infertility is more than a health problem, it is also a psychosocial problem. The prevalence is very high in sub-Saharan Africa, where one third of couples are affected, with a male responsibility of about 40% [2]. A disorder of spermatogenesis in men (oligospermia, azoospermia) is thought to be present in 1/3 of cases of male infertility [3]. The deterioration in sperm quality, observed for several decades, poses the problem of declining fertility in men [4].

Several causes of male infertility are listed, including hormonal disorders, hereditary diseases, gonadotoxins such as drugs, insecticides, alcohol consumption [5]. Among these causes, one of the factors related to the man's lifestyle is strongly involved. Alcohol is known to be one of the human lifestyle factors that severely impairs male reproduction and whose effects are further developed by physiological, genetic and environmental variations, psychosocial factors and the man's lifestyle [4, 6]. The treatment of idiopathic male infertility involves many medical treatments, with varying probabilities of success. None of these treatments has yet shown a real therapeutic effect [7]. Further, the side effects of these substances are so marked. It is therefore essential to combine efforts to propose new therapeutic solutions adapted to reduce the suffering of couples where infertility is perceived as a fatality for the whole family. In the search for solutions to this human-related infertility problem, the consumption of certain plants has been shown to have an effect on reproduction [8]. The treatment of male infertility with natural herbs is now an increasingly popular treatment method.

Some authors have shown that the leaves of *Anthocleista liebrechtsiana* are used in the traditional Cameroonian pharmacopoeia, particularly in the town of Bangangté, in western Cameroon, for the effective treatment of reproductive difficulties in men [9]. In addition, other authors reported that the same plants was used in maceration in the Democratic Republic of Congo for the treatment of azoospermia [10]. Based on these results, the leaves of *Anthocleista liebrechtsiana* could well be a source of bioactive molecules that could correct male infertility disorders. The aim of our work was to perform an *invivo* pharmacological trial of this plant in order to propose an alternative solution for male infertility.

MATERIAL AND METHODS

Plants material

The fresh of *Anthocleista liebrechtsiana* were harvest on December 5,2018, in Akonolinga (center region) along the Nyong River. The botanical identification of the plant was carried out at the National Herbarium of Cameroon (NHC) in Yaoundé in comparison to another sample registered at No.55963/HNC. The leaves were shade-dried and then pulverised using an electric mill. This,2.3 kg of the powder obtained after spraying were used for the preparation of the hydralcoholic extract by maceration of the powder for 48 hours in a percolator with 6.9 L of solvent (80% ethanol at 95 °C (v/v) and 20% distilled water). After filtration, the filtrate was taken up for 24 hours with the same solvent. The extract concentrate was obtained in a rotary evaporator and then dried in an oven at 45 °c. Thus,149.37 g of crude plant extract in the form of powder of dark brown a colour was obtained, corresponding to an extraction yield of 6.49%.

Qualitative phytochemical screening of the plant extract

A qualitative analysis of the various secondary metabolites (alkaloids, tannins, flavonoids, coumarins, saponins, terpenoids and reducing sugars) present in the extract was carried out according to protocol described by Odebiyi and Sofowara [11]

Animal material

The animals used were Wistar albino male rats aged 3 months and weighing approximately 300g. These animals were housed in plastic cages and given food and tap water ad libitum. All animal treatment procedures used in the present study were approved by the Cameroon National Ethics Committee (Ref.No.FWIRB 00001954 **).**

Experimental protocol

After randomization of the animals, different group were established at the rate of 5 animals per group: a normal control group receiving only distilled water, a negative control group receiving alcohol (6g/kg/day) and treated after an hour's interval with distilled water (10 mg/kg) and two test groups ET +E150 and ET +E300 mg/kg receiving alcohol and treated after an hour's interval with the plant extract at a dose of 150 and 300 mg/kg, respectively. Acclimatization was done over two weeks. The experiment began with the administration of 40 ethanol by gavage at a dose of 6 g/kg/day for 21 days for the induction of oxidative stress and spermatic disorders. On day 22, animals of test groups were treated respectively with the extract at doses of 150 and 300 mg/kg/day for 54 days. The administration of alcohol was continued until the 75th day of the experiment. On day 76th the animals were sacrificed, the reproductive organs (testis, epididymis, seminal vesicles, prostate, vas deferens, penis elevator muscle and penis) were removed and weighed. The left testis prostate and epididymis were kept in bouin liquid for later histological sections. The relative weight of organs, the number, the mobility, and the viability of the sperm were determined. The blood collected was used for the preparation of the serum, which was stored in aliquot in the freezer (-40 °c) for testosterone assay.

Study of the effects of *Anthocleista liebrechtsiana* on fertility parameters

The weight parameters of each sex organ were determined by calculating the relative weight of each organ compared to the weight of corresponding animal. The number, mobility and visibility of spermatozoa was determined. Histological sections were stained using haematoxylin-eosin and observations were made using a microscope (OLYMPUS) assisted by a Compaq nx9010 computer connected to a digital microscope camera (DCM35 350K pixels). Data were observed on 4 slides from each group.

