ABSTRACT

Objective: The sexual stimulatory effect of aqueous stem bark extract of *Xylopi a aethiopica* which is commonly used traditionally for the treatment of fertility-related problems in males is reported in this work.

Methods: The present study investigated the effect on 14 days treatment of 1 ml/kg distilled water (control), 100, 200 and 300 mg/kg of body weight of the stem bark extract on some fertility parameters of the Swiss male rats. All the target organs (testes, epididymis, vas deferens, seminal vesicle and prostate) were weighed. The sperm motility and sperm count in testis, epididymis and vas deferens were determined. Serum and tissue protein, as well as cholesterol, were evaluated. The serum testosterone level was also assessed.

Results: The extract did not produce significant change in the mobility of sperm, the sperm count in vas deferens, the relative weight of body and accessory organs at all doses. The daily sperm production (DSP) and the weight of prostate significantly increased at a dose of 100 mg/kg (p<0.01) while the sperm count in cauda epididymis increased at a dose of 200 mg/kg. The testicular and serum cholesterol significantly increased (p<0.001) at the dose of 100 mg/kg whereas the testicular and serum proteins increased (p<0.001) at the dose of 200 mg/kg. The serum testosterone level increased following 14 d of treatment (p<0.01) at the dose of 100 mg/kg.

Conclusion: The results suggest that *Xylopi a aethiopica* may have the potential of being developed into a male fertility enhancing drug.

Keywords: Spermatogenesis, *Xylopi a aethiopica*, Testosterone level, Fertility

INTRODUCTION

Since time immemorial, all civilizations have always relied on plants and their products in the field of treatment and cure of diseases. Although the last centuries, the development of scientific research has provided many facilities in the domains of healthcare, infertility remains one of the problems of human society in which 40% of these problems are due to male factor [1].

There are many herbal drugs that have been used traditionally to treat sperm abnormalities, erectile and ejaculatory dysfunction [2-4]. *Xylopi a aethiopica* (*Annonaaceae*) is a tree over 20 m in height and 60-75 cm in diameter which grows in forested areas and especially along the rivers in arid zones [5]. In Cameroon, *Xylopi a aethiopica* has been identified in the South in the locality of Ebondi and locally known as ‘Iting’. Many therapeutic effects such as the treatment of a cough, bronchitis, rheumatism, dysentery and infertility are already been found when using the aerial parts of *Xylopi a aethiopica* [6].

Previous phytochemical investigations of *Xylopi a aethiopica* yielded alkaloids, saponins, tannins, terpenes [7], flavonoids and glycosides [8]. The increasing incidence of male infertility is always necessitating scientific research into plants with fertility enhancing potentials. This work is intended to study the effect of *Xylopi a aethiopica* on sperm cells formation and androgen level of male rats.

MATERIALS AND METHODS

Chemicals and reagents

Reagents used were procured from Polypharmacy (Douala, Cameroon). The standard drugs were purchased from SIGMA-ALDRICH Chemical Co (St. Louis, MO, USA) and DIAGNOSTIC Products Co (Los Angeles, CA, USA). All other reagents were of analytical grade.

Plant material

The fresh stem bark of *Xylopi a aethiopica* was collected during the month of May in the locality of to Ebondi of the southern region of Cameroon. The botanical identification of the plant was made in the national Herbarium of Cameroon at Yaoundé by GHOGUE Jean Paul, Senior Researcher/Environmentalist in comparison to the sample N°JJ 7931 (De Wilde).

Extract preparation

The stem bark of *Xylopi a aethiopica* was cut into small pieces and air-dried in the shade away from the dust and then crushed. The extraction is done in accordance with the requirements of traditional physicians. A 500 g of the powdered stem bark was suspended in 6 l of distilled water, heated and boiled under reflux for 45 min. After filtration by the Whatman N° 3 filter paper, the obtained decoction was stored at-20 °C for lyophilisation. The crude yield of the lyophilised material was approximately 29.1 g (5.82 %).

