International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2021; 6(4): 26-33

www.veterinarypaper.com
Received: 07-04-2021
Accepted: 10-05-2021
Publice: 20-05-2021

Boufack Judith Laure
Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box 188, Dschang, Cameroon

Guiekep Nounamo Arthénice Jémima
College of Technology, University of Bamenda, P.O.Box 39, Bambili, Cameroon

Vemo Bertin Narcisse
Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O.Box 188, Dschang, Cameroon

Fonou Tadiesse Lavoisier
Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O.Box 188, Dschang, Cameroon

Tsambou Megnimeza Martine Astride
Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O.Box 188, Dschang, Cameroon

Menkem Brice
Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O.Box 188, Dschang, Cameroon

Ngaleu Djieudeu Claude Cédric
Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O.Box 188, Dschang, Cameroon

Yidjieu Nana Ferry
Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box 188, Dschang, Cameroon

Kenfack Augustave
Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O.Box 188, Dschang, Cameroon

Corresponding Author:
Boufack Judith Laure
Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box 188, Dschang, Cameroon

Effects of ethanol extract of *Afrostyrax lepidophyllus* seeds on reproductive parameters and oxidative stress markers in male guinea pigs (*Cavia porcellus*) exposed to Paraquat

Boufack Judith Laure, Guiekep Nounamo Arthénice Jémima, Vemo Bertin Narcisse, Fonou Tadiesse Lavoisier, Tsambou Megnimeza Martine Astride, Menkem Brice, Ngaleu Djieudeu Claude Cédric, Yidjieu Nana Ferry and Kenfack Augustave

DOI: https://doi.org/10.22271/veterinary.2021.v6.i4a.363

Abstract
Paraquat is a contact herbicide, particularly effective for weeds control. This study aimed at evaluating the effects of ethanol extract of *Afrostyrax lepidophyllus* (EEAL) seeds on reproductive toxicity and oxidative stress caused by paraquat in male Guinea pigs. 60 male guinea pigs were randomly assigned to 6 treatment groups (n = 8) which orally received varied concentration of solutions for 115 days. Group 1 (G1) received distilled water; group 2 (G2) was given 4 mg kg^{-1} of body weight of paraquat (PQ), group 3 (G3) received 4 mg kg^{-1} bw of PQ and 100 mg kg^{-1} bw of Vitamin C (Vit C) while groups 4, 5 and 6 were co-treated with PQ (4 mg kg^{-1}) plus EEAL at doses of 50, 100 and 200 mg kg^{-1} bw respectively. Serum testosterone, total cholesterol, the reaction time, sexual organ weights, sperm count, motility and anomalies (minors and majors), spermatozoa with entire plasma membrane, testicular malondialdehyde (MDA) level, superoxide dismutase (SOD), catalase (CAT) and total peroxidases activities were assessed. Result showed that the co-administration of paraquat and EEAL significantly (p<0.05) reduced the reaction time, serum concentrations of testosterone and total cholesterol, sperm anomalies and testicular levels of MDA, SOD, CAT, peroxydases and total proteins compared to the receiving paraquat only group. The co-administration of paraquat and EEAL significantly (p<0.05) alleviated sperm count and motility, percentage of spermatozoa with the normal plasma membrane, seminal vesicle and prostate weights with reference to guinea pigs given PQ only. In conclusion, paraquat negatively affected animal reproductive function and induced oxidative stress. The co-administration of EEAL alleviated the toxic effects of paraquat on the reproductive function of male guinea pigs.

Keywords: *Afrostyrax lepidophyllus*, guinea pig, oxidative stress, Paraquat, reproductive parameters

1. Introduction
Paraquat is a contact herbicide, particularly effective for weeds control [1]. However it is extremely toxic to those who use it [2]. It is one of the most widely used herbicides in the world, sold in more than 120 countries [1]. It is used mainly in the tropics, where weeds growth is extremely fast and makes manual weeding techniques very inefficient, or even the use of a large number of other phytosanitary products [2]. It is used in floriculture and in certain woodlots to weed or prepare the soil for a hundred crops of cereals (corn, wheat, barley, rye, rice, etc.), soybeans, potatoes, fruits (apple, orange, banana), plants intended for the manufacture of beverages (coffee, tea, cocoa) and crops such as that of cotton, oil palm, sugar cane and rubber [3]. However, paraquat is extremely toxic to humans and animals in many aspects [4]. The number of patients worldwide who had been poisoned by paraquat was over 650,000 in 2016 [5], and is predicted to exceed 1 million by 2025 [6]. In animal reproduction, many studies demonstrated the toxicity of paraquat [7]. Suggested that paraquat changes the chromatin configuration in mouse oocytes and deteriorates the embryonic development potential, by affecting the transcriptional activity of oocytes. Paraquat can transform the hypothalamic–pituitary–gonadal axis to affect the reproductive function in Japanese quails [8].

Male rats were given paraquat by oral administration for a short time, which could retard the regeneration of Leydig cells from stem/progenitor Leydig cells, decrease the production of testosterone, and block the spermatogenesis [9]. In addition, paraquat can reduce the *in vitro* fertilization outcomes from oxidative stress [10, 11]. Oral administration of paraquat caused significant reductions in sperm parameters (count, mobility and morphology), sera testosterone levels, as well as marked alterations in the testicular histoarchitecture [12, 13].

Natural plant products have been used to enhance male fertility and management of some physiological disorders [14]. This is because they are rich in many natural antioxidants like phenols, flavonoids, terpenoids, tannins and xanthons. Also, they are easily accessible, cheap and relatively safe [15].