Biochemical test

The serum testosterone level was determined by the solid phase enzyme-linked immunosorbent assay (**ELISA**) using the CYPRESS diagnostic kit 2015 which is based the competition principle

Statistical analyses

Statistical analyses of the values obtained were performed using Graph Pad Prism 7.0 software. Results were expressed as the mean ± standard error of the mean (SEM) and the different values were compared using the ANOVA one-way analysis of variance test, followed by the multiple comparison Turkey's post-test. The differences were considered significant at $p<0.05$

RESULTS

Phytochemical screening

Table 1 shows the results of the screening of the different groups of secondary metabolites of the hydroalcoholic extract of the leaves of *A. liebrechtsiana*. It shows that it contains alkaloids, polyphenols (tannins, flavonoids, coumarins), saponins, terpenoids, and reducing sugars. Steroids have not been detected in the alcoholic extract of the leaves of *A. liebrechtsiana.*

Table 1: Phytochemical composition of the hydroalcoholic extract of the leaves of *A. liebrechtsiana*.

Compounds	Results
Alkaloids	+
Tannins	+
Flavonoids	+
Coumarins	+
Saponins	+
Triterpenoides	+
Steroids	-
+: presence	–: absence

Effects of the hydroalcoholic extractbof *Anthocleista liebrechtsiana* on body weight

Figure 1 shows the body weight variation of the animals during the experimental period. It appears that the administration of ethanol to the negative control group resulted in a significant loss of body weight from the 35th day (p <0.05) and until the 75th day (p <0, 05) by 15.83% and 19.75%, respectively, compared to the normal group. The hydroalcoholic extract of *A. liebrechtsiana* administered at different doses (150 and 300 mg/kg) did not induce any significant variation in body weight compared to the negative control group

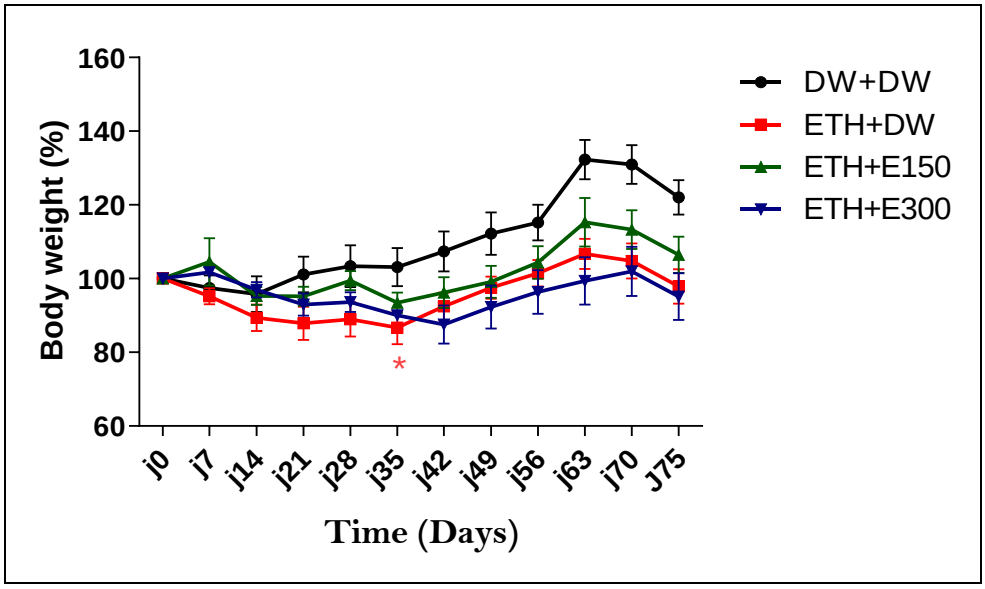

Figure 1: Body weight evolution of different treated groups during the experiment.

Each point represents the mean ±SEM of the percentage of weight change; (n=5);*(p<0.05) significantly different compared to the normal control group; DW+DW: Normal control receiving distilled water twice; ET+DW: Negative control receiving ethanol at the dose of 6 g/kg and distilled water; ET+E150 and ET +E300:test group A receiving ethanol 6 g/kg and the hydroalcoholic extract of *Anthocleista liebrechtsiana* at the respective doses of 150 mg/kg and 300 mg/kg.

Effects of the hydroalcoholic extract of *Anthocleista liebrechtsiana* on the relative weight of the androgenic-depend sexual organs

Table 2 shows the impact of treatment with hydroalcoholic extract of *A. liebrechtsiana* on androgen-dependent sex organ weights. It appears that the administration of ethanol to the negative control group resulted in a significant decrease in the relative weights of the reproductive organs compared to normal control group. A significant decrease (p<0.05) in the weight of the testis. (p<0.01) of the epididymis, (p<0.001) of the prostate and seminal vesicles was observed in the negative group and aa non-significant decrease in the relative weights of the Vas deferens, penis, and penis elevator muscle compared to the normal group.

