Original Research Article

Spermatozoa characteristics, serum metabolites and testicular oxidative stress traits in guinea pigs (*Cavia porcellus*) fed on *Zanthoxyliun leprieurii* fruit

ABSTRACT

Zanthoxyliun leprieurii belong to aromatic plants. Its fruit is commonly used as spice in soups and as medicine in many African countries. Because of its phytochemical composition, it cans also be used as antioxidant. In the present study, effects of aqueous extract of *Z. leprieurii* fruit on reproductive function in male guinea pig (*Cavia porcellus*) was evaluated. Fifty male guinea pigs with an average weight of 320.56 ± 30 g, aged 4 months were used. They were divided into 5 groups (G1, G2, G3, G4 and G5) of 10 animals each. During 60 days, animals of G1 were daily given distilled water orally, while G2 received 100 mg/kg body weight (bw) of vitamin C. In the other hand, G3, G4 and G5 received respectively by the same method 50, 100 and 200 mg/kg bw of aqueous extract of *Z. leprieurii*. At the end of the treatment, all animals were sacrificed for evaluating the genital organs weight, sperm characteristics, serum levels of reproductive hormones and stress biomarkers. Results revealed that the weight of testes, epididymis, vas deferens and accessory glands did not significantly affect ($p>0.05$) in cavies exposed to different treatments compared to control animals. There was a significant ($p<0.05$) increase in serum content of FSH at 100 mg/kg. bw (26.67 ± 3.51 ng ml $^{-1}$) and LH at 50 mg/kg. bw (10.71 ± 2.42 ng ml $^{-1}$) in animals exposed to aqueous extract of *Z. leprieurii* with reference to the control groups. In addition, there is a non-significant increase ($p>0.05$) of the level of testosterone in the treated cavies compared to controls. Aqueous extract induced significant ($p<0.05$) increase in sperm mobility and sperm count in treated cavies with respect to the controls. The testicular activities of superoxide dismutase, catalase and peroxidase increased significantly ($p<0.05$) in guinea pigs exposed to aqueous extract of *Z. leprieurii* compared with those of control (G1). The reverse effect was observed concerning the concentration of malondialdehyde. In conclusion, the aqueous extract of *Z. leprieurii* fruit efficiently improves male reproductive characteristics by increasing level in reproductive hormones and an improvement of anti-oxidative enzymes activities.

Keywords: Aqueous extract, Z. leprieurii, male guinea pig, reproduction.

1. INTRODUCTION

In Africa especially in tropical region, the use of plants and its extract for the treatment and management of diseases has been in existence since ancient times. Factors such as poverty and illiteracy still militate against availability and accessibility of conventional medical services. A larger number of tropical plants and their extract possess diverse bioactive molecules such as phenols, terpenes, tannins, alkaloids rich in various properties: anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial and aphrodisiac [1]. These properties can be used in animal production to neutralize the effects of endogenous and exogenous in benefic of growth and productivity. Many studies in the area of animal production have shown that the decrease in animal growth and productivity ~~in~~ is linked to poor quality of feed and water, climate changes, psychological and hormonal disorders and drug [2-5]. As a solution, the breeders previously used in animal diet, antibiotics as food additives to enhance their performances. Unfortunately, the use of that feed additive have been prohibited due to its side effects attached to microbial resistance, cell apoptosis and reprotoxicity [6].

Nowadays, natural plants products with marked pharmacological activities and available all year round, cheap, accessible and often with minimal side effects [7, 8] are massively used to improve animal performances. Many plants among which *Zanthoxylium leprieurii* due to their bioactive molecules (phenols, alkaloids and terpenes) have been reputed to improve animal reproduction characteristics [9-11].

