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Evaluation of the fertility activity of the aqueous leaves extract of *Zanthoxylum macrophylla* (Rutaceae) on male rats

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ABSTRACT

Zanthoxylum macrophylla, one of the useful medicinal plants in Cameroon, was scientifically conducted to find its aqueous extract leaves effect in some fertility parameters of male rats. 24 rats were randomly divided into 4 groups: Control, A, B and C group of six animals each. They received respectively 0, 100, 200 and 400 mg/kg body weight of aqueous extract of *Z. macrophylla leaves* on daily basis for 14 days. There were no significant changes on the body weight of treated animals. However, the weight of testis and the Daily Sperm Production respectively increased at dose of 200 mg/kg ($P< 0.05$ and $P< 0.001$) and 400 mg/kg ($P< 0.01$ and $P< 0.001$) while the weight of seminal vesicle and prostate also increased respectively at dose of 200 mg/kg ($P< 0.05$) and 400 mg/kg ($P< 0.01$) when compared to the control. The serum testosterone level significantly increased at the dose of 200 mg/kg ($P< 0.01$) and 400 mg/kg ($P< 0.001$) while the total cholesterol decreased at dose of 400 mg/kg ($P< 0.05$) when compared to the control. The serum protein decreased respectively at dose of 200 mg/kg ($P< 0.01$) and 400 mg/kg ($P< 0.01$) when compared to the control. The histological sections of the testis did not show any structural abnormalities in all treated animals. These results indicate that *Zanthoxylum macrophylla* could improve male reproductive activities.

Keywords: Fertility, *Zanthoxylum macrophylla*, Androgene level, Sperm count.

INTRODUCTION

In traditional medicine, the search for plants with properties that increase fertility as well as those that have anti fertility potentials to improve reproductive health is now topical. In the third world countries, in the face of financial precariousness, the notorious inaccessibility to essential drugs as well as its adverse effects, associated to the high cost of modern therapies, populations are increasingly resorting to the use of traditional medicine as an alternative [1]. So far, medicinal plants that have shown a great deal of success are still being used empirically and sometimes develop unexpected and deleterious effects on some functions of the organism [2]. Then, it is necessary to carry out scientific studies of the plants in order to identify these harmful effects and to prevent the traditional physicians and the patients. *Alangium salvifolium* is an African plant, known for its therapeutic properties against fever, helminthiasis, syphilis, hemoparasitosis and spasms, but it has a toxic effect on male fertility [3]. Similarly, *Moringa oleifera*, a plant widely used in Africa on the side of its many therapeutic effects, produces anti spermatogenic effects [4]. *Zanthoxylum macrophylla* is a plant used in folklore medicine to treat digestive disorders, gonorrhea, malaria, pneumonia and tuberculosis and male relative abnormalities. No scientifically studies have be done to evaluate the effect the plant on male reproduction functions. Previous phytochemical studies of leaves of *Zanthoxylum macrophylla* yielded alkaloids, flavonoids, saponins, tanins and sterols [5, 6]. Then the objective of this investigation was to determine the effects of aqueous extract of *Zanthoxylum macrophylla leaves* on some fertility parameters of male rats.

MATERIALS AND METHODS

Plant material

Collection and identification

The fresh leaves of *Zanthoxylum macrophylla* were collected at the locality of the Littoral region of Cameroon. Botanical identification of the plant was made at the National herbarium in comparison with

sample No. 2710/SRFK/CAM.

Extraction

50 dry leaves corresponding to 650 g were brought to a boil in two liters of distilled water for 30 minutes. The resulting preparation was filtered using a Whatman filter paper No. 1; then the collected filtrate was lyophilized to obtain the final raw powder extract. The extraction yield was 3.29%.