The lyophilised extract was further diluted to obtain different concentrations in 1 ml. Obtained solutions were kept at 4 °C until further used.

Screening of the plant

According to the Guidelines for the appropriate use of herbal medicines [9] (WHO, 1998), Different oral doses up to 2 g/kg body weight (BW) of an extract of *Xylopi a aethiopica* was given to different groups of mice and observe 48 h later to determine any sign of toxicity. No toxic effect was observed during the period of the test (data not shown).

Animals

Our study was conducted with 24 adult male rats from the Wistar strain Albino weighing between 150-250 g obtained from the vivarium at the University of Douala. The animals were housed six
animals per cage in a room with controlled temperature (temperature 24-28 °C) and lighting (12 h light-dark cycles). The Ethics Committee of the University of Douala in accordance with the internationally accepted principles for laboratory use and care of European Community (EEC directive of 1986; 86/609/EEC) approved the protocol for these experiments. In an attempt to further validate scientifically the traditional claims, the rats were divided into the following 4 groups (6 per group) and orally treated with 1 ml/kg distilled water (control), 100, 200 and 300 mg/kg body weight (BW) per day of *Xylopia aethiopica* for 14 d. The *Xylopia aethiopica* extract diluted in water or vehicle (water) was administered by gavages. One day after the last treatment, the animals were sacrificed and a blood sample was collected for testosterone assay and biochemical analysis. Testis, seminal vesicles, epididymis and ventral prostate were removed and cleared of attached fat and connective tissue and weighed. The testis, vas deferens and epididymis were used for biochemical analysis, sperm count and motility.

**Sperm count**

Homogenization-resistant epididymal sperm from non-perfused rats were counted as described previously [10]. Sperm counts were measured in caput/corpus and cauda of the left epididymis. Homogenization was performed in 5 ml of saline (NaCl 0.9%). Homogenates were kept refrigerated at 4 °C for 24 h to allow sperm counted in 25 squares for 4 times. The average sperm count of each homogenate was added to 7 ml (dilution 1/8) of eosin (0.2%) and a sample was placed in a Neubauer chamber. Heads per cent sperm were determined by ELISA method.

**Sperm count in vas deferens**

The vas deferens was cut in two parts corresponding to the large and thin one. Each part was homogenized with 1 ml of saline (0.9%). An aliquot was diluted with two parts of eosin (0.2%). Homogenization resistant sperm heads were counted in the 25 squares of the Neubauer chamber. Four chambers were measured in each sample, and they were averaged. Results from the large and the thin part were multiplied by 0.03 and defined as sperm x 10⁶ per part of vas deferens. Data were expressed as the total amount of sperms in vas deferens (sperm count in the large and the thin part of vas deferens).

**Daily sperm production**

The capsule of the left testis was removed and the parenchyma was homogenized in 10 ml of 0.9% saline/0.05% Triton X-100 (v/v) solution for 1 min by a homogenizer [11] and then diluted into 1/10 with saline. The number of homogenization-resistant elongated spermatid nuclei per testis was determined with a hemocytometer. Counts for 4 hemocytometer chambers were averaged. The daily sperm production (DSP) were determined by the division of the elongated spermatid count per testis and spermatids per gram of testis by 6.3, which represents, the duration of steps 17 to 19 elongated spermatid count per testis and spermatids per gram of testis by 6.3, which represents, the duration of steps 17 to 19 spermatids in the seminiferous epithelial cycle for rats [12]. The epididymal sperm transit rate was calculated by dividing the cauda epididymal sperm number by the DSP [13].

**Sperm motility determination**

The cauda epididymis was separated and minced using a pair of small scissors, to release the sperm into 10 ml of warmed physiological saline. The sperm suspension was placed in an incubator at 37 °C for 10 min prior to total motility and progressive motility assessment. The aliquot of the sperm suspension was then placed on Makler counting chamber and motile sperm was counted under a light microscope (OEM1, OLYMPUS). Nine microscopic fields were observed per sample and averaged. Progressive and total sperm motility was expressed as a percent of motile sperm of the total sperm count.

