

Original Research Article

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

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ABSTRACT

Zanthoxylium 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 can also be used as antioxidant. In the present study, we evaluated the effects of aqueous extract of *Z. leprieurii* fruit on reproductive function in male guinea pig (*Cavia porcellus*). 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. It is responsible for the increased level in reproductive hormones and an improve 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 these tropical plants and their extracts 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 in animal production to neutralize the effects of endogenous and exogenous in benefic of growth and productivity. Many studies carried y out in the animal production have shown that the decrease in animal growth and productivity in linked to poor quality of food 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 th additive have been prohibited due to its side effects attached attributed to microbial resistance, cell apoptosis and

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reprotoxicity [6]. **n** 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 *Zanthoxylum leprieurii* due to their bioactive molecules (phenols, alkaloids and terpenes) have been reputed to improve animal reproduction characteristics [9-11]. The findings of Etuk [12]. **recorded reported that in** male rats receiving the aqueous extracts of *Lophira lanceolata* at doses of 100, 200 and 300 mg/kg body weight for 40 days **an** increased in the sperm concentration in spermatozoa. Similar results have been observed by Sariah [13] in male rats treated at 50, 100 and 150 mg doses of *Anacyclus pyrethrum* aqueous extract/kg body weight for a period of 28 days. Oral administration of the aqueous extract of *Zanthoxylum macrophylla* at a dose of 400 mg/kg body weight for 14 consecutive days resulted in a significant improvement in sperm characteristics in rats.¹⁴ In addition, the rats **fed with administered** aqueous extract of *Psidium guajava* at doses of 200 and 300 mg/kg body weight for 70 days revealed an increase in spermatozoa mobility and viability.¹⁵

The seeds of *Zanthoxylum 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, raynaud disease, arthritis, rheumatism, neuralgia, fever, toothache and gum diseases [9]. Abdou [16] showed that the fruit of this plant possess compounds such as phenols, flavonoids, tannins, vitamins A, E and C.

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

Animals, lodging and 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). 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 [17] 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 (Fibre) (%) : 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	

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Comment [PE6]: Male reproductive function in all animals? Please, specify the specie you worked on.

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Sal	0.50
Premix10% *	5.00

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

64 **Plant material**

66 The seed of *Zanthoxylum* were bought from Dschang (Menoua division in Cameroon) local market. They were dried
67 sheltered from the sun, and then grinded at the mill. The obtained powder was used for extractions, using 6 liters of
68 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.*
69 *leprieurii* aqueous extract at different concentrations.

70 **Phytochemical screening of *Z. leprieurii***

72 The phytochemical screening of *Z. leprieurii* aqueous extract (Table 2) was done as described by Ngbede [18] and
73 Ngbede [19].

74 **Table 2 :** Phytochemical constituents of *Z. leprieurii* aqueous extract

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

76 **Experimental design**

78 Animals were distributed into 5 groups (G1, G2, G3, G4 and G5) of 10 animals each, comparable in term of body weight.
79 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
80 diluted in distilled water, while G3, G4 and G5 received respectively 50, 100 and 200 mg/kg bw of aqueous extract of *Z.*
81 *leprieurii*, dissolved in distilled water. The animal's body weight was recorded weekly and the doses of vitamin C and *Z.*
82 *leprieurii* aqueous extract adjusted accordingly.

83 **Blood and organs collection**

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

88 | The cauda epididymis of each animal were was minced in 10 ml of 0.9% NaCl solution (37 °C) for sperm characteristics
 89 | evaluation. For sperm mobility, a drop of the obtained solution was placed on a slide and observed at magnification 400x
 90 | under the light microscope (Leica) and the mobility score was attributed according to the method described by Boiti.²⁰ The
 91 | sperm count was done using the Thomas haemocytometer, while the integrity of the plasma membrane was evaluated
 92 | using the hypo-osmotic test [21].

93 | **Evaluation of oxidative stress indicators**

94 | A 15% (weight/volume) homogenate was prepared using left testis of each animal. Thus, the testis was crushed in cold
 95 | 0.9% NaCl solution followed by a centrifugation (3000 rpm, 30 min). The supernatant obtained was used to evaluate the
 96 | testes content in oxidative stress indicators. The malondialdehyde concentration was determined using the thiobarbituric
 97 | acid method, [22] the superoxide dismutase activity was evaluated according to the method described by Dimo [23]. The
 98 | catalase (CAT) activity was assessed using the chromic acetate method [24] and the glutathione peroxidases (GPx)
 99 | activity was determined by the potassium iodate method.²⁵

100 | **Evaluation of reproductive hormones**

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

103 | **Statistical analysis**

104 | Data analysis were performed using one-way Analysis of Variance (ANOVA), followed by Duncan's test at 5 %
 105 | significance. All analyses were done using SPSS IBM statistics software 20.0. Obtained results are expressed as mean ±
 106 | standard deviation.