Afrostyrax lepidophyllus is a plant of the Huaceae family and is commonly found in Equatorial and Tropical Africa [16, 17, 18]. This plant is used in Congo as an antiseptic and in traditional medicine for the treatment of gastroenteric diseases [19]. In the Central African Republic and in Cameroon, the seeds of this plant are traditionally used as a spice. Moreover, pharmacological studies have been carried out by several researchers and have shown that the extracts of seeds of *Afrostyrax lepidophyllus* possess very interesting properties. [20] demonstrated antifungal activity and identified Afrostyraxthioside A, Afrostyraxthioside B and Afrostyraxthioside C [21]. showed that seed extracts possess antioxidant properties [22]. Reported that the essential oil of *Afrostyrax lepidophyllus* seeds reduces free radicals. Total pholyphenols, flavonoids, tannins and anthocyanins were also quantified in the work of [18]. The IC50 (inhibitory concentration which reduces free radicals by 50%) of the antioxidant activity of their extracts are respectively of the order of 3890.5±4.3 (cyclohexane extract), 3313.8±3.5 (dicloromethane extract), 1872.8±1.0 (ethyl acetate extract) and 248.4±0.1 (methanol extract) mg/L [18]. However, the effects of *Afrostyrax lepidophyllus* seeds against paraquat-induced reproductive toxicity in male animals have not been reported so far. Hence the objective of this study was to evaluate the effects of the ethanol extract of *Afrostyrax lepidophyllus* seeds on reproductive parameters and oxidative stress in male guinea pig exposed to paraquat.

2. Materials and Methods

2.1 Animals and lodging

Sixty adult male guinea pigs (*Cavia porcellus*) aged 4 months and averagely weighing 370.31 ± 39.43 g, reared at the Teaching and Research Farm of the University of Dschang were used. The lodges were equipped with feeders and drinkers. Their floors were covered with white woodchip litter, replaced every 7 days.

2.2 Feeding, pesticide, vitamin C and plant material

During the whole trial, animals received drinking water, fresh pre-wilted forage (*Pennisetum purpureum*) and provender *ad libitum.*

The studied pesticide was paraquat, commercially named Almoxone super. It is a dark green solution containing 276 g of paraquat and 200 g of paraquat ions per liter, produced by Agro one. In guinea pigs, the oral LD50 is 20-40 mg/kg bw.

Vitamin C was obtained from commercial sources (Shalina, Nariman point, Mumbai, India. A/Em/At: Plot No. E-2, M.I.D.C. Jejuri; Tal: Purandar. Dist: Pune, Maharashtra, India).

Dried seeds of *Afrostyrax lepidophyllus* were bought at the Dschang market, they were pulped and then grinded at the mill. The obtained powder was used for extractions, using 5 liters of ethanol for 1 kilogram of powder. The filtrate was dried in the rotary evaporator at 70 °C to obtain ethanol extract of *Afrostyrax lepidophyllus* seeds.

2.3 Experimental design

The animals were randomly distributed into 6 groups (G1, G2, G3, G4, G5 and G6) of 10 animals each, comparable in body weight. During 115 days, animals of G1 were given distilled water orally (1 ml/kg bw), while the other groups (G2-G6) received 4 mg/kg of body weight (bw) of paraquat. In addition, G3 received 100 mg kg^{-1} of vitamin C; while G4, G5 and G6 received respectively 50, 100 and 200 mg/kg bw of ethanol extract of *Afrostyrax lepidophyllus* seeds, dissolved in 1 ml of distilled water. The animal's body weight was recorded weekly and the doses of pesticide and ethanol extract adjusted accordingly.

2.4 Studied parameters and data collection

2.4.1 Serum testosterone and total cholesterol concentrations

Blood was collected by cardiac puncture and put into test tubes free from anticoagulant. 8 hours later, serum was collected and distributed in labeled microtubes, then stored at -20°C for the dosage. The serum concentrations of total cholesterol and testosterone were determined using CHRONOLAB kit (Barcelona, Spain) and Omega Diagnostic ELISA kit (Scotland, England) respectively.

2.4.2 Reaction time and percentage of males reacting in the presence of a female

A week prior to the breeding, a female was presented to each of the experimental males. The stopwatch was started as soon as the male and female were placed together and stopped when a reaction from the male was observed (attempt to mount). The time recorded was considered as the reaction time. The maximum waiting time was 5 minutes. The proportion of males that reacted in the presence of a female was then calculated in each group, using the following formula:

$$\% \text{ of reaction} = \frac{\text{Number of males having reacted}}{\text{Total number of males presented to the females}} \times 100$$

2.4.3 Sexual organs weight and sperm characteristics

At the end of the trial, animals were anesthetised using ether vapour and then dissected. The testes, epididymides, vas deferens, and sex accessory glands were removed, freed from fat and weighed using a scale of capacity 160 g and precision 10^{-3}g. The cauda epididymides were then minced in 5 ml of 0.9% NaCl solution (at 37 °C) for sperm count, mobility, plasma membrane integrity and morphology evaluation. A drop of the obtained solution was placed on a slide and observed under the microscope, and a mobility score was attributed according to [23], using a scale from 0 to 5. The sperm count was done using the Thoma haemocytometer, while sperm morphological abnormalities (small and big heads, coiled tails) and the integrity of the plasma membrane were evaluated using an eosin-nigrosin solution and the hypo-osmotic test [24] respectively.

2.4.4 Oxidative stress indicators

A 15% (W/V) homogenate was prepared using a testis of each animal. Thus, a testis was crushed in cold 0.9% NaCl followed by a centrifugation (3000 rpm, 30 min) and the supernatant was used for biochemical analyses. The determination of malondialdehyde concentration was done by the thiobarbituric acid method [25], while the superoxide dismustase activity was evaluated according to [26]. The catalase (CAT) activity was assessed using the chromic acetate method as described by [27] and the total peroxydases (POX) activity was determined by the potassium iodate method [28].