Treatment with hydroalcoholic extract of *A.liebrechtsiana* at the dose of 150 mg/kg resulted in a significant increase in the weight of seminal vesicles (p<0.001), prostate (p<0.01), epididymis (p<0.01), on testis (p<0.05), penis elevator muscle(p<0.05), and a non significant increase in Vas deferens, and penis compared to the negative group. Treatment of the hydroalcoholic extract of *A.liebrechtsiana* at the dose of 300 mg/kg resulted in a significant increase in the relative weight of the prostate (p<0.001),seminal vesicles (p<0.01),epididymis (p<0.05),on the one hand ,and on the other hand, a non significant increase in the testis, the penis elevator muscle and the penis compared to the negative control group.

Table 2: Effects of the hydroalcoholic extract of *A. liebrechtsiana* on the relative weight of male reproductive organs.

Relative weight (%)	Normal group (DW+DW)	Negative group (ETH+DW)	ETH+E150	ETH+E300
Testicles	1.02 ± 0.02	0.89 ± 0.08*	1.22 ± 0.03^{a}	1.05 ± 0.03
Epididymis	0.36 ± 0.01	0.22 ± 0.01**	0.36 ± 0.02^{aa}	0.34 ± 0.03^{a}
Prostate	0.36 ± 0.04	0.16 ± 0.01***	0.22 ± 0.01^{aa}	0.30 ± 0.02^{aaa}
Vas deferens	0.11 ± 0.01	0.09 ± 0.00	0.09 ± 0.00	0.11 ± 0.01
Seminal vesicles	0.52 ± 0.11	0.23 ± 0.05***	0.54 ± 0.03^{aaa}	0.44 ± 0.02^{aa}
PEM	0.42 ± 0.03	0.31 ± 0.04	0.43 ± 0.05^{a}	0.35 ± 0.03
Penis	0.13 ± 0.01	0.14 ± 0.01	0.12 ± 0.10	0.15 ± 0.01

Each values represents the mean of the relative weight ± SEM of the reproductive organ (n=5); ***p<0.001**p<0.01;;*p<0.05 significantly different compared to the normal group ,[aa]p<0.01,[a]p<0.05significantly different compared to the negative group; PEM :penis elevator muscle. DW+ DW: Normal control receiving twice distilled water; ET +DW: Negative control receiving 6 g/kg ethanol and distilled water; ET+E150 and ET +E300:test group A receiving ethanol 6 g/kg and the hydroalcoholic extract of *Anthocleista liebrechtsiana* at the respective doses of 150 mg/kg and 300 mg/kg.

Effects of *Anthocleista liebrechtsiana* hydroalcoholic extract on sperm count

Figure 2 illustrates the effects of *A. liebrechtsiana* on sperm count. It appears that the administration of ethanol at the dose of 6 g/kg to negative group caused a significant decrease (p<0.001) of 76.40%in the number of spermatozoa compared to the normal group. The hydroalcoholic extract of *A. liebrechtsiana* caused a significant increase in the number of spermatozoa, which amounted to 200.86 % (p<0.001) for the dose of 150 mg/kg and to 160.86% (p<0.01) for the dose of 300 mg/kg, compared to the negative control group.

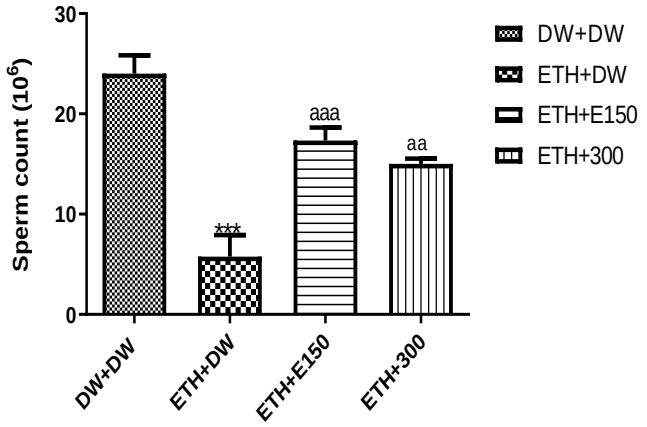

Figure 2: Effect of *A. liebrechtsiana* hydroalcoholic extract on sperm count

Each bar represents the mean ±SEM (n=5) of number of sperm per group. ***p<0.001 significantly different compared to the negative batch; DW+DW: Normal control receiving twice distilled water, ET+DW: Negative control receiving ethanol at a dose of 9 g/kg and distilled water; ET+E150 and ET +E300:test group A receiving ethanol 6 g/kg and the hydroalcoholic extract of *Anthocleista liebrechtsiana* at the respective doses of 150 mg/kg and 300 mg/kg.

Effects of hydroalcoholic extract of *Anthocleista liebrechtsiana* on the mobility of spermatozoa

Figure 3 shows the effects of the hydroalcoholic extract of *Anthocleista liebrechtsiana* on sperm mobility. It was found that the administration of ethanol at the dose of 6 g/kg to the negative group caused a significant decrease in mobility of 34.13% (p<0.01) compared to the normal group. Besides, the treatment with the hydroalcoholic extract of *A. liechrectsiana* at the respective doses of 150 mg/kg and 300 mg/kg caused a significant increase in sperm mobility respectively by 55.29% (p<0.01) and 56.75% (p<0.01) as compared to the negative group.