Animals

A total of 24 sexually experienced Wistar rats, aged at least 3 months and weighing between 190 g and 250 g, were housed in clean metabolic cages (6 per cage) contained in well ventilated standard housing conditions (temperature: 28–31°C; photoperiod: 12 hours natural light and 12 hours dark). The experiments were performed to minimize animal suffering in accordance with the internationally accepted principles for laboratory use and care of European Community (EEC directive of 1986; 86/609/EEC). The animals were acclimatized for one week under laboratory conditions before the beginning of the experiment. The rats were completely randomized into four groups and orally treated with 1 mL/kg distilled water (control), 100, 200 and 400 mg/kg body weight (BW) per day of *Zanthoxylum macrophylla* for 14 days. During the treatment period the body weight changes was recorded. One day after the last treatment, the animals were sacrificed and blood sample was collected for biochemical analysis and androgen assay. Testis, seminal vesicles, epididymis and ventral prostate were removed and cleared of attached fat and connective tissue and weighed. The left testis and epididymis were used for sperm count while the right testis was kept to histological analysis.

Sperm count in testis (DSP)

The capsule and the blood vessel of the left testis were removed, and the parenchyma was homogenized in 10 mL of 0.9% saline-0.05% (v/v) Triton X-100 solution for 1 minute by a homogenizer, and then diluted into 1/10 [7]. The number of spermatid per testis was determined using hemocytometer. The daily sperm production (DSP) and its efficiency (DSP per weight of testis) were determined by division of the number of spermatid per testis and spermatids per gram of testis by 6.3. The value 6.3 represents the duration of steps 17 to 19 spermatids in the seminiferous epithelial cycle for Holtzman rats [8]. The epididymal sperm transit rate was calculated by dividing the caudaepididymal sperm number by the DSP [9].

Sperm count in epididymis

The spermatozoa in epididymis were counted by method described by Gonzales *et al*. 2004 with modification which consisted to evaluate separately the number of spermatozoa in the cauda epididymis and caput/corpus. 5 mL of saline (NaCl 0.9%) was performed for homogenization of resistant epididymal sperm. After 24h in a fridge at 4°C, 10 mL of the refrigerated homogenate was added to 70 mL of eosin (2%), and a sample was placed in a Neubauer chamber. Head sperms were counted in 25 squares for four times. The average sperm count of each rat was multiplied by 0.06 (sperm × 10^6/mL) and then by 5 mL (sperm × 10^6 per caput/corpus or cauda). Data are referred as sperm per caput/corpus or cauda epididymis.

Biochemical analysis

Total protein levels were determined in the serum and sexual organ (testis and epididymis) using colorimetric methods described by Gornal *et al*. [10] and Bradford [11] respectively. The cholesterol levels in the testis were determined using the colorimetric method described by Forbes [12].

Hormonal assay

Serum testosterone levels were determined by ELISA method using a commercial kit (Diagnostic Products Co., Los Angeles, CA, USA).

Histological analysis.

The right testis was fixed in Bouin medium and was embedded in paraffin, and then subjected to haematoxylin–eosin staining. The pathological observations of the right testis were performed on gross and microscopic bases.

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). Differences between pair of means were assessed by the Least Significant Difference (LSD) test. When variance was not homogeneous a non-parametric analysis was performed. A value of $P < 0.05$ was considered as statistically significant.

RESULTS

Effect of Zanthoxylum macrophylla on body and reproductive organs weight

There was no significant difference on body weight of treated animals, however, at dose of 200 mg/kg and 400 mg/kg, the testis ($P < 0.05$; $P < 0.01$) and weight of accessory organs (prostate $P < 0.01$; seminal vesicle$P < 0.05$) significantly increased when compared to the control [Table 1].