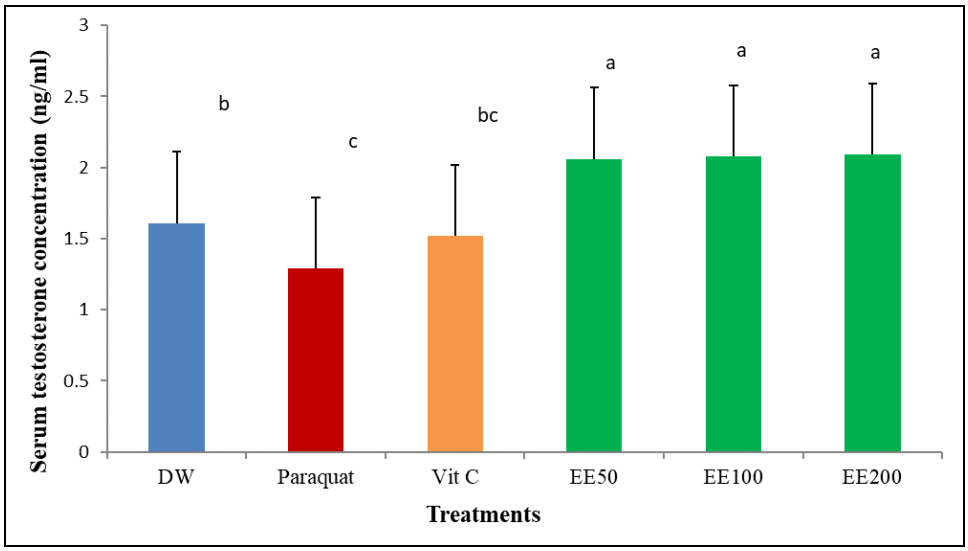
2.5 Statistical analysis

Results were expressed as mean ± standard deviation. Differences among groups were assessed using one way ANOVA, followed by the Duncan's test at 5% significance. All the analyses were performed using the SPSS 20.0 software.

3 Results

3.1 Blood Testosterone Concentration

The serum testosterone concentration decreased significantly ($p<0.05$) in guinea pigs treated with paraquat only compared to the control receiving distilled water (Figure 1). The co-administration of vitamin C to paraquat exposed animals did not significantly ($p>0.05$) repair the serum testosterone concentration. But, the ethanol extract of *Afrostyrax lepidophyllus* at all doses let to a significant ($p<0.05$) increase in testosterone concentration, with reference to distilled water, paraquat and paraquat+vitamin C groups (Figure 1).

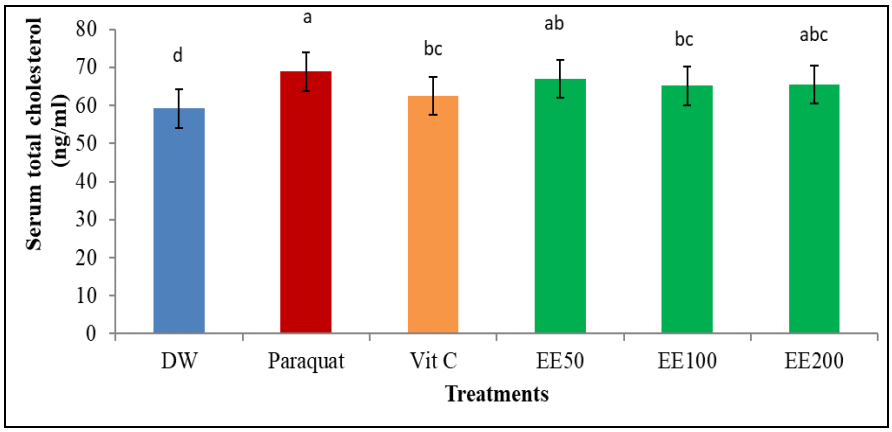


DW: distilled water, Vit C: vitamine C, EE50, EE100, EE200: ethanolic extract of *Afrostyrax lepidophyllus* at doses 50, 100 and 200 mg/kg of body weight. a, b, c: histograms values with the same letters are not significantly ($P>0.05$) different.

Fig 1: Effects of the ethanol extract of *Afrostyrax lepidophyllus* seeds on the serum content of testosterone in male guinea pig exposed to paraquat

3.2 Serum total cholesterol

The serum total cholesterol concentration significantly ($p<0.05$) increased in animals treated with paraquat only compared to distilled water-treated animals (Figure 2). Only animals treated with paraquat and vitamin C or 100 mg/kg bw of ethanol extract of *Afrostyrax lepidophyllus* showed a significant ($p<0.05$) decrease of total cholesterol concentration, though still significantly ($p<0.05$) higher when referring to distilled water-treated animals (Figure 2)



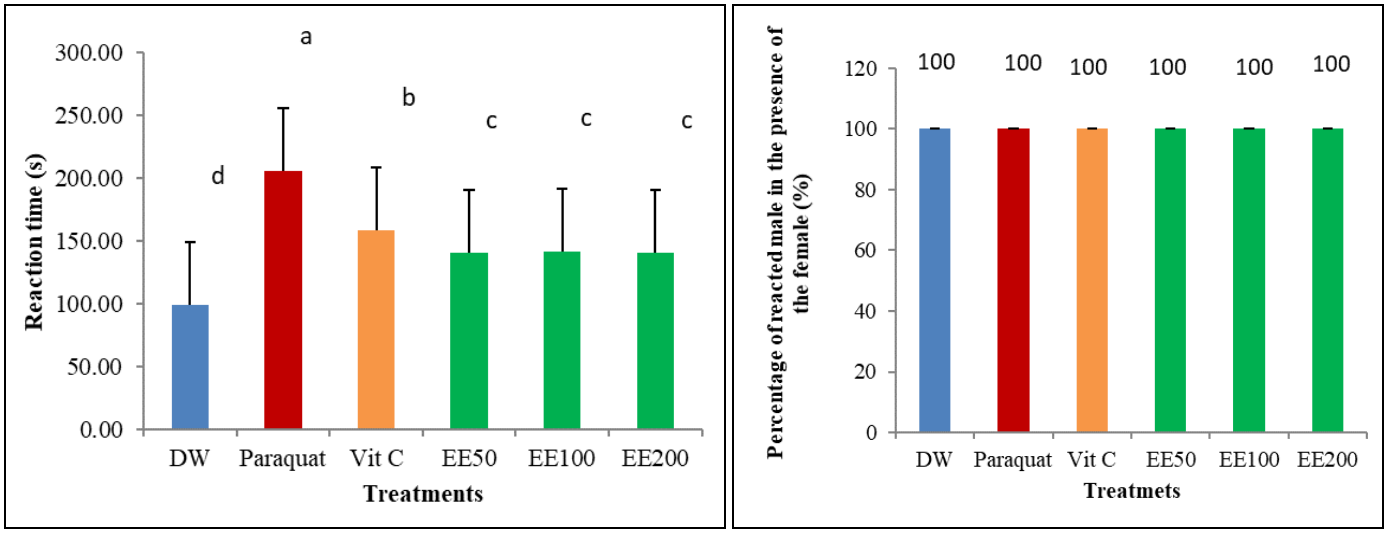
DW: distilled water, Vit C: vitamine C, EE50, EE100, EE200: ethanolic extract of *Afrostyrax lepidophyllus* at doses 50, 100 and 200 mg/kg of body weight. a, b, c, d: histograms values with the same letters are not significantly ($p>0.05$) different.