107 | **3. RESULTS AND DISCUSSION**

108 | **Weight of genital organs in male guinea pigs**

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

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

Relative weight of genital organs (g/100 g bw)	Controls		Doses of <i>Z. leprieurii</i> extract (mg/kg bw)			P-value
	T0 ⁻ (n=12)	T0 ⁺ (n=12)	50 (n=12)	100 (n=12)	200 (n=12)	
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

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

119 | **Characteristics of spermatozoa in guinea pigs**

Comment [PE10]: Write these hormones in full then abbreviate in bracket.

Comment [PE11]: The level of significance is attributable to ANOVA. Duncan is compares pairs of treatment means, and this does not have its own separate level of significance as noted here. Moreso, 5% level of significance are not acceptable in scientific results. Carryout your analysis at either 95% or 99% level of significance.

Comment [PE12]: This increase was not statistically significant and therefore of no consequence. Present and discuss results from a statistical stand point and nominal differences.

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

Sperm characteristics	Controls		Doses of <i>Z. lepreurii</i> 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 ^c	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

127 a, b, c, d, e, within the same row, values with the same letters superscripts are not significantly ($p > 0.05$) different. N, Number of
 128 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;
 129 EPM, entire plasma membrane.

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

132 As shown in Table 5, the total cholesterol decreased significantly ($p < 0.05$) in *Z. lepreurii* aqueous extract treated guinea
 133 pigs irrespective of the dose as compared to the negative control. The oral administration of *Z. lepreurii* aqueous extract
 134 in guinea pigs did not significantly ($p > 0.05$) affect the serum content in total proteins. With respect to the controls, the
 135 serum content in FSH increased in cavies exposed to *Z. lepreurii* aqueous extract, but that increase was significant only
 136 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
 137 *Z. lepreurii* aqueous extract compared to control (T0-). The level of testosterone increased but not significantly ($p > 0.05$) in
 138 guinea pigs treated with aqueous extract of *Z. lepreurii* at doses of 100 and 200 mg/kg bw compared to the controls.
 139

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

Serum metabolites	Controls		Doses of <i>Z. lepreurii</i> 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

141 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.
 142 T0-, group receiving 1 mL/kg bw of distilled water. T0+, group receiving 100 mg/kg bw of vitamin C; FSH, follicle stimulating
 143 hormone; LH, luteinizing hormone.

Comment [PE14]: Talk of those with different and same superscripts.

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

145 As shown in Table 6, the testicular activities of catalase (CAT), superoxide dismutase (SOD) and peroxidase glutathione
 146 (GPx) significantly increased ($p < 0.05$) in cavies treated with aqueous extract of *Z. lepreurii* compared to the controls. The

147 concentration of malondialdehyde (MDA) significantly decreased ($p < 0.05$) in animals treated with the aqueous extract of
 148 *Z. lepreurii* irrespective of the dose compared to the controls.

149

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

Oxidative stress indicators	Controls		Doses of <i>Z. lepreurii</i> 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±0.14 ^b	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

151 a, b, c, d, within the same line, values with the same letters are not significantly ($P > 0.05$) different. n, Number of cavies. bw: body
 152 weight. T0⁻, group receiving 1 mL/kg bw of distilled water. T0⁺, group receiving 100 mg/kg bw of vitamin C; MDA,
 153 Malondialdehyde. CAT, Catalase; SOD, Superoxide dismutase; GPx, Glutathione peroxidase.

154

155 3.2. Discussion

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

160 In this study, the effects of aqueous extract of *Z. lepreurii* fruit on reproductive parameters in male guinea pigs were
 161 determined. The weights of the testis, epididymis, vas deferens and accessory sex glands increased dose-dependently in
 162 animals **submitted subjected** to aqueous extract of *Z. lepreurii* compared to the controls. These results are comparable
 163 to those found in male rats treated with ethanol extract of the fruits of *Xylopi aethiopic a* at doses of 30, 100 and 300
 164 mg/kg bw [26]. In rats treated with aqueous extracts of *Psidium guajava* leaves (100, 200 and 300 mg/kg bw) [27, 28] and
 165 in male guinea pigs treated with essential oil of *Psidium guajava* leaves (80, 100 and 120 μl /kg bw), [29] the similar results
 166 were also recorded. This observation might be explained by the action of antioxidant compounds such as phenols,
 167 flavonoids, tannins, terpenoids and saponins revealed in this extract following the phytochemical tests [12]. These
 168 molecules could have neutralized free radical attacks [30] or inhibiting enzymes responsible **of for** their production and
 169 then protecting cells against oxidative stress. The weight, size and secretory function of testes, epididymis and seminal
 170 vesicle are closely regulated by androgens. [31]. In fact, Androgens, especially testosterone have anabolic properties
 171 which are characterized by an increased synthesis of proteins and therefore muscle mass. Androgens then contribute to
 172 the increased volume and weight of the testis and epididymis by stimulating protein synthesis [31, 32] The increase in the
 173 serum content of testosterone observed in the present study would induce that of the sexual organ weights.