Table 1: Effect of *Zanthoxylum macrophylla* on body weight (g) and reproductive organs weight (g)

Treatment groups mg/kg	Organsweight (g)				
	Body	Testis	Epididymis	Prostate	Seminalvesicle
Control	20.580 ± 3.480	0.470 ± 0.200	0.172 ± 0.008	0.071 ± 0.005	0.295 ± 0.019
100	14.120 ± 5.040	0.590 ± 0.080	0.170 ± 0.200	0.090 ± 0.020	0.331 ± 0.060
200	16.370 ± 3.140	0.600 ± 0.020*	0.173 ± 0.005	0.093 ± 0.0003	0.466 ± 0.013*
400	14.300 ± 4.420	0.690 ± 0.020**	0.174 ± 0.007	0.118 ± 0.004**	0.390± 0.490

Value are mean ± SE; n=6; *$P<0.05$ with respect to control; **$P<0.01$ with respect to control

Effect of Zanthoxylum macrophylla on sperm count

14 days after aqueous extract treatment of *Zanthoxylum macrophylla* leaves, no significant differences were observed in photomicrographs of cross section of the right testis [Figure 1] as well as in sperm count in epididymis and its transit in cauda. The daily sperm production significantly increased at dose of 200 mg/kg and 400 mg/kg with *P<0.001*[Table 2].

Table 2: Effect of *Zanthoxylum macrophyllaon* sperm count ($x10^6$)

Parameters	Treatment groups (mg/kg)			
	Control	100	200	400
DSP	15.23 ± 0.06	16.73 ± 0.31	21.40 ± 0.08***	20.08 ± 0.80***
DSP/w.testis	12.99 ± 0.82	16.67 ± 1.55	19.39 ± 1.03	18.25 ± 1.14*
Epididymis	71.68 ± 6.38	65.60 ± 8.83	61.22 ± 7.72	79.95 ± 9.78
Sperm transit in cauda	4.70 ± 0.48	3.92 ± 0.54	3.35 ± 0.44	3.97 ± 0. 40

Value are mean ± SE; n=6; *P<0.05 with respect to control; ***P<0.001 with respect to control

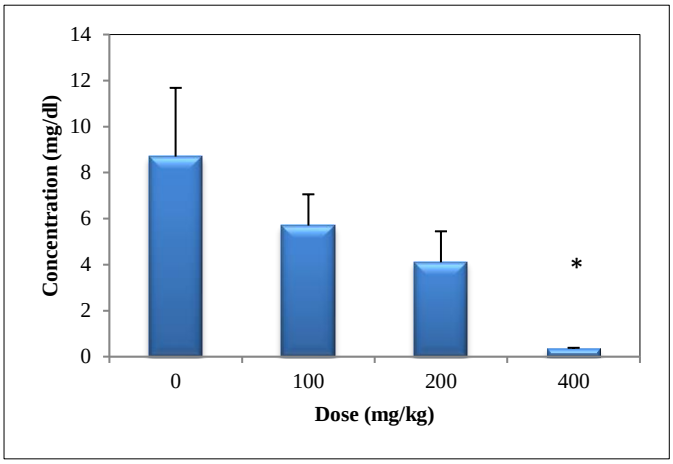
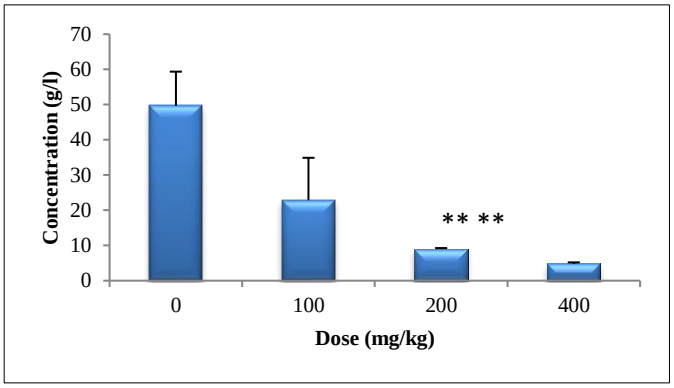