**Biochemical analyses**

Biochemical analyses were determined using commercial kits. Total protein levels were determined in the serum and sexual organ (testis and epididymis) using colourimetric methods described by Gornal et al. [14] and Bradford [15] respectively. The cholesterol levels in the tests were determined using the colourimetric method described by Forbes [16]. Serum testosterone levels were determined by ELISA method.

**Statistical analysis**

All statistical analysis was conducted using the STATGRAPHICS PLUS SOFTWARE (version 5.0). Results were expressed as mean±SEM (standard error of the mean). Differences between groups were assessed by one-way analysis of variance (ANOVA). When the differences were significant, the classification of Tukey test was used to locate the differences between the averages. When variance was not homogeneous a non-parametric analysis was performed. A value of P<0.05 was considered as statistically significant.

**RESULTS**

**Effects of aqueous extract of Xylopia aethiopica on body and accessory organs weight**

After 14 d of treatment, no significant change was observed on the body weight of all treated group as compared with control group. However, the relative weight of prostate significantly increased (P<0.01) at the single dose 100 mg/kg as compared to the control.

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**Table 1: Effects of aqueous extract of Xylopia aethiopica on relative organs weight**

| Parameters       | Doses (mg/kg) | control       | 100           | 200           | 300           |
|------------------|---------------|---------------|---------------|---------------|---------------|
|                  |               | 0.570±0.020   | 0.620±0.030   | 0.590±0.030   | 0.580±0.010   |
| Testis           |               | 0.171±0.008   | 0.172±0.010   | 0.164±0.020   | 0.155±0.009   |
| Epididymis       |               | 0.024±0.002   | 0.024±0.003   | 0.026±0.002   | 0.030±0.003   |
| Vas deferens     |               | 0.280±0.050   | 0.390±0.008   | 0.280±0.040   | 0.270±0.060   |
| Seminal vesicle  |               | 0.106±0.015   | 0.155±0.080   | 0.96±0.10     | 0.10±0.005    |
| Prostate         |               | 0.286±0.002   | 0.390±0.008   | 0.280±0.040   | 0.270±0.060   |

Value are mean±SEM; n=6; value is statistically significant at P<0.01 level of significance. **=compared with control P<0.01

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*Fig. 1: Effects of aqueous extract of Xylopia aethiopica on relative body weight, value are mean±SEM; n=6*
Effect of aqueous extract of *Xylopia aethiopica* on sperm count and sperm motility

After 14 d of treatment, aqueous extract of *Xylopia aethiopica* significantly affected the daily sperm production (DSP) in testis at dose of 100 mg/kg whereas there were no significant changes concerning the sperm motility in epididymis at all doses. At the other hand, we noticed a significant increase in sperm count (P<0.05) and transit (P<0.01) in epididymis at the dose of 200 mg/kg (table 2). Regression analysis showed a significant correlation (r^2 = 0.90) between the sperm transit in the cauda epididymis and epididymal proteins.

**Table 2: Effect of aqueous extract of *Xylopia aethiopica* on sperm count (x10^6) and sperm motility (%)**

| Parameters                | Control     | 100         | 200         | 300         |
|---------------------------|-------------|-------------|-------------|-------------|
| DSP                       | 19.40±1.58  | 22.78±0.71**| 15.52±0.76  | 18.24±1.92  |
| DSP/weight testis         | 14.67±1.53  | 16.33±0.92  | 11.71±0.86  | 13.60±1.63  |
| Epididymis                | 101.13±16.89| 102.13±21.13| 126.88±18.31*| 105.88±17.52|
| Vas deferens              | 0.53±0.10   | 0.57±0.10   | 0.62±0.13   | 0.76±0.21   |
| Sperm transit             | 3.07±0.55   | 3.59±0.15   | 7.19±0.32** | 3.41±0.50   |
| Sperm motility            | 56.71±0.84  | 63.75±4.76  | 51.54±3.66  | 40.98±1.67  |

Value are mean±SEM; n=6; values are statistically significant at P<0.001 level of significance. **-compared with control P<0.001, *-compared with control P<0.05.