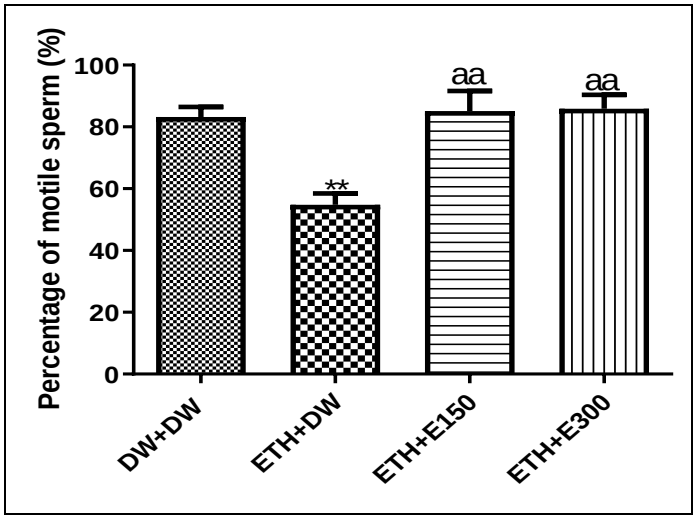

Figure 3: Effect of the hydroalcoholic extract of *A. liebrechtsiana* on the mobility of spermatozoa.

Each bar represents the mean ± SEM (n=5) of the mobility of sperm per group.**p<0.01; significantly different compared to the normal group; [aaa]p<0.001,significantly different compared to the negative group ;DW+DW: Normal control receiving twice distilled water, ET+DW: Negative control receiving ethanol at a dose of 6 g/kg and distilled water; ET+E150 and ET +E300:test group A receiving ethanol 6 g/kg and the hydroalcoholic extract of *Anthocleista liebrechtsiana* at the respective doses of 150 mg/kg and 300 mg/kg.

Effects of the hydroalcoholic extract of *Anthocleista liebrechtsiana* on the vitality of spermatozoa

Figure 4 shows the effects of the hydroalcoholic extract of *Anthocleista liebrechtsiana* on sperm vitality. It shows that the administration of ethanol at the dose of 6 g/kg to the negative group caused a significant decrease in sperm vitality of the order of 68.42% (p<0.001) compared to the normal group. In addition, hydroalcoholic extract of *Anthocleista liebrechtsiana* at the dose of 150 mg/kg caused a significant increase in the vitality of spermatozoa, which was of the order of 198.37% (p<0.001) compared to the negative group. At the dose of 300 mg/kg,the hydroalcoholic extract of *A. liebrechtsiana* caused a non significant increase of 92.68% in the vitality of spermatozoa compared to the negative group

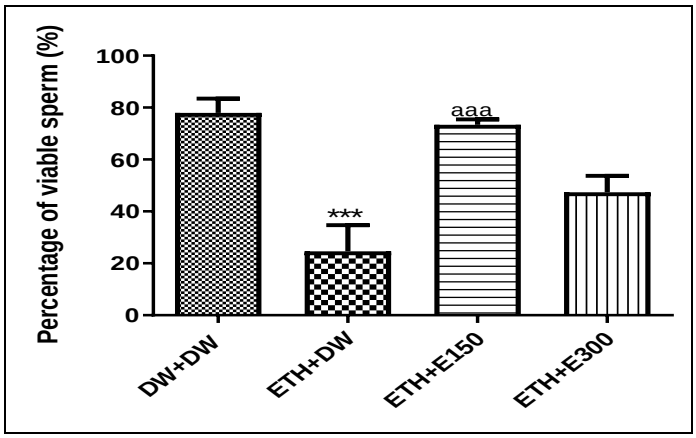

Figure 4: Effects of the hydroalcoholic extract of *A.liebrechtsiana* on the vitality of spermatozoa.

Each bar represents the mean ± SEM (n=5) of sperm vitality per group.***p<0.001:significantly different compared to the normal group;[aaa]p<0.001, significantly different compared to the negative group; DW+DW: Normal control receiving twice distilled water; ET+DW: Negative control receiving ethanol at the dose of 6 g/kg and distilled water, ET+E150 and ET +E300:test group A receiving ethanol 6 g/kg and the hydroalcoholic extract of *Anthocleista liebrechtsiana* at the respective doses of 150 mg/kg and 300 mg/kg.

Effects of hydroalcoholic extract of *Anthocleista liebrechtsiana* on serum testosterone levels

Figure 5 shows the effects of the hydroalcoholic extract of Anthoclesita liebrechtsiana on the serum testosterone level in rats after 75 days of treatment. It displays that the administration of ethanol at the dose of 6 g/kg to the negative group caused a significant decrease of 96.08% (p<0.001) in serum testosterone levels compared to the normal group. The hydroalcoholic extract of *A.liebrechtsiana* at the doses of 150 mg/kg and 300 mg/kg caused a non-significant increase in serum testosterone levels which was of 1006.66% and 1066.66%, respectively

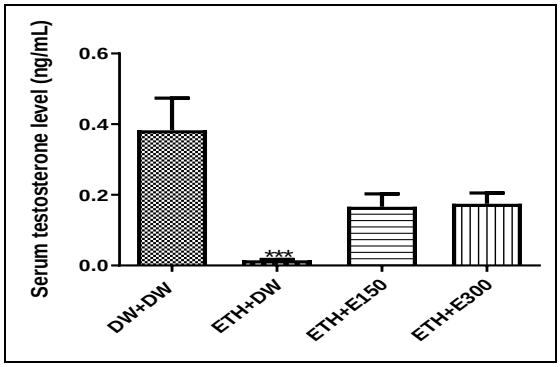

Figure 5: Effects of the hydroalcoholic extract of *A.liebrechtsiana* on the serum testosterone level.