Figure 1

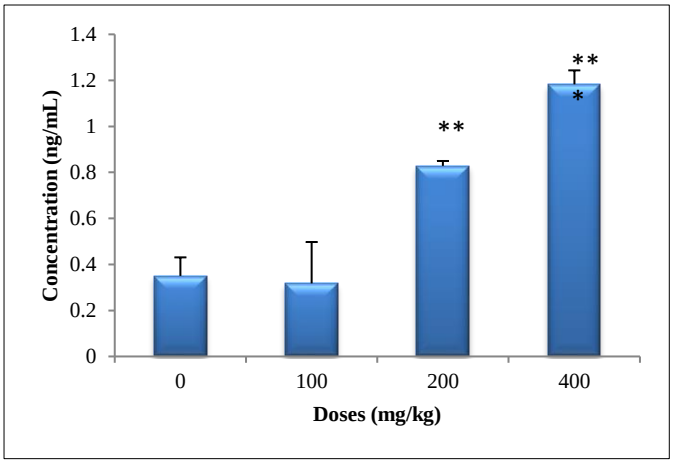


Values are mean ± SE; n = 6.** *(P <0.01)* with respect to control*;*
*** *(P <0.001)* with respect to control.

Figure 3: Effect of the aqueous extract of Z. *macrophylla* leaves on testosterone level

Effect of Zanthoxylum macrophylla on biochemical parameters

After treatment with the aqueous extract of *Zanthoxylum macrophylla*, we observed a significant reduction of total cholesterol levels ($P < 0.05$) at dose of 400 mg/kg [figure 2] and total proteins ($P < 0.01$) at dose of 200 and 400 mg/kg [Figure 3].



Values are mean ± SE; n = 6.*(P <0.05)* with respect to control.

Figure 2: Effect of the aqueous extract of Z. *macrophylla* leaves on total cholesterol level.

Effect of Zanthoxylum macrophylla on testosterone level.

According to [Figure 4], the serum testosterone concentration significantly increased at dose of 200 mg/kg and with ($P < 0.01$) and at dose of 400 mg/kg with ($P < 0.001$) following 14 days of treatment with the aqueous extract of *Zanthoxylum macrophylla* leaves.

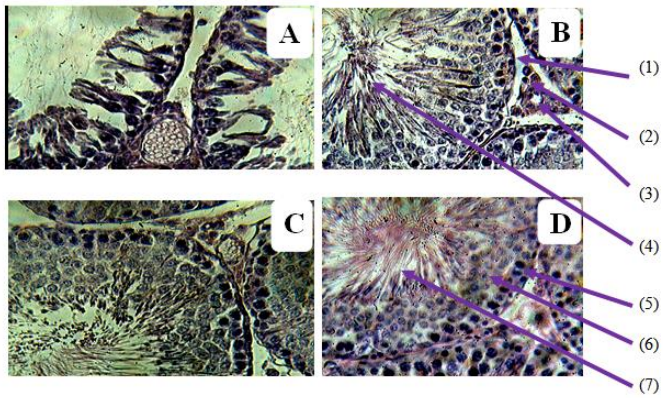


Figure 1 : Photomicrographs of cross sections of the testis of rats treated with distilled water (A), and z. macrophylla extract 100 mg/kg (B); 200 mg/kg (C); 400 mg/kg (D).
(1) is the tube wall seminiferous, (2) = Leydig cell, (3) = blood vessel, (4) = the seminiferous tube light, (5) is Spermatogonie, (6) = Spermatocyte, (7) = sperm

Values are mean ± SEM; n = 6. ** (P <0.01) with respect to control

Figure 4: Effect of the aqueous extract of Z. *macrophylla* leaves on the total protein level.

DISCUSSION

The development of additional methods and the active pursuit of research into new approaches to fertility control could provide enormous benefits in the area of social and public health.

In the present work, the administration of the aqueous extract of *Zanthoxylum macrophylla* did not significantly alter the body weight of the treated animals. However, we noticed a significant increase in the relative weight of the androgenic dependent organs (prostate and seminal vesicles) in rats receiving 200 mg/kg and 400 mg/kg of extract. These results are similar to those reported by Varsha *et al*., 2013 [13]. These results would be derived from the increase in the rate of testosterone generated by the extract. Indeed, it has been reported that accessory sexual organs are developed under the action of testosterone (seminal vesicles) and dihydrotestosterone (prostate). By fixing to their specific receptors, those hormones activate the transcription of the genes necessary for the cellular proliferation of androgenic dependent organs and consequently increase their size [14].