Biochemical profile in the serum and sexual organs

Administration of aqueous extract of *Xylopia aethiopica* significantly affected the serum and testicular cholesterol (P<0.001) at a dose of 100 mg/kg while the serum, testicular and epididymal proteins significantly increased (P<0.001) at the dose of 200 mg/kg. Multiple correlation analysis showed that the secretion of testosterone was related to the testicular cholesterol (r^2 = 0.90) (table 3).

**Table 3: Effects of aqueous extract of *Xylopia aethiopica* on biochemical (total cholesterol and protein) parameters**

| Biochemical parameters                  | Control     | 100         | 200         | 300         |
|----------------------------------------|-------------|-------------|-------------|-------------|
| Total serum protein (g/dl)             | 5.52±0.82   | 6.05±0.36   | 8.68±0.14***| 6.22±0.18   |
| Total testicular protein (g/dl)        | 1.59±0.12   | 1.67±0.31   | 3.93±0.08***| 1.71±0.19   |
| Total epididymal protein (g/dl)        | 1.49±0.26   | 1.70±0.10   | 305±0.06*** | 1.73±0.15   |
| Total serum cholesterol (mg/dl)        | 69.14±1.97  | 75.05±7.2   | 73.25±5.19  | 60.55±2.26  |
| Total testicular cholesterol (mg/dl)   | 54.88±5.91  | 104.91±0.96***| 60.55±2.26  | 58.51±3.81  |

Value are mean±SEM; n=6; values are statistically significant at P<0.001 level of significance. ***-compared with control P<0.001.

Effect of aqueous extract of *Xylopia aethiopica* on sex hormone level

In this study, the highest testosterone level was observed at the dose of 100 mg/kg of *Xylopia aethiopica* (P<0.01). Multiple correlation analysis showed that testosterone level was related to the serum cholesterol (r^2 = 0.83) (fig. 2).

**Fig. 2: Effect of aqueous extract of *Xylopia aethiopica* on testosterone level. Value are mean±SEM; n=6; value is statistically significant at P<0.01 level of significance. **-compared with control P<0.01.

DISCUSSION

The use of plants in folk medicine still plays an important role in developing countries and particularity in Cameroon. Nowadays, the practical utilization of traditional medicines has received remarkable consideration and a large number of plants have been screened for their fertility enhancing properties [17]. In the present study, neither significant difference was observed among groups in body weight gain nor did weight of some accessory organs. However, it was noted at the dose of 100 mg/kg, a significant increase (p<0.01) of the relative weight of the prostate which is a body whose activity is linked to the secretion of androgen. This finding concerning the absence of weight gain is in line with the earlier report showing that aerial parts of this plant possess a hypolipidemic activity [18]. The increase of weight of prostate is similar to work reported by Güldeniz et al. 2009 [19]. This would suggest that the bark aqueous extract of *Xylopia aethiopica* would have androgenic properties. Indeed, it is known that androgens are steroids possessing anabolic activity at target tissues [20]. It has also been shown in rats that any increase in the testosterone level or any Androgen therapy induces secretory activity and elevated sexual organ weight [21]. It is why we observed an increase in testosterone levels at the same dose (100 mg/kg) in this work.

In the present investigation, the observed increase in daily sperm production to animals treated at a dose of 100 mg/kg might be due to an increase in the pattern of androgen secretion which increased at that dose and also to the presence of flavonoids in the extract. In fact, increased production of sperm cells in the seminiferous tubules is always associated with an increase in testosterone secretion that interacts with follicle stimulating hormone (FSH) during the process of spermatogenesis [22]. On the other hand, several lines have indicated that flavonoids possess reproductive and pharmacological
properties [23-25]. Firstly, these flavonoids could directly act as antioxidants at the target cells and by this way contribute to the improvement of fertility [26]; secondly, they could maintain the synthesis of androgens by inhibiting 17β estradiol aromatase, which is the enzyme involve for the conversion of testosterone to estrogen [27].