Fig 2: Effects of the ethanol extract of *Afrostyrax lepidophyllus* seeds on the serum total cholesterol concentration in male guinea pig exposed to paraquat

3.3 Reaction time and percentage of males reacting in the presence of a female

The reaction time increased significantly ($p<0.05$) in paraquat treated animals compared to the control receiving distilled water. The administration of vitamin C or ethanol extract of *Afrostyrax lepidophyllus* at all doses, to guinea pigs exposed to paraquat induced a significant ($p<0.05$) in the reaction time, yet not at the level of control animals receiving distilled water(Figure 3A).

All the treatments were comparable ($p>0.05$) for the percentages of reaction in the presence of female (Figure 3B).



(A) (B)

DW: distilled water, Vit C: vitamine C, EE50, EE100, EE200: ethanolic extract of *Afrostyrax lepidophyllus* at doses 50, 100 and 200 mg/kg of body weight. a, b, c, d: histograms values with the same letters are not significantly ($p>0.05$) different.

Fig 3: Effects of the ethanol extract of *Afrostyrax lepidophyllus* seeds on the reaction time (A) and percentage of males reacting in the presence of the female (B) in male guinea pig exposed to paraquat

3.4 Sexual organs weight

Table 1 presents the effects of ethanol extract of *Afrostyrax lepidophyllus* seeds on the relative weights of genital organs in male guinea pigs exposed to paraquat. Globally, treatments were comparable ($p>0.05$) for the weights of sexual organs.

Table 1: Effects of the ethanol extract of *Afrostyrax lepidophyllus* seeds on the relative weight of genital organs in male guinea pig exposed to paraquat.

Relative weights of genital organs (g/100 g bw)	Controls and Ethanol extracts of *Afrostyrax lepidophyllus* seeds						
	Controls			Ethanol extracts (mg/kg bw)			
	DW (n = 8)	Paraquat (n= 8)	Vit C (n = 8)	50 (n = 8)	100 (n= 8)	200 (n = 8)	p
Testis	0.38± 0.11	0.38± 0.08	0.44± 0.08	0.39± 0.10	0.55± 0.09	0.44± 0.08	0.263
Epididymis	0.09± 0.04	0.09± 0.01	0.09± 0.03	0.10± 0.02	0.09± 0.01	0.09± 0.03	0.809
Seminal vesicle	0.27 ± 0.09^{ab}	0.29 ± 0.11^{ab}	0.18 ± 0.07^{b}	0.22 ± 0.09^{ab}	$0.38\pm .10^{a}$	0.21 ± 0.09^{ab}	0.048
Bulbo uretral gland	0.02± 0.01	0.0 3± 0.01	0.03± 0.01	0.03± 0.01	0.03± 0.01	0.02 ± 0.01	0.619
Vas deferens	0.05± 0.01	0.05 ± 0.01	0.04± 0.02	0.04± 0,02	0.04± 0.02	0.04 ± 0.02	0.737
Prostate	0.13± 0.07	0.13 ± 0.08	0.13± 0.07	0.13± 0.06	0.15± 0.03	0.14 ± 0.03	0.969

a, b, c: within the same line, values with the same letters are not significantly ($p>0.05$) different. n: Number of observations. bw: body weight. DW: control group receiving 1 mL/kg bw of distilled water. Paraquat: control group receiving 4 mg/kg bw of paraquat. Vit C: control group receiving 4 mg/kg bw of paraquat and 100 mg/kg of Vitamin C. 50, 100, 200: doses of ethanol extracts of *Afrostyrax lepidophyllus*. p: probability.

3.5 Characteristics of cauda epididymis sperm

The mobility of sperm, the sperm number/tails of epididymis, the sperm number/g of epididymal tails and the percentage of spermatozoa with integrated plasma membrane decreased significantly ($p<0.05$) in guinea pigs exposed to paraquat only compared to the control receiving distilled water. These same characteristics increased significantly ($p<0.05$) in guinea pigs submitted to paraquat and treated with ethanol extract of *Afrostyrax lepidophyllus* seeds or vitamin C referring to males receiving paraquat only.

The percentage of major spermatozoa anomalies (big and small head) increased insignificantly ($p>0.05$) in guinea pigs exposed to paraquat only, compared to distilled water-treated animals. The administration of vitamin C and ethanol extract of *Afrostyrax lepidophyllus* seeds led to a decrease in this characteristic, but the difference was significant ($p<0.05$) only with male receiving 100 mg kg^{-1} bw of ethanol extract of *Afrostyrax lepidophyllus* seeds. The percentage of minor anomalies (spermatozoa with coiled and double tails) increased significantly ($p<0.05$) in paraquat control compared to the control receiving distilled water.