Each bar represents the mean ± SEM concentration (n=5) of serum testosterone levels per group.***p<0.01:significantly different compared to the normal group ;DW+DW :Normal control receiving twice distilled water; ET+DW: Negative control receiving ethanol at the dose of 6 g/kg and distilled water; ET+E150 and ET +E300:test group A receiving ethanol 6 g/kg and the hydroalcoholic extract of *Anthocleista liebrechtsiana* at the respective doses of 150 mg/kg and 300 mg/kg.

Effects of *Anthocleista liebrechtsiana* hydroalcoholic extract on the histology of the testis, prostate and epididymis

1-Effects of the hydroalcoholic extract of *Anthocleista liebrechtsiana* on the histology of testis

Histological study of the testicles revealed normal architecture in normal control group (DW+DW). Figure 6 shows tight seminiferous tubules with a lumen filled with sperm as well as cell succession representing a normal course of spermatogenesis which proceeds centripetally from the wall to the lumen. Mature sperm fill the lumen of the seminiferous tubules through their flagella. The histological architecture of the testes of animals treated with ethanol at the dose of 6 g/kg (ETH+DW) shows a reduction of spermatogenesis, sperm degeneration and vacuolation of the testicular epithelium. The histology of the testes of the animals treated with the hydroalcoholic extract of *A. liebrechtsiana* at the respective doses of 150 mg/kg and 300 mg/kg shows a resumption of spermatogenesis, with the presence of necrotic cells in the lumen of the seminiferous tubes.

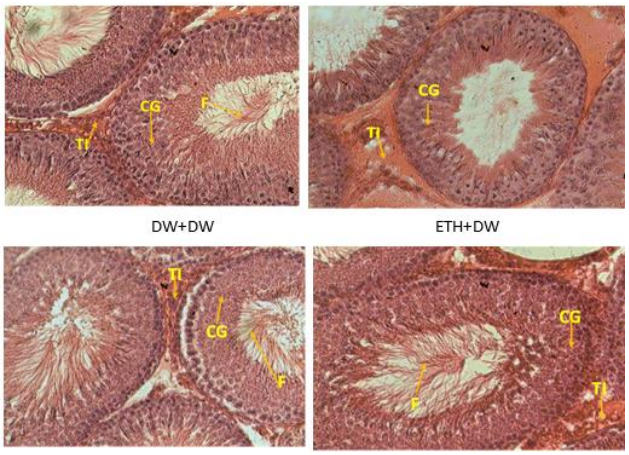

Figure 6: Photomicrograph of the testis of animals from different groups (Haematoxylin-Eosin 100X).

DW+DW: Normal control receiving twice distilled water; ET+DW; Negative control receiving ethanol at a dose of 6 g/kg and distilled water; ET+E150 and ET +E300:test group receiving ethanol 6 g/kg and the hydroalcoholic extract of *Anthocleista liebrechtsiana* at the respective doses of 150 mg/kg and 300 mg/kg. GC: Germ Cells; TI: Interstitial tissue; F: Flagellum.

2-Effects of the hydroalcoholic extract of *Anthocleista liebrechtsiana* on the histology of the epididymis

Figure 7 shows the effect of the hydroalcoholic extract of *Anthocleista liebrechtsiana* on the histology of the epididymis of rats after 75 days of treatment. It shows that the histology of the epididymis in the animals of the normal control group (DW+DW) reveals normal architecture. It shows epididymis ducts with lots of spermatozoa distributed evenly and lined by a simple prismatic epithelium with stereocilia. The epididymal ducts are surrounded by connective tissue. In the negative group (ET+DW), the histology shows an aggregation of sperm on one side of the lumen of the epididymis and an absence of stereocilia on the epithelial cells. The histology of the epididymis of the animals treated with the hydroalcoholic extract of *A. liebrechtsiana* at the respective doses of 150 mg/kg and 300 mg/kg shows a normal architecture with a homogeneous presence od spermatozoa in the lumen.

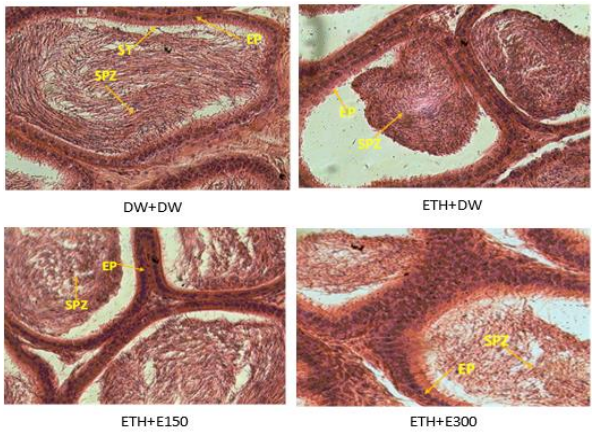

Figure 7: Photomicrograph of the epididymis of animals from different groups (Haematoxylin-Eosin 100X).