The seeds of *Zanthoxylium leprieurii* are largely used as spices by the people of Cameroon and especially those from west region. It belongs to the family Rutaceae and it is an aromatic, spiny, thicket forming deciduous shrub or tree. Ethnomedically, it is used in the treatment and management of muscle spasm, varicose vein, raynauld disease, arthritis, rheumatism, neuraligia, fever, toothache and gum diseases [9]. Abdou [12] showed that the fruit of this plant possess compounds such as phenols, flavonoids, tannins, vitamins A, E and C. These molecules possess diverse activities including antioxidant properties which can be exploited to reduce the reactive oxygen species attacks in membrane and nucleic acids of animal cell tissues in which the spermatozoa. This effect would subsequently increase the thickness of spermatozoa membrane, their mobility, their viability and their concentration [13]

Despite frequent and regular use, there is no reported work related to the effect of this plant on male reproductive function. The study was thus intended to bridge the gap in our continued efforts to establish the effects of local food spices and medications on male reproductive function.

2. MATERIAL AND METHODS

Selection of Animals and experimental feeding

Fifty male guinea pigs (*Cavia porcellus*) reared at the Teaching and Research Farm of the University of Dschang were used. Their average body weight was 320.56±30 g at the beginning of the assay. They were identified individually using numbered earrings and housed in identical cages of 100 cm x 80 cm x 60 cm (length, width and height) under standard conditions with free access to water and feed (Table 1). The average ambient temperature of the experimental housing was 22,5°C. All experiments were carried out in compliance with the recommendations Guide of the National Academy of Sciences on the care and use of laboratory Animals [14] and approved by the department of animal science.

Table 1: Composition and chemical characteristics of the experimental diet

Ingredients	Quantities (kg)	Chemical Characteristics

Corn	26.50	Metabolizable energy (kcal/kg) :	2600.00
Bran wheat	25.00	Crude protein (%) :	19.00
Rice bran	12.00	Crude cellulose (%) :	14.18
Soy beans cake	6.00	Calcium (%) :	10.05
Cotton cake	6.00	Phosphorus (%) :	0.68
Palm kernel cake	13.00	Sodium (%) :	0.28
Fish meal	2.00	Lysine (%) :	1.01
Sea shells	2.00	Methionine(%):	0.40
Palm oil	2.00		
Sal	0.50		
Premix10% *	5.00		

∗Premix 5%: mixture of vitamins A, B complex, D, K, and E plus iron, Cu, Zn, Se, Mn, methionine, lysine principally and incorporated at 5% in diet.

Plant material

The seed of *Zanthoxylium* were bought from Dschang (Menoua division in Cameroon) local market. They were dried sheltered from the sun, and then grinded at the mill. The obtained powder was used for extractions, using 6 liters of distilled water for 1 kilogram of powder. The filtrate was dried in the oven at 45 °C to obtain a paste used to prepare the *Z. leprieurii* aqueous extract at different concentrations.

Phytochemical screening of *Z. leprieurii*

The phytochemical screening of *Z. leprieurii* aqueous extract (Table 2) was done as described by Ngbede [15] and Ngbede [16].

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Table 2 : Phytochemical constituent of *Z. leprieurii* aqueous extract

Constituents	**(+) present ; (-) absent**
Alkaloïds	-
Phenols	+
Tannins	+
Saponins	+
Flavonoids	+
Steroids	-
Triterpenes	+

Experimental design

Animals were distributed into 5 groups (G1, G2, G3, G4 and G5) of 10 animals each, comparable in term of body weight. During 60 days, animals of G1 were daily given orally distilled water (1 ml/kg bw), G2 received 100 mg/kg bw of vitamin C

diluted in distilled water, while G3, G4 and G5 received respectively 50, 100 and 200 mg/kg bw of aqueous extract of *Z. leprieurii*, dissolved in distilled water. The animal's body weight was recorded weekly and the doses of vitamin C and *Z. leprieurii* aqueous extract adjusted accordingly.