This study also shows that the significant increase in testicular weight observed after administration of the extract at doses 200 mg/kg and 400 mg/kg is parallel to the significant increase in daily sperm production per testis at the same doses. The increase in the relative weight of the testis would be due to the increase in spermatogenesis [15] which is a consequence of the activity of testosterone and the presence of flavonoids in the extract of *Zanthoxylum macrophylla* [16]. It is well known that the regulation of spermatogenesis is under the control of testosterone and androgen receptors (AR) of Sertoli cells. Testosterone deprivation studies performed in rodents have established that testosterone is required for germ cells to progress beyond meiosis and that testosterone is required for the release of mature spermatids during stage VIII in rats [17]. Then, the withdrawal of testosterone or knock out of AR in Sertoli cells results in three major impairments to fertility. First, it exposes post meiotic germ cells to autoimmune attack and cytotoxic factors [18, 19]. Second, it causes the premature detachment of round spermatids from Sertoli cells [20-22]. Third, fully mature spermatozoa cannot be released from Sertoli cells and the germ cells are phagocytized by the Sertoli cells [20]. It is then clear thatSertoli cells are thought to be the major cellular target for the testosterone signaling that is required to support male germ cell development and survival [23, 24]. On the other hand, flavonoids stimulate testicular androgenesis and spermatogenesis by acting on LH receptors and FSH, which in turn activate respectively the biosynthesis of testosterone by Leydig cells and spermatogenesis by Sertoli cells [25, 26]. Flavonoids can also activate the Nrf_2 transcription pathway, which acts as mediator of the induction of NFE_2-related factors genes and play a major role in defending testicular tissues against oxidative stress that leads to testicular alterations [27, 28]. It is why the analyses of the histological sections of the testes in the extract-treated rats when compared to the control showed no structural and morphological abnormalities. Sperm cells particularly spermatozoa was normal in the seminiferous tubes of treated animals when compared to the control. We can therefore say that the *Zanthoxylum macrophylla* extract could improve spermatogenesis in rats and has no substances that can produce toxic effects on intra-testicular structures.

As we were not expected, we noticed a significant decrease of serum cholesterol at the high dose (400 mg/kg) while at the same dose the level of serum testosterone increased. This contrast could be explained as follow: According to Rommerts 1998 [29], cholesterol required for the synthesis of testosterone arrives at the testis and the adrenal gland by two pathways: the main or direct route is to extract cholesterol fixed to plasma lipoproteins including the low density fraction and the indirect route or local synthesis pathway consists of producing cellular cholesterol from acetate, necessary for the synthesis of testosterone. It should therefore be thought that, the elevated testosterone level found in higher dose was related to the tissue cholesterol used for the synthesis of androgen. This testosterone would come from the indirect synthesis by both testes and/or the adrenal gland. On the other hand, sterols and Flavonoids found in the plant could interfere for androgen synthesis. Indeed, sterol are lipid substances that could be used as a substrate for the synthesis of testosterone or for the synthesis of one of these precursors [30] while Flavonoids are substances that could inhibit the synthesis of cholesterol by interacting with 3-hydroxy-3 methylglutaryl CoA (HMG-CoA) reductase, enzyme catalyzing the conversion of HMG-CoA to mevalonic acid [31]. One could say that *Zanthoxylum macrophylla* contains several secondary metabolites leading to testosterone metabolism and consequently to the improvement of spermatogenesis.

Similarly, the administration of the extract resulted in a significant decrease of the total serum protein level at dose of 200 mg/kg and 400 mg/kg in animals. The presence of sitosterol in the plant may be the cause of this significant decline since sitosterol would act by inhibiting the synthesis of proteins in tissues [32].

CONCLUSION

Based on the above obtained results, it may be concluded that the aqueous extract *Zanthoxylum macrophylla* leaves could improve the male reproductive functions, but information regarding exact mechanism of action requires further investigations.

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