DW+DW: Normal control receiving twice distilled water; ET+DW: Negative control receiving ethanol at the dose of 6 g/kg and distilled water; ET+E150 and ET +E300:test group A receiving ethanol 6 g/kg and the hydroalcoholic extract of *Anthocleista liebrechtsiana* at the respective doses of 150 mg/kg and 300 mg/kg. EP: epithelium; SPZ: spermatozoa; ST: stereocilia.

3-Effects of the hydroalcoholic extract of *Anthocleista liebrechtsiana* on the histology of the prostate

Histological study of prostate reveals normal architecture both in normal control animals (DW+DW), as well at those treated with ethanol at a dose of 6 g/kg (ET+DW) and those treated with *A. liebrechtsiana* hydroalcoholic extract at the respective doses of 150 mg/kg and 300 mg/kg. There is a prostate which is formed by several tubule-alveolar glands surrounded by a connective stroma. The glands are coated by a simple epithelium of prism cells. The secretion product is stored as an amorphous mass (eosinophilic secretions).

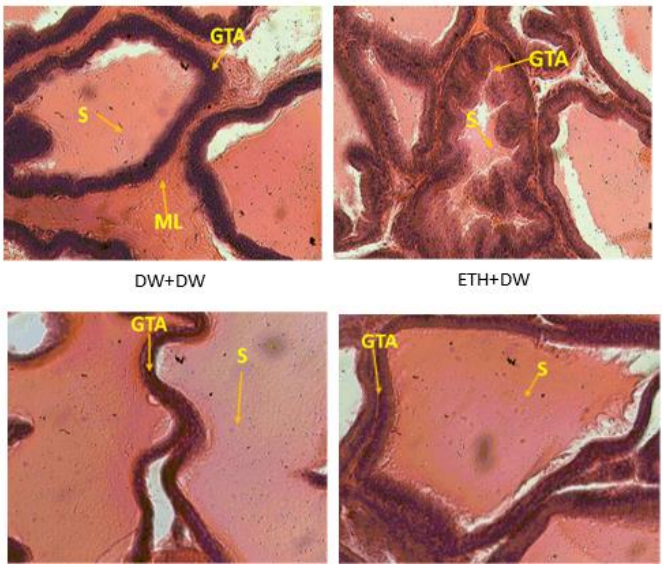

Figure 8: Photomicrograph of the Prostate of animals from different groups (Haematoxylin-Eosin 100X).

DW+DW: Normal control receiving distilled water twice; ET+DW: Negative control receiving ethanol at the dose of 6 g/kg and distilled water; ET+E150 and ET +E300:test group A receiving ethanol 6 g/kg and the hydroalcoholic extract of *Anthocleista liebrechtsiana* at the respective doses of 150 mg/kg and 300 mg/kg; GTA: Tubulo-alveolar Glands; ML: Smooth muscle, S: Secretion.

DISCUSSION

This study was carried out to determine the effects of the hydroalcoholic extract of *Anthocleista liebrechtsiana* leaves (Longamiceae) on sperm disorders induced by ethanol in rats. The animals of the negative group showed after 35 days of experience a significant decrease in weight gain compared to the animals of the normal group. This decrease becomes more pronounced on the 75th day of the experience. Besides, the administration of ethanol (40°) also resulted in a significant decrease in the weight of the reproductive organs. There was a significant decrease between the normal control group and in the weight of the testes, epididymis, seminal vesicles and prostate. According to some studies, ethanol caused a significant and dose-dependent decrease in testis and epididymis weights in male rabbits [12]. Ethanol is known to have toxic effect on male reproductive functions. Due to its amphiphilic property, it diffuses into all tissues and affects their vital functions [12]. The main mechanism involved here is tissue oxidation as a process occurs as part of the metabolism of alcohol and generates by-products called reactive oxygen species (ROS) which can contribute to cell damage and thus play a role in tissue damage, caused by alcohol [13].

Treatment with the hydroalcoholic extract of *A. liebrechtsiana* resulted in a significant increase in the relative weight of the seminal vesicles, prostate, testis, epididymis and penis compared to the negative control group [14]. All of these glands depend on androgens and we have observed an increase in serum testosterone levels during treatment with *A. liebrechtsiana*; which may suggest that the hydroalcoholic extract of *A. liebrechtsiana* contains nutritional factors capable of counteracting the harmful effects of ethanol on the weight of the reproductive organs [6, 14]