In the present study, the doses of *Z. leprieurii* were selected in reference of those used in the work of Kpomah et *al*. [17]) on diherbal mixture of *Zanthoxylum leprieurii* and *Piper guineense* on male wistar rats. In fact, the authors showed that the mixture of *Zanthoxylum leprieurii* and *Piper guineense* at doses of 100 and 150 mg/kg body weight increases the serum content in testosterone and progesterone, the mount, the intromission and the ejaculatory frequency.

Blood and organs collection

Twenty-four hours after the last administration of the vitamin C and aqueous extract solutions, animals were anesthetized using ether vapor and blood samples were collected by cardiac puncture for the measurement of hormonal concentrations. After the sacrifice, testes, epididymis, vas deferens and sexual accessory glands were excised out and weighed.

The cauda epididymis of each animal were minced in 10 ml of 0.9% NaCl solution (37 °C) for sperm characteristics evaluation. For sperm mobility, a drop of the obtained solution was placed on a slide and observed at magnification 400x under the light microscope (Leica) and the mobility score was attributed according to the method described by Boiti [18]. The sperm count was done using the Thomas haemocytometer, while the integrity of the plasma membrane was evaluated using the hypo-osmotic test [19].

Evaluation of oxidative stress indicators

A 15% (weigh/volume) homogenate was prepared using left testis of each animal. Thus, the testis was crushed in cold 0.9% NaCl solution followed by a centrifugation (3000 rpm, 30 min). The supernatant obtained was used to evaluate the testes content in oxidative stress indicators. The malondialdehyde concentration was determined using the thiobarbituric acid metho, [20] the superoxide dismustase activity was evaluated according to the method describe by Dimo [21]. The catalase (CAT) activity was assessed using the chromic acetate method [22] and the glutathione peroxidases (GPx) activity was determined by the potassium iodate method [23].

Evaluation of reproductive hormones

The serum concentration in testosterone, FSH and LH were measured using the instructions from ELISA kit (Omega Diagnostics) (Scotland, United Kingdom).

Statistical analysis

Data analysis were performed using one-way ANOVA, followed by Duncan's test at 5 % significance. All analyses were done using SPSS IBM statistics software 20.0. The results are expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

Weight of genital organs in male guinea pigs

The effects of the aqueous extract of *Z. leprieurii* on the weight of sexual organs in guinea pig are presented in table 3. The weights of testes, epididymis, vas deferens and accessory glands were comparable (p>0.05) among treatments. Although the values of these characteristics were statistically comparable, an increase was observed in the animals treated with aqueous extract with reference to the negative control (T0-).

Table 3: Effects of the aqueous extract of Z. *leprieurii* fruit on the relative weight of genital organs in cavy

Relative weight of	Control	Doses of *Z. leprieurii* extract (mg/kg bw)	

genital organs (g/100 g bw)	T0$^-$ (n=12)	T0$^+$ (n=12)	50 (n=12)	100 (n=12)	200 (n=12)	P-value
Testis	0.43±0.03^a	0.48±0.05^a	0.54±0.08^a	0.47±0.05^a	0.48±0.08^a	0.26
Epididymis	0.08±0.01^a	0.09±0.02^a	0.10±0.03^a	0.10±0.01^a	0.09±0.02^a	0.52
Vas deferens	0.42±0.09^a	0.40±0.08^a	0.43±0.08^a	0.43±0.04^a	0.46±0.08^a	0.89
Accessory glands	0.04±0.01^a	0.04±0.01^a	0.05±0.01^a	0.04±0.01^a	0.05±0.00^a	0.67

a: within the same raw, values with the same letters are not significantly different (p>0.05) n: Number of cavy. Bw, body weight; T0-, group receiving 1 mL/kg bw of distilled water; T0+, group receiving 100 mg/kg bw of vitamin C; P, Probability.