The mammalian testis is under the overall control of pituitary gonadotropins but the utilisation of these signals to achieve normal testicular function involves complex local interactions between the Sertoli, germ and Leydig cells. These interactions serve to control the complex but orderly sequence of events that constitute the spermatogenic cycle. This process involves multiplication, differentiation and translocation of the germ cells. One of the factors involved in controlling the formation of sperm cells, is testosterone which is the result of the activity of Leydig cells [28]. Among the aqueous extract of Xylopia aethiopica treated animals, group treated at dose of 100 mg/kg produced a significant increase in testosterone level. This may be as a result of the ability of the aqueous extract of Xylopia aethiopica at the given doses, to stimulate the activity of Leydig and Sertoli cells. It is well established that both Leydig and Sertoli cells are required for testosterone synthesis in the mouse fetal testis [29]. Indeed, Leydig cells secrete testosterone and are able to synthesize cholesterol obtained by lipoproteins uptake relative to synthesis from acetate [30]. In the present study, there was a significant correlation ($r^2 = 0.90$) between the production of testosterone and testicular cholesterol at dose of 100 mg/kg, showing in this case, the direct action of some secondary metabolites, mainly flavonoids which are present in the extract and that can modulate the synthesis of testosterone [31]. On the other hand, earlier reports demonstrated the important role of testicular proteins in sperm maturation [32]. The testicular androgen-binding protein (ABP) is synthesized in Sertoli cells as a response to FSH stimulation. Androgen binding protein (ABP) is transported with the testicular fluid into the caput epididymis, where it is partly taken up by the lining epithelial cells where it serves as an important store of androgenic hormones (mainly testosterone and dihydro-testosterone) that are necessary for initiation and maintenance of spermatogenesis [33].

The present work also showed that, at a dose of 200 mg/kg, there was no significant ($p=0.05$) decrease of daily sperm production as well as the decrease of testosterone at the same dose when compared to control. Nevertheless, at that dose, we noticed a remarkable increase ($p<0.001$) of sperm count and transit in epididymis. The significant increase in sperm count and transit in this work was likely linked to the significant increase ($p<0.001$) of the epididymal proteins observed at the same dose (200 mg/kg). It is why there was a high correlation ($r^2 = 1$) between the sperm transit and epididymal proteins. It has been already postulated that some proteins of testis and epididymis ensure the sperm maturation and transit. Indeed, epididymal channel participates in the maturation and the transit of sperm, or by secreting enzymatic proteins (glycogenases or glycosyl transferases) that are absorbed into the plasma membrane of the sperm during their stay in the luminal tube of epididymis [34]; either through the caging of testicular proteins (fertilin beta) pre-existing on the surface of the plasma membrane of the sperm [35]. These proteins are necessary and essential to ensure to male gametes their optimal fertilizing ability [36], although in the present work we observed a decline of sperm motility, one of a very important parameter involved for evaluating male fertility potential. It is then clear that the 200 mg/kg dose could influence the maturation of the spermatozoa in the male rats, which might also be a contributory factor to the increase of the overall sperm count of the epididymis.

CONCLUSION

In conclusion, these results indicate that treatment with the aqueous extract of Xylopia aethiopica could improve the fertility of male rats. Nevertheless, further investigations on animals with impaired reproductive capabilities are required in this study to better confirm traditional claims concerning the use of this plant on male infertility.

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AUTHORS CONTRIBUTIONS

BRICE LANDRY KOLOKO: Designed the work.

CYPRIEN MAGLOIRE HAMBE: Data collection.

HUBERT KENMOGNE and MARIE NGAH  NJILA: Analysis of data.

DIEUDONNE MASSOMA LEMBE: Wrote the manuscript and contributed the materials/analysis tools/reagents.

CONFLICT OF INTERESTS

Authors declare no conflict of interest.

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