Chronic administration of ethanol at the dose of 6 g/kg caused a significant decrease in sperm count. This result is similar to that of Srikanth *et al* (2001) who showed that the spermatozoon content in the epididymis of rats decreased significantly following treatment with ethanol [14] . This result is due to the fact that the chronic use of ethanol causes dysfunctions in the gonads; which suppresses spermatogenesis, reduces the activation and proliferation of spermatogonia at each level of the seminiferous tubule cycles [6]. Another study of 80 alcoholics showed that alcohol reduced sperm count. Alcohol also induces oxidative stress which can lead to testicular damage which impedes spermatogenesis and therefore leads to a decrease in sperm count [15]. *A. liebrechtsiana* caused a significant increase in the number of sperm at the tail of the epididymis, at the dose of 150 mg/kg and 300 mg/kg, respectively, compared to the negative control. This would reflect the ability of the extract to correct the deleterious effects induced by ethanol. Kameni *et al*. explained this action by the presence of polyphenols, compounds also present in the hydroalcoholic extract which could promote an increase in the number of epididymal spermatozoa[16]. Administration of ethanol at a dose of 6 g/kg also caused a significant decrease in sperm mobility and vitality compared to the normal group.It has been founf a dose-dependent and significant decrease in sperm vitality and mobility in ethanol (20%) treated group and a highly significant decrease in the groups treated with 25% and 30% ethanol[12]. These results may be due to the direct toxic effect of ethanol on the epididymis. Indeed, when sperm leave the testis in an immature state, they complete their maturation and acquire progressive mobility during their transit through the epididymis. Concerning the vitality of the spermatozoa compared to the control group. The increase in the number of malformed sperm is a consequence of the alteration of epididymal secretions. This result is due to the fact that alcohol affects the mitochondrial functions of spermatozoa thus resulting in reduced mobility of those spermatozoa [17]. The hydroalcoholic extract of *A. liebrechtsiana* at the doses of 150 mg/kg and 300 mg/kg caused a significant increase in the mobility of sperm. This suggests that *A.liebrechtsiana* may have a direct effect by inhibiting the action of alcohol on mitochondrial energy metabolism. One factor that promotes the movement of sperm is the ability of our extract to increase the concentration of testosterone in rats because sperm mobility is androgen-dependent. The presence of secondary metabolites such as saponins and polyphenols whose beneficial properties on the mobility of spermatozoa pass respectively through an androgen-mimetic effect and an improvement in oxidative status[17, 18]. In addition, the metabolism of sugars present in our extract would lead to pyruvate production. Pyruvate is known to be the preferred and

essential substrate for sperm activity and survival [19]. Administration of ethanol at the dose of 6 g/kg caused a significant decrease in serum testosterone level compared to the normal control. Many authors obtained similar results when administering ethanol in rabbits [12]. Their testosterone results showed a significant decrease in testosterone in all ethanol-treated groups compared to the control group. The decline in testosterone concentration may be due to the effect of ethanol on the steroidogenic enzymes that take place in the Leydig cell [1, 12]. This decrease in the concentration of testosterone induced by ethanol would be the consequence of chronic exposure to ethanol which would cause the decline of LH synthesis by the pituitary gland and affects the hypothalamus-pituitary-gonadal axis in the humans and rats by decrease GnRH secretion [12]. The hydroalcoholic extract of *A.liebrechtsiana* at doses of 150 mg/kg and 300 mg/kg reversed the harmful effects of alcohol by increasing testosterone levels. The result of the phytocheminal screening of *A.liebrechtsiana* showed the presence of various secondary metabolites (polyphenols and saponins) in the plant. These compounds are well known for their wide range of pharmacological properties, including the antioxidant activity of polyphenols which blocks the deleterious action of alcohol on Leydig cells and the presence of compounds of terpene nature in this extract which would increase the pool in cholesterol, a precursor metabolite of androgen synthesis [1]. The histological architecture of the testis of animals treated with ethanol at the dose of 6 g/kg (ET+DW) shows a reduction of spermatogenesis, sperm degeneration and vacuolation of the testicular epithelium. Our data are similar to those obtained by Uygur *et al*. Indeed, they observed changes in testicular histopathology such as the immaturity of germ cells in lumen, acidophilic cells and vacuolation of the testicular epithelium in ethanol (40%)-treated animals at a dose of 3 g/kg for 8 weeks [20]. Servio *et al*. explained these changes by the direct harmful action of ethanol on germ cells or by lipid peroxidation of membranes. The histology of the testis of the animals treated with the hydroalcoholic extract of *A. liebrechtsiana* at the doses 150 mg/kg and 300 mg/kg showed a resumption of spermatogenesis, with the presence of nerotic cells in the lumen of the seminiferous tube[21]. This reflects the ability of the extract to block the harmful effect of alcohol on germ cells. Khan et Ahmed. obtained similar results when administering *Digera muricata* in male rats [22]. They explained this result by the presence of constituents such as flavonoids and saponins in the plant which, directly or indirectly, improve testicular damage and thus normalize their function [17]. More over, some authors, suggested that this could result from the presence of flavonoids in the plant. These compounds, also present in our hydroalcoholic extract, are powerful antioxidants that can increase the production of testosterone, the key hormone involved in the production and maturation of sperm in the seminiferous tubules of testis.