Characteristics of spermatozoa in guinea pigs

Table 4 summarizes the effects of the aqueous extract of Z. leprieurii on caudal epididymal sperm characteristics in male guinea pigs. The sperm mobility, sperm count per cauda and per gram of epididymis and spermatozoa with entire plasma membrane increased significantly (p<0.05) in animals treated with aqueous extract of Z. leprieurii compared to controls (T0- and T0+). However, the highest sperm mobility and the sperm count was observed in animals exposed to 200 mg/kg bw compared to the others.

Table 4: Effects of the aqueous extract of *Z. leprieurii* fruit on the caudal epididymal sperm characteristics in male guinea pigs

Sperm characteristics	Controls		Doses of *Z. leprieurii* extract (mg/kg bw)			P value
	T0$^-$ (n=12)	T0$^+$ (n=12)	50 (n=12)	100 (n=12)	200 (n=12)	
Mobility (%)	67.50±1.73^e	76.67±0.58^c	80.00±0.00^b	74.00±1.41^d	88.33±1.15^a	0.00
Number/cauda epididymis (10^7)	06.50±0.17^c	04.67±0.63^d	07.81±0.38^b	10.50±0.43^a	10.42±0.38^a	0.00
Number/g of cauda epididymis (10^7)	29.83±1.25^c	39.55±2.37^b	55.51±1.23^a	37.23±1.38^b	54.88±1.71^a	0.00
Spermatozoa with EPM (%)	60,0±0,50^c	52,5±0,58^d	75,0±0,82^b	72,67±0,58^a	75,33 ±0,58^a	0,00

$^{a, b, c, d, e}$, within the same raw, values with the same letters are not significantly (p>0.05) different. N, Number of guinea pigs. Bw, body weight. T0-, group receiving 1 mL/kg bw of distilled water. T0+, group receiving 100 mg/kg bw of vitamin C; EPM, entire plasma membrane.

Effect of aqueous extract of *Z. leprieurii* on serum metabolites in male guinea pigs

As shown in Table 5, the total cholesterol decreased significantly (p<0.05) in Z. leprieurii aqueous extract treated guinea pigs irrespective of the dose as compared to the negative control. The oral administration of Z. leprieurii aqueous extract in guinea pigs did not significantly (p>0.05) affect the serum content in total proteins. With respect to the controls, the serum content in FSH increased in cavies exposed to Z. leprieurii aqueous extract, but that increase was significant only at dose of 100 mg/kg bw. The serum content in LH increased significantly (p<0.05) in cavies treated with 50 mg/kg bw of Z. leprieurii aqueous extract compared to control (T0-). The level of testosterone increased but not significantly (p>0.05) in guinea pigs treated with aqueous extract of Z. leprieurii at doses of 100 and 200 mg/kg bw compared to the controls.

Table 5: Effects of aqueous extract of *Z. leprieurii* fruit on serum metabolites in male guinea pigs

Serum metabolites	Controls		Doses of *Z. leprieurii* extract (mg/kg bw)			P-value
	T0^{-} (n=12)	T0^{+} (n=12)	50 (n=12)	100 (n=12)	200 (n=12)	
Total cholesterol (mg/dl)	104.66±5.45^{a}	90.03±7.34^{bc}	85.36±10.74^{c}	90.03±7.72^{bc}	101.16±7.57^{ab}	0.02
Total proteins (g/dl)	3.76±0.65	4.16±0.94	3.63±0.6	4.04±0.18	3.78±0.42	0.81
FSH (mIU/ml)	14 ±3.67^{b}	12±3.74^{b}	16.5±4.81^{b}	26.67±3.51^{a}	18.25±4.72^{b}	0.00
LH (mIU/ml)	9.74±1.17^{ab}	7.92±1.71^{ab}	10.71±2.42^{a}	7.14±0.33^{b}	8.71±1.89^{ab}	0.04
Testosterone (ng/ml)	1.36±0.38	1.43±0.31	1.21±0.12	1.53±0.46	1.57±0.32	0.54

a, b, within the same line, values with the same letters are not significantly (P>0.05) different. n: Number of cavies. bw: body weight. T0-, group receiving 1 mL/kg bw of distilled water. T0+, group receiving 100 mg/kg bw of vitamin C; FSH, follicle stimulating hormone; LH, luteinizing hormone.