174 Sperm count, mobility and entire plasma membrane are considered to be important factors that affect fertility [33]. The
 175 sperm with high density of spermatozoa could contained an important proportion of active spermatozoa capable to fertilize
 176 an ovum [34]. It can also be diluted to have a high volume used to fertilize a large number of females in the case of
 177 artificial insemination. **In On** the other hand, spermatozoa with high mobility rate move rapidly in female genital tract **k** and
 178 has an advantage **to of meet fertilizing** an ovum at the ampullary-isthmic junction, **and fuse with it** [34]. In the present
 179 study, the *Z. lepreurii* markedly increased **the** sperm count and **the** spermatozoa mobility in guinea pigs. This effect
 180 would be a result of *Z. lepreurii* bioactive molecules with antioxidant properties. These molecules reduce the spermatozoa
 181 membrane and nucleic acid attack by reactive oxygen species. This action subsequently decreases the death in the
 182 spermatozoa and increases the spermatozoa mobility which is beneficial for reproduction.

183 The serum content of FSH in *Z. lepreurii* treated guinea pigs significantly increased at doses of 100 and 200 (mg/kg bw)
 184 with respect to the controls. Some bioactive molecules of *Z. lepreurii* would have a possible action on pituitary gland cells
 185 synthesizing the FSH. The elevated sperm count recorded in this study would result in FSH action. FSH has a key role in
 186 **the** spermatogenesis. Together, FSH and testosterone support meiosis, exhibit an anti-apoptotic action on spermatocytes
 187 and round spermatids. They act co-operatively to promote spermatid maturation and sperm release [35]. Testosterone is
 188 the main male gonadal hormone produced by the interstitial cells of the Leydig in the testes in response to LH [36]. In this
 189 study, both serum content in LH and testosterone tend to increase in *Z. lepreurii* treated guinea pigs. The *Z. lepreurii* rich

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190 in bioactive molecules (phenols, alkaloids...) with antioxidant properties would protect the pituitary gland cells specialized
191 in the production of LH. The testosterone levels recorded would be a result of the relationship between LH and
192 testosterone.

193 Many environmental insults (poor quality of food and water intake, fluctuation of temperature, highly density in
194 breeding...) induce overproduction of reactive oxygen species responsible for animal cell membrane and nucleic acids
195 impairment. According to Tchoffo, [34] the substances with antioxidant properties inhibit the reactive oxygen species
196 attacks and subsequently improve the animal cell characteristics. In the present study, the malondialdehyde which is the
197 major resulting product from the membrane lipid peroxidation decreased significantly ($p < 0.05$) in *Z. lepreurii* aqueous
198 extract treated male guinea pigs. Inversely, the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase
199 glutathione (GPx) increased significantly ($p < 0.05$) in male guinea pigs exposed to aqueous extract of *Z. lepreurii*
200 compared to the controls. The phytochemical test on *Z. lepreurii* revealed that it contains bioactive molecules as phenols
201 and flavonoids. These molecules possess antioxidant properties responsible for *Z. lepreurii* aqueous extract effects. In
202 male animals, the spermatozoa membrane is characterized by a highly presence of polyunsaturated fatty acids rendering
203 them susceptible to lipid peroxidation [34]. The *Zanthoxylum* due to their molecules with antioxidant properties
204 could have protected the spermatozoa from reactive oxygen species attacks and subsequently improve their
205 characteristics as recorded in the present study.

206 207 4. CONCLUSION

208 The results of this study revealed that the *Z. lepreurii* fruit aqueous extract efficiently improved reproductive
209 characteristics. It is responsible for the increased level in reproductive hormones. It also induced an increase in testicular
210 antioxidant enzymes (SOD, catalase, peroxidase) activities and a decrease in MDA level. Based on these effects, the
211 *lepreurii* fruit aqueous extract can be used in male animals guinea pigs to improve its reproductive performances.

213 ETHICAL APPROVAL

214 All experiments were carried out in compliance with the recommendations Guide of the National Academy of Sciences on
215 the care and use of laboratory Animals and approved by the department of animal science, FASA, University of Dschang
216

217 COMPETING INTERESTS

218 The authors declare that they have no conflict of interest.
219
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221

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