CONCLUSION

The hydroalcoholic extract of *A.liebrechtsiana* at the doses of 150 mg/kg and 300 mg/kg. Significantly corrected the relative weight of the reproductive organs and annexed glands, the number of spermatozoa, the mobility and vitality of the spermatozoa on the one hand and on the other hand engendered an increase although not significant in testosterone levels and body weight compared to spermatic and androgenic activity in human patients suffering from infertility and androgen deficiency.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest

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REFERENCES

1. Moundipa PF, Beboyl NSE, Zelefack F, Ngouela S, Tsamo E, Schill W-B, *et al*. Effects of Basella alba and Hibiscus macranthus extracts on testosterone production of adult rat and bull Ley dig cells. Asian Journal of Andrology. 2005;7:411-7.
2. Kirakoya B, Barnabé Z, Karim PA, Aristide KF, Clotaire Y, Amélie N. Epidemiological and Clinical Profile of Male Hypofertility in Consultation at the Urology-Andrology of Yalgado Ouedraogo Teaching Hospital (Burkina Faso). Advances in Sexual Medicine. 2014;5:1-6.
3. Wallerand H, Chabannes E, Bittard H. Infertilité masculine idiopathique et récepteur aux androgènes. Progrès en Urologie. 2001.
4. Ramakrishna A, Ravishankar GA. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal Behav. 2011;6:1720-31.
5. Gül T, Yılmaz G, Bayram S, Dolgun ZN, Ege S. The Effect Of Occupational Groups And Use Of Alcohol And Smoking In Thrace On Semen Parameters. Turkish Medical Student Journal. 2014;1:7–11.
6. Kadiri, Dereddy Nagalinga Reddy2, Gundala Harold Philip. Abnormalities in Semen Parameters of Men Due to Consumption of Alcohol. IJSR. 2016;5:216-20.
7. Demoulin A. Les traitements médicaux de l'oligoasthénozoospermie idiopathique. Androl. 1998;8:47-56.
8. Armel A, Souleymane K, Laure P. F, Balira O. K. Towards A "Deep" Ontology for African Traditional Medicine. Intelligent Information Management. 2011;2011.
9. Djoko E, Tchantchou C, Kanmangné F-M, Foutse Y, Kwetche P, Wouessidjewe D. Formulation d'un médicament traditionnel amélioré à base de Kalanchoe crenata (Andr) haw. HEGEL - HEpato-GastroEntérologie Libérale. 2016;N° 4.
10. Koto-te-Nyiwa N, . Mubindukila REN, Mpiana PT, Tshibangu DST, Ashande MC, Nzongola W-X, *et al*. Phytochemical Screening, Antibacterial and Antioxidant Activities of Anthocleista liebrechtsiana Wild & T. Durand (Gentianaceae) Originated from Democratic Republic of the Congo. Journal Of Advancement In Medical And Life Sciences. 2014;1:1-6.
11. Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medicinal plants II. Lloydia. 1978;41:234-46.
12. Saihia A, Khelili K, Boulakoud MS. Effets d'éthanol sur la fertilité du lapin mâle adulte Oryctolagus cuniculus. International Journal of Biological and Chemical Sciences. 2015;9:1910.
13. Emmanuel B. Initiation à la recherche sur les substances naturelles: extraction - analyse - essais biologiques. Presse Universitaire de Dakar. 2012, 150.
14. Srikanth V, Malini T, Arunakaran J, Govindarajulu P, Balasubramanian K. Effects of Ethanol Treatment on Epididymal

Secretory Products and Sperm Maturation in Albino Rats. J Pharmacol Exp Ther. 1999;288:509-15.
15. Kefer JC, Agarwal A, Sabanegh E. Role of antioxidants in the treatment of male infertility. International Journal of Urology. 2009;16:449-57.
16. Kameni M. Evaluation des effets de l'extrait aqueux des fleurs de Nymphaea lotus Linn. (Nymphéacées) sur la fonction de reproduction des rats normoglycémique et diabétique de type 1. Thèse de doctorat (Phd). 2011;99:2011.
17. Akpantah AO, Oremosu AA, Ajala MO, Noronha CC, Okanlawon AO. The Effect of Crude Extract of Garcinia Kola Seed on the Histology and Hormonal Milieu of Male Sprague-Dawley Rats' Reproductive Organs. Nigerian Journal of Health and Biomedical Sciences. 2003;2:40-6.
18. Ngoula F, Watcho P, Dongmo M-C, Kenfack A, Kamtchouing P, Tchoumboué J. Effects of pirimiphos-methyl (an organophosphate insecticide) on the fertility of adult male rats. African Health Sciences. 2007;7:3.
19. Salman TM, Adesokan AA. Sperm quality of male rats treated with aqueous extract of Enantia chlorantha stem bark. African Journal of Biotechnology. 2008;7.
20. Uygur R, Aktas C, Tulubas F, Uygur E, Kanter M, Erboga M, *et al*. Protective effects of fish omega-3 fatty acids on doxorubicin-induced testicular apoptosis and oxidative damage in rats. Andrologia. 2014;46:917-26.
21. Siervo GEML, Vieira HR, Ogo FM, Fernandez CDB, Gonçalves GD, Mesquita SFP, *et al*. Spermatic and testicular damages in rats exposed to ethanol: Influence of lipid peroxidation but not testosterone. Toxicology. 2015;330:1-8.
22. Khan M, Yi F, Rasul A, Li T, Wang N, Gao H, *et al*. Alantolactone induces apoptosis in glioblastoma cells via GSH depletion, ROS generation, and mitochondrial dysfunction. IUBMB Life. 2012;64:783-94.

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