Effect of *Z. leprieurii* extract on some oxidative stress indicators in male guinea pigs

As shown in Table 6, the testicular activities of catalase (CAT), superoxide dismutase (SOD) and peroxidase glutathione (GPx) significantly increased ($p<0.05$) in cavies treated with aqueous extract of Z. leprieurii compared to the controls. The concentration of malondialdehyde (MDA) significantly decreased ($p<0.05$) in animals treated with the aqueous extract of Z. leprieurii irrespective of the dose compared to the controls.

Table 6: Effects of aqueous extract of *Z. leprieurii* on oxidative stress indicators in male guinea pigs

Oxidative stress indicators	Controls		Doses of *Z. leprieurii* extract (mg/kg bw)			P-value
	T0^{-} (n=12)	T0^{+} (n=12)	50 (n=12)	100 (n=12)	200 (n=12)	
MDA(µM/g of testis)	1.23±0.05^{a}	1.14±0.02^{b}	0.68±0.08^{d}	0.64±0.04^{d}	0.95±0.03^{c}	0.00
CAT(µM/min/g of testis)	6.68±0.33^{c}	6.21±0.04^{d}	7.89±0.09^{b}	7.72±0.05^{b}	12.25±0.02^{a}	0.00
SOD(U/min/mg of testicular protein)	0.32±0.01^{c}	0.47±0.03^{c}	0.81±0.16^{b}	0.87±0.14b^{a}	1.04±0.07^{a}	0.00
GPx (µmol/min/g of testis)	26.99±0.68^{c}	25.84±0.95^{d}	30.05±0.44^{b}	32.83±0.55^{a}	29.44±0.3^{b}	0.00

Superscripts $^{a, b, c, d}$ within the same raw differ significantly ($P<0.05$), n, Number of cavies. bw:,body weight. T0-, group receiving 1 mL/kg bw of distilled water. T0+, group receiving 100 mg/kg bw of vitamin C; MDA, Malondialdehyde. CAT, Catalase; SOD, Superoxide dismutase; GPx, Glutathione peroxidase.

3.2. Discussion

The chemical screening of *Z. leprieurii* aqueous extract carried out in the present study revealed the presence of phenols, tannins, triterpenes, saponins flavonoids. These molecules have been reputed to have diverse activities (antioxidants, antibacterial, anti-inflammatory, antiseptic, antiparasitic, and immunomodulatory properties) susceptible to improve the animal reproductive characteristics [6, 5]

In this study, the effects of aqueous extract of *Z. leprieurii* fruit on reproductive parameters in male guinea pigs were determined. The weights of the testis, epididymis, vas deferens and accessory sex glands increased dose-dependently in animals submitted to aqueous extract of *Z. leprieurii* compared to the controls. These results are comparable to those found in male rats treated with ethanol extract of the fruits of *Xylopia aethiopica* at doses of 30, 100 and 300 mg/kg bw [24]. In rats treated with aqueous extracts of *Psidium guajava* leaves (100, 200 and 300 mg/kg bw) [25, 26] and in male guinea pigs treated with essential oil of *Psidium guajava* leaves (80, 100 and 120µl /kg bw), [29] the similar results were also recorded. This observation might be explained by the action of antioxidant compounds such as phenols, flavonoids, tannins, terpenoids and saponins revealed in this extract following the phytochemical tests [27]. These molecules could

have neutralized free radical attacks [28] or inhibiting enzymes responsible of their production and then protecting cells against oxidative stress. The weight, size and secretory function of testes, epididymis and seminal vesicle are closely regulated by androgens. [29]. In fact, Androgens, especially testosterone have anabolic properties which are characterized by an increased synthesis of proteins and therefore muscle mass. Androgens then contribute to the increased volume and weight of the testis and epididymis by stimulating protein synthesis [29, 30] The increase in the serum content of testosterone observed in the present study would induce that of the sexual organ weights.