The administration of vitamin C or 50 mg/kg bw of extract to guinea pigs exposed to paraquat induced a significant ($p<0.05$) in this characteristic with reference to animals given paraquat only.

Table 2: Effects of the ethanol extract of *Afrostyrax lepidophyllus* seeds on epididymal sperm characteristics in male guinea pig exposed to paraquat

Epididymals sperm Characteristics	Controls and Ethanolics extracts of *Afrostyrax lepidophyllus* seeds						p
	Controls			Ethanolic extracts (mg/kg bw)			
	DW (n = 8)	Paraquat (n= 8)	Vit C (n = 8)	50 (n = 8)	100 (n= 8)	200 (n = 8)	
Mobility (%)	91.11±6.01^{a}	65.56±7.27^{b}	90.00±7.39^{a}	88.18±7.51^{a}	90.00±8.17^{a}	89.00±5.68^{a}	0.000
Number/tails of epididymis (x10^{7})	13.57±2.13^{a}	9.04±2.34^{b}	9.94±2.99^{b}	12.50±1.64^{a}	12.13±1.86^{a}	11.96±1.29^{a}	0.000
Number/g of epididymis tails (x10^{7})	49.95± 8.52^{a}	33.81± 5.51^{d}	38.17± 8.25^{cd}	48.28± 8.40^{ab}	46.60± 3.26^{ab}	43.00± 6.23^{bc}	0.000
Spermatozoa with IPM (%)	92.81± 1.67^{a}	85.76± 3.51^{b}	93.69± 1.27^{a}	91.37± 3.91^{a}	91.57± 2.41^{a}	92.12± 3.15^{a}	0.000
Major anomalies (%)	0.83± 0.35^{ab}	1.17±0.56^{a}	0.93± 0.41^{ab}	0.96± 0.24^{ab}	0.71± 0.44^{b}	1.09±0.45^{ab}	0.048
Minor anomalies (%)	0.83± 035^{bc}	1.22± 0.50^{a}	0.53± 0.38^{d}	0.66± 0.30^{cd}	1.15± 0.26^{ab}	0.90± 0.21^{ab}	0.000

a, b, c: within the same line, values with the same letters are not significantly (p>0.05) different. n: Number of observations. bw: body weight. DW: control group receiving 1 mL/kg bw of distilled water. paraquat: control group receiving 4 mg/kg bw of paraquat. Vit C: control group receiving 4 mg/kg bw of paraquat and 100 mg/kg of Vitamin C. 50, 100, 200: doses of ethanol extracts of *Afrostyrax lepidophyllus*. p: probability.

3.6 Breeding performance

Table 3 presents the effect of ethanol extract of *Afrostyrax lepidophyllus* (p>0.05) seeds on breeding performance in male guinea pigs exposed to paraquat. Globally, treatments were comparable (p>0.05) for the breeding performance.

Table 3: Effects of the ethanol extract of *Afrostyrax lepidophyllus* seeds on breeding performances in male guinea pig exposed to paraquat

Breeding performances	Controls and ethanolics extracts of *Afrostyrax lepidophyllus* seeds						p
	Controls			Ethanolic extract (mg/kg bw)			
	DW (n = 8)	Paraquat (n= 8)	Vit C (n = 8)	50 (n = 8)	100 (n= 8)	200 (n = 8)	
Litter size at parturition	1.67±1.16	1.00±0.00	1.33±0.58	1.00±0.00	1.60±0.55	1.33±0.58	0.694
Kid weight (g)	71.33±3.22	56.50±3.54	70.67±1.16	63.00±7.00	58.20±12.07	60.33±11.59	0.229
Kid viability rate (%)	100.00± 0.00	100.00± 0.00	66.67± 57.74	100.00± 0.00	80.00± 44.72	100.00± 0 00	0.720
Fertility rate (%)	75.00± 50.00	66.67±57.74	75.00± 50.00	60.00± 54.77	83.33± 40.83	75.00±50.00	0.923

n: Number of observations. bw: body weight. DW: control group receiving 1 mL/kg bw of distilled water. paraquat: control group receiving 4 mg/kg bw of paraquat. Vit C: control group receiving 4 mg/kg bw of paraquat and 100 mg/kg of Vitamin C. 50, 100, 200: doses of ethanol extracts. P: probability.

3.7 Oxidative stress indicators

The testicular activities of superoxide dismutase (SOD), catalase (CAT) and total peroxydases (POX) and concentration of malondialdehyde (MDA) (Table 4) decreased significantly (p<0.05) in guinea pigs exposed only to paraquat compared to the control receiving distilled water. These same indicators decreased significantly (p<0.05) in animals submitted to paraquat and treated with vitamin C or ethanol extract of *Afrostyrax lepidophyllus* seeds referring to those submitted to paraquat only.

The concentration of testicular total proteins (TTP) was comparable (p>0.05) among the different treatments.

Table 4: Effects of the ethanol extract of *Afrostyrax lepidophyllus* seeds on oxidatives stress biomarkers in male guinea pig exposed to paraquat