Sperm count, mobility and entire plasma membrane are considered to be important factors that affect fertility [31]. The sperm with high density of spermatozoa could contained an important proportion of active spermatozoa capable to fertilize an ovum [13]. It can also be diluted to have a high volume used to fertilize a large number of females in the case of artificial insemination. In the other hand, spermatozoa with high mobility rate move rapidly in female genital track and has an advantage to meet an ovum at the ampullary-isthmic junction and fuse with it [13]. In the present study, the *Z. leprieurii* markedly increased the sperm count and the spermatozoa mobility in guinea pigs. This effect would be a result of *Z. leprieurii* bioactive molecules with antioxidant properties. These molecules reduce the spermatozoa membrane and nucleic acid attack by reactive oxygen species. This action subsequently decreases the death in the spermatozoa and increases the spermatozoa mobility which is beneficial for reproduction.

The serum content of FSH in *Z. leprieurii* treated guinea pigs significantly increased at doses of 100 and 200 (mg/kg bw) with respect to the controls. Some bioactive molecules of *Z. leprieurii* would have a possible action on pituitary gland cells synthesizing the FSH. The elevated sperm count recorded in this study would result in FSH action. FSH has a key role in the spermatogenesis. Together, FSH and testosterone support meiosis, exhibit an anti-apoptotic action on spermatocytes and round spermatids. They act co-operatively to promote spermatid maturation and sperm release [32]. Testosterone is the main male gonadal hormone produced by the interstitial cells of the Leydig in the testes in response to LH [33]. In this study, both serum content in LH and testosterone tend to increase in *Z. leprieurii* treated guinea pigs. The *Z. leprieurii* rich in bioactive molecules (phenols, alkaloids…) with antioxidant properties would protect the pituitary gland cells specialized in the production of LH. The testosterone levels recorded would be a result of the relationship between LH and testosterone.

Many environmental insults (poor quality of food and water intake, fluctuation of temperature, highly density in breeding…) induce overproduction of reactive oxygen species responsible for animal cell membrane and nucleic acids impairment. According to Tchoffo, [13] the substances with antioxidant properties inhibit the reactive oxygen species attacks and subsequently improve the animal cell characteristics. In the present study, the malondialdehyde which is the major resulting product from the membrane lipid peroxidation decreased significantly ($p<0.05$) in *Z. leprieurii* aqueous extract treated male guinea pigs. Inversely, the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase glutathione (GPx) increased significantly ($p<0.05$) in male guinea pigs exposed to aqueous extract of *Z. leprieurii* compared to the controls. The phytochemical test on *Z. leprieurii* revealed that it contains bioactive molecules as phenols and flavonoids. These molecules possess antioxidant properties responsible for *Z. leprieurii* aqueous extract effects. In male animals, the spermatozoa membrane is characterized by a highly presence of polyunsaturated fatty acids rending them susceptible to lipid peroxidation [13]. The *Zanthoxylium* due to their molecules with antioxidant properties could have protect the spermatozoa from reactive oxygen species attacks and subsequently improve their characteristics as recorded in the present study.

4. CONCLUSION

The results of this study revealed that the *Z. leprieurii* fruit aqueous extract efficiently improved reproductive characteristics by increasing level in reproductive hormones. It also induced an increase in testicular antioxidant enzymes (SOD, catalase, peroxidase) activities and a decrease in MDA level. Based on these effects, the *Z. leprieurii* fruit aqueous extract can be used in male animals to improve its reproductive performances.

ETHICAL APPROVAL

All experiments were carried out in compliance with the recommendations Guide of the National Academy of Sciences on the care and use of laboratory Animals and approved by the department of animal science, FASA, University of Dschang

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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