Oxidatives stress indicators	Controls and ethanolics extracts of *Afrostyrax lepidophyllus* seeds						p
	Controls			Ethanolic extracts (mg/kg bw)			
	DW (n=8)	Paraquat (n=8)	VitC (n=8)	50 (n=8)	100 (n=8)	200 (n=8)	
SOD (UI/mg)	0.30 ±0.04^{b}	0.39 ±0.05^{a}	0.31 ±0.06^{b}	0.32 ±0.05^{b}	0.31 ±0.07^{b}	0.29 ±0.07^{b}	0.009
Catalase (UI/mg)	1.24± 0.19^{c}	2.05 ±0.45^{a}	1.47 ±0.27^{bc}	1,60 ±0.29^{b}	1.58 ±0.36^{b}	1.44 ±0.24^{bc}	0.000
MDA (nM/mg)	1.48± 0.17^{c}	2.22 ±0.26^{a}	1.76 ±0.25^{b}	1.79 ±0.36^{b}	1.56 ±0.21^{bc}	1.72 ±0.22^{b}	0.000
POX (µM/mg)	32.52 ±5.20^{bc}	39.06 ±4.05^{a}	35.03 ±3.99^{b}	34.09 ±4.49^{bc}	33.31 ±4.98^{bc}	30.52 ±3.65^{c}	0.002
TTP (mg/ml)	2.20 ±0.17	2.13 ±0.12	2.29 ±0.23	2.31 ±0.25	2.17 ±0.17	2.29 ±0.34	0.418

a, b, c, d: within the same line, values with the same letters are not significantly (p>0.05) different. n: Number of observations. bw: body weight. DW: control group receiving 1 mL/kg bw of distilled water. Paraquat: control group receiving 4 mg/kg bw of paraquat. Vit C: control group receiving 4 mg/kg bw of paraquat and 100 mg/kg of Vitamin C. 50, 100, 200: doses of ethanol extracts. P: probability.

4. Discussion

Exposure of animals to pesticides is detrimental to their reproductive potential [29, 30, 7, 13]. Results of this study showed that the serum testosterone concentration increased significantly in guinea pigs exposed to paraquat and treated with ethanol extract of *Afrostyrax lepidophyllus* compared to the control submitted to paraquat only. The higher testosterone levels and decrease reaction time (libido) observed in groups that received a co-administration of paraquat and different doses of ethanol extract of *Afrostyrax lepidophyllus* seed might be as a result of the photochemical present in *Afrostyrax lepidophyllus* seed extract especially pholyphenols, flavonoids, tannins, glucosids, saponins, steroids and anthocyanins [31, 17, 18]. This result is in agreement with the work of [32] in male albino wistar rats which received the co-administration of paraquat (20 mg/kg of body weight) and ethanol extract of *Afromomum melegueta* at 200 and 400 mg/kg of body weight for four weeks. The cholesterol being the precursor of testosterone, its increase in animals treated with ethanol extract might have resulted in an increase in testosterone and consequently that of libido. The lower testosterone levels observed in paraquat group might be as a result of the deleterious effect of paraquat which produces volumes of free radicals [12] that react with macromolecules causing damages to various organs including the testis and also inhibits biochemical activities such as enzymes and hormones synthesis [33, 9].

The characteristics of epididymal sperm such as mobility, count, viability of sperm and morphology increased in guinea pigs submitted to paraquat and treated ethanol extracts of *Afrostyrax lepidophyllus*. This result is in agreement with the study of [34] in guinea pigs orally exposed to cypermethrin (137.5 mg/kg of body weight) and treated with ethanol extract of *Bersama engleriana* at 100 and 200 mg/kg bw and [30] in guinea pigs orally exposed to acetamiprid at 80 mg/kg bw and treated with extract of *Mangifera indica* for 90 days. This could be due not only to the antioxidant compounds present in the *Afrostyrax lepidophyllus* seeds (polyphenols, flavonoids and tannins), but also to the androgenic properties of molecules such as steroids and saponins. In fact, androgens have been reported to be important modulators of male sexual behavior including libido and spermatogenesis [35]. Also, the antioxidant molecules might have protected the structure and function of the testes leading to the increase of androgens concentration and thus their effects on the spermatogenesis. The administration of *Afrostyrax lepidophyllus* seeds markedly protected against the adverse effects of paraquat by normalizing the sperm count, mobility and plasma membrane integrity and decreasing the percentage of minor and major anomalies of spermatozoa. The decrease of its concentration might explain the decrease of breeding performances such as litter size at parturition, kid weight and fertility rate [29, 7].

The decrease in testicular concentration of malondialdehyde and activities of superoxide dismutase, catalase and total peroxydases in guinea pigs exposed to paraquat and treated with ethanol extract of *Afrostyrax lepidophyllus* seeds in the present study is similar to the observations of [36] in mice exposed to 13.8 mg/kg bw of cypermethrin and 150 or 300 mg/kg bw of *Cedrelopsis grevei*, [37] on Japanese quail orally exposed to 75 mg/kg bw of Antouka Super (insectide) and treated with 100 or 200 mg/kg bw of *Persea Americana* for 60 days and the work of [38] in adult mice co-administratered 5 mg/kg of paraquat and 200 mg/kg bw of crocin (CCN) by intraperitoneal route for 35 days. This might be as a result of the action of antioxidant compounds contained in the ethanolic extract of *Afrostyrax lepidophyllus* seeds. The antioxidant molecules could have neutralized free radicals or inhibited enzymes responsible of their production such as aldose reductase, lipoxygenase and phospholipase [39] resulting in cytoprotection against paraquat induced oxidative stress. Indeed, phenolic compounds found in *Afrostyrax lepidophyllus* directly contribute to antioxidative actions as they are regarded to be the most important antioxidative components [40, 41, 43]. Such molecules and their actions can prevent the cell membrane lipid peroxidation, thereby reducing the MDA concentration and antioxidant enzymes (SOD, CAT and peroxidases activities [42].

5. Conclusion

Results from this study showed that ethanolic extract of *Afrostyrax lepidophyllus* seeds can alleviated the toxic effects of paraquat on the reproductive system and oxidative stress inmale guinea pigs. This plant can be a source of bioactive compounds with anti-toxicity potential and against oxidative stress exposure.

6. Ethical Approval

Experimental protocols used in this study were strictly conformed with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986.

Competing Interests

Authors have declared that no competing interests exist

7. References

1. Dinis-Oliveira RJ, Duarte JA, Sanchez-Navarro A. Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. Critical Reviews in Toxicology 2008;38:13-71.
2. Nuber S, Tadros D, Fields J. Environmental neurotoxic challenge of conditional alpha-synuclein transgenic mice predicts a dopaminergic olfactory-striatal interplay in early PD. *Acta Neuropathologica* 2014;127:477-94.
3. Gawarammana IB, Buckley NA. Medical management of paraquat ingestion. British Journal of Clinical Pharmacology 2011;72:745-57.
4. Kumar M, Padula MP, Davey P, Pernice M, Jiang Z, Sablok G *et al*. Proteome Analysis reveals extensive light stress-response reprogramming in the seagrass *Zostera muelleri* (Alismatales. zosteraceae) metabolism. Front. Plant Sci 2016;7:2023. doi: 10.3389/fpls.2016.02023
5. Nunes ME, Muller TE, Braga MM, Fontana BD, Quadros VA, Marins A *et al*. Chronic treatment with paraquat induces brain injury, changes in antioxidant defenses system, and modulates behavioral functions in Zebrafish. *Mol. Neurobiol* 2017;54:3925-3934. doi: 10.1007/s12035-016-9919-x
6. Ding YW, Zhao GJ, Li XL, Hong GL, Li MF, Qiu QM *et al*. SIRT1 exerts protective effects against paraquat-induced injury in mouse type II alveolar epithelial cells by deacetylating NRF2 *in vitro*. Int. J. Mol. Med 2016;37:1049-1058. doi: 10.3892/ijmm.2503
7. Sun Y-L, Wang X-L, Yang L-L, Ge Z-J, Zhao Y, Luo S-M, *et al*. Paraquat Reduces the Female Fertility by Impairing the Oocyte Maturation in Mice. Front. Cell Dev. Biol 2021. | https://doi.org/10.3389/fcell.2020.631104
8. Soni R, Haldar C, Chaturvedi CM. Paraquat induced impaired reproductive function and modulation of retinal and extra-retinal photoreceptors in Japanese quail (*Coturnix coturnix* japonica). *Comp.* Biochem. Physiol. C Toxicol. Pharmacol 2019;224:108568. doi: 10.1016/j.cbpc.2019.108568
9. Li H, Zhu Q, Wang S, Huang T, Li X, Ni C, *et al*. Paraquat exposure delays stem/progenitor Leydig cell regeneration in the adult rat testis. Chemosphere 231:60-71. doi: 10.1016/j.chemosphere.2019.05.104
10. Pang YW, Jiang XL, Wang YC, Wang YY, Hao HS, Zhao SJ, *et al*. Melatonin protects against paraquat-induced damage during *in vitro* maturation of bovine oocytes. J. Pineal Res 2019;66:e12532. doi: 10.1111/jpi.12532
11. Kamali FS, Shahrooz R, Najafi GHR, Razi M. Ameliorative effect of crocin on sperm parameters and *in vitro* fertilization in mice under oxidative stress induced by paraquat. Intern. J Fertil. Steril 2020;13:307-314. doi: 10.22074/ijfs.2020.5617
12. Boufack JL, Guiekep NAJ, Nantia AE, Vemo BN, Kenfack A, Menkem B, *et al*. Reproductive Toxicity of Paraquat in Male Guinea Pig (Cavia porcellus). AJRAVS 2021;7(2):15-24. Article no. AJRAVS.65383.
13. Ofoego UC, Eze ED, Nweke EO, Obiesie IJ, Mbagwu IS, Okafor EC, *et al*. AlliumCepa (Onion) Extract Enhances and Protects Testicular Function and Architecture against Paraquat Induced Oxidative Damage. Int. J. Life Sci.

Pharma Res 2021.11(1):194-203. http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.1.L194-203
14. Nantia AE, Moundipa FP, Monsees KT, *et al*. Medicinal plants as potential male anti infertility agents: A review. Basic Clin Androl 2009;19(3):148-158.
15. Sharif MDM, Banik GR. Status and utilization of medicinal plants in Rangamati of Bangladesh. Res J Agric Biol Sci 2006;2(6):268-273.
16. Cronquist A. An Integrated System of Classification of Flowering Plants; Columbia University Press: New York 1981, 396-397.
17. Moukette BM, Constant AP, Prosper CNB, Vicky JAM, Eustace B, Jeanne YN. Afrostyrax lepidophyllus extracts exhibit *in vitro* free radical scavenging, antioxidant potential and protective properties against liver enzymes ion mediated oxidative damage. BMC Res Notes 2015. https ://doi.org/10.1186/s1310 4-015-1304-8
18. Namkona AF, Bolevane OSF, Moustapha F, Worowounga X, Ngaissona P, Koane JN, *et al*. Biological activities and phytochemical analysis of extracts Afrostyrax lepidophyllus Mildbr. seeds. The Journal of Phytopharmacology 2017;6(2):102-106. www.phytopharmajournal.com
19. Bouquet A. Féticheurs et médecines traditionnelles du Congo (Brazzaville); O.R.S.T.O.M: Paris 1969, 234-235.
20. Ngono NA. Ph.D. Thesis, Université de Reims Champagne-Ardenne, n_212. Contribution a` l'étude des propriétés antifongiques et analyse phytochimique de cinq plantes médicinales camerounaises 1999.
21. Agbor GA, Oben JE, Ngogang JY, Xinxing C, Vinson JA. Antioxidant Capacity of Some Herbs/Spices from Cameroon: A Comparative Study of Two Methods. J Agric. Food Chem 2005;53:6819-6824.
22. Fogang HPD, Maggi F, Tapondjou LA, Womeni HM, Papa F, Quassinti L, *et al*. *In vitro* Biological Activities of Seed Essential Oils from the Cameroonian Spices Afrostyrax lepidophyllus Mildbr. and Scorodophloeus zenkeri Harms Rich in Sulfur-Containing Compounds. Chemistry & Biodiversity 2014;11(1):161-169.
23. Baril G, Chemineau P, Cognie Y, Guerin Y, Leboeuf B, Orgeur P, *et al*. Manuel de formation pour l'insémination artificielle chez les ovins et les caprins. FAO. Station de la physiologie de la reproduction institut national de la recherche agronomique (INRA) 1993;83:125.
24. Kumi-Diaka J. Subjecting canine semen to the hypoosmotic test. Theriogenology 1993;39(6):1279-1289.
25. Nilsson UA, Olsson LI, Carlin G, Bylund-Fellenius AC. Inhibition of lipid peroxidation by spin labels. The Journal of Biological Chemistry 1989;264:11131-11135.
26. Misra HP, Fridovich I. The generation of superoxide radical during the autoxidation of hemoglobin. The Journal of Biological Chemistry 1972.247:6960-6962.
27. Sinha AK. Colorimetric assay of catalase. Analytical Biochemistry 1972;47:389-394. DOI: http://dx. org/10.1016/0003-2697(72)90132-7.
28. Kodjio N, Atsafack SS, Njateng GSS, Sokoudjou JB, Kuiate J-R, Gatsing G. Antioxidant effect of aqueous extract of Curcuma longa rhizomes (Zingiberaceae) in the typhoid fever induced in Wistar rats model. Journal of Advances in Medical and Pharmaceutical Sciences 2016;7(3):1-13.
29. Kenfack A, Nantia AE, Ngoula F, Guiekep NAJ, Kamtchouing P, Tchoumboue J. Post Exposure Effects of Propoxur (Agricultural Pesticide) on Male Fertility in Wistar Rat. Iranian Journal of Toxicology 2018;12(3).
30. Guiekep NAJ, Kenfack A, Ngoula F, Vemo BN, Nguemmeugne KS, Tedonkeng PE. Attenuating effects of Mangifera indica leaves ethanolic extract against acetamiprid induced reproductive toxicity in male guinea pigs. Journal Homepage: vrf.iranjournals.ir. Veterinary Research Forum 2019;10(3):187-192. Doi: 10.30466/vrf.2019.95154.2292.
31. Ngane AN, Marie L, Seraphin D, Landreau A, Richomme P. Three 1-thio-β-d-glucopyranosides from the seeds of *Afrostyrax lepidophyllus* Mildbr. Carbohydr Res 2003;341(17):2799-2802.
32. Ofoego UC, Ekwujuru EU, Ireka MI, Ofoego AN. Ameliorative effect of *Aframomum melegueta* (alligator pepper) against paraquat induced testicular damage. World Journal of Pharmaceutical Research 2020;9(5):2105-2124. DOI: 10.20959/wjpr20205-17442
33. Ofoego UC, Ekwujuru EU, Nwakamma A, Mbagwu IS, Anibeze CIP. "Protective and ameliorating effects of Methanolic seed extract of *Mucuna pruriens* on paraquat induced testicular damage. Advances in Life Science and Technology 2018;63:8-16.
34. Vemo BN, Kenfack A, Ngoula F, Akono NE, Ngaleu NCC, Guiekep NAJ *et al*. Toxicity and reproductive parameters impairment of cypermethrin in male Guinea pig (*Cavia porcellus*). Turkish Journal of Agriculture-Food Science and Technology 2018;6(2):130-135.
35. Ahangarpour A, Oroojan AA, Heydari H. Effect of hydro-alcoholic extract of *Dorema aucheri* on serum levels of testosterone, FSH and sperm count in nicotinamide STZ-induced diabetic rat models. J Adv Med Biomed Res 2013;21(87):22-31.
36. Mossa AH, Heikal TM, Belaiba M, Raoelison EG, Ferhout H, Bouajila J. Antioxidant activity and hepatoprotective potential of *Cedrelopsis grevei* on cypermethrin induced oxidative stress and liver damage in male mice. BMC, Complementary and Alternative Medicine 2015;15(251):1-10. DOI: 10.1186/s12906-015-0740-2.
37. Ngoumtsop VH, Ngoula F, Ngouateu KOB, Kenfack A, Nguemmogne TG, Mutwedu V *et al*. Oxidative Damage and reproductive toxicity associated with Antouka Super® in male Japanese quail (*Coturnix coturnix* japonica): The protective effects of hydroethanolic leaves extract of *Persea americana*. Annual Research and Review in Biology 2017;14(1):1-12. no.ARRB.34098.ISSN:2347-566X, NLMD ID: 101632869.
38. Kamali FS, Shahrooz R, Golamreza N, Mazdak R. Ameliorative effects of crocin on paraquat-induced oxidative stress in testisof adult mice: An experimental study. International Journal of Reproductive Bio Medicine. 2019;17:11, https://doi.org/10.18502/ijrm.v17i10.5490.
39. Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del-Rio JA. Uses and properties of Citrus flavonoids. Journal of Agricultural and Food Chemistry 1997;45:4505-4515. DOI: 10.1021/jf970373s.
40. Wang YP, Yang L, Chen X, Ye TT, Zhong B, Liu RJ, *et al*. Major latex protein-like protein 43 (MLP43) functions as a positive regulator during abscisic acid responses and confers drought tolerance in Arabidopsis thaliana. J Exp Botany 2016;67:421-34. Doi: 10.1093/jxb/erv477.

41. Liu ZH, Shi XY, Li S, Zhang LL, Song XY. Oxidative stress and aberrant programmed cell death are associated with pollen abortion in isonuclear alloplasmic male-sterile wheat. Front Plant Sci 2018;9:595. Doi: 10.3389/fpls.2018.00595.
42. Huang CP, Qin NN, Sun L, Yu MY, Hu WZ, Qi ZY. Selenium improves physiological parameters and alleviates oxidative stress in strawberry seedlings under low-temperature stress. Int J Mol Sci 2018;19:1913. Doi: 10.3390/ijms19071913.
43. Madkour NK. Protective effect of curcumin on oxidative stress and DNA fragmentation against lambda cyhalothrin-induced liver damage in rats. Journal of Applied Pharmaceutical Science 2012;2(12):076-081. DOI: 10.7324/JAPS.2012.21214