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Effects of ethanol extract of *Bersama engleriana* leaves on oxidative stress and reproductive parameters in male Guinea pig (*Cavia porcellus*) exposed to cypermethrin

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ABSTRACT

Pesticides are used to improve agricultural yields; meanwhile they have detrimental effects on human and animal reproduction. This study aimed at evaluating the protective effects of ethanol extract of *Bersama engleriana* leaves against cypermethrin-induced oxidative stress and reproductive toxicity. Fifty male guinea pigs were divided into 5 groups (G1, G2, G3, G4 and G5) of 10 animals each. During 90 days, animals of G1 were given distilled water orally, while other groups received 137.50 mg/kg body weight (bw) of cypermethrin. In addition, G3, G4 and G5 received respectively 50, 100 and 200 mg/kg bw of ethanol extract of *Bersama engleriana* leaves. The testicular concentration of malondialdehyde, the activities of superoxide dismutase and catalase decreased significantly ($P<0.05$) in guinea pigs exposed to cypermethrin and ethanol extract of *B. engleriana* leaves compared with those exposed to cypermethrin only (G2), while the reverse effect was observed concerning the activity of peroxidases. The time of reaction of male guinea pig in the presence of females and the percentage of abnormal spermatozoa decreased significantly ($P<0.05$) in animals treated with the insecticide and the ethanol extract of *B. engleriana* leaves with respect to G2 animals. The weight of testes, the serum level of testosterone, the sperm count, mobility and the percentage of spermatozoa with normal plasma membrane increased significantly ($P<0.05$) in guinea pigs treated with ethanol extract of *B. engleriana* leaves compared with those submitted to cypermethrin only. The histological sections of testes in animals exposed to cypermethrin and ethanol extract of *B. engleriana* leaves showed a normal structure compared with those exposed to cypermethrin only, of which sections of testes revealed the presence of immature germinal cells in the seminiferous tubules lumen. Hence, ethanol extract of *B. engleriana* leaves prevent the induction of oxidative stress and reproductive parameters impairment by cypermethrin in male guinea pig.

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Keywords: *Bersama engleriana*, cypermethrin, natural antioxidants, reproductive toxicity, sperm characteristics.

INTRODUCTION

Although pesticides permit the improvement of agricultural yields, they have detrimental effects on mammals and their persistency in the environment is a serious public health concern. They are responsible of serious environmental pollutions, with fatal consequences on animal and human health (Rudant et al., 2007; Amin and Hashem, 2012). In fact, they are considered as risk factors of many diseases such as cancer, congenital malformations and infertility (Rudant et al., 2007). Pesticides damage reproduction of animals and some reported effects are the reduction in testosterone production and/or action (Bustos-Obregon and Gonzalez-Hormazabal, 2003) and the decrease in sperm count and mobility (Naravana et al., 2005; Ngoula et al., 2007; Kenfack et al., 2015; Nwozo et al., 2016). One of the mechanisms by which they perform their toxicity is the generation of high production of free radicals and then of the oxidative stress (Banerjee et al., 2001; Amin and Hashem, 2012). The oxidative stress results from the imbalance between oxidants and antioxidants (Jahanian et al., 2014; Sarr et al., 2016; Dieng et al., 2017) in the favour of the formers. The damages of the oxidative stress on the reproduction have been broadly studied by many authors (Agarwal et al., 2006; Jackson and McArdle, 2011; Jahanian et al., 2014). Sharma et al. (2014) reported that the decrease in male rats' fertility, exposed to Cypermethrin might be due to the high production of free radicals it induced. To face the oxidative stress, antioxidant molecules such as Selenium, Vitamins E and C, BHT, BHA are usually used (Yousef, 2010; Djeffal, 2014; El-dakak, 2015). But, their high costs and their availability constitute limits for their use in developing countries. Hence, medicinal plants appear as an alternative solution, since they are rich in many natural antioxidants like phenols, flavonoids, terpenoids, xanthons, etc. Also, their toxicity is very low (Vijayakumar et al., 2012; Ikpeme et al., 2014). *Bersama engleriana* is one the medicinal plants. It is found in Sub-Saharan Africa (Bosch, 2008).

Unlike many medicinal plants (pawpaw tree, guava tree, tea plant...), it is not used as food for human. It is empirically used to treat many diseases such as malaria, headache, diabetes, typhoid, haemorrhoid etc. (Bosch, 2008; Lather et al., 2010). The study of Kuete et al. (2008) showed that extracts from all parts (leaves, stem bark and roots) of this plant have antioxidant effects. Mangiferin they contain has an antioxidant activity higher than that of vitamins E and C (Martinez et al., 2000; Sanchez et al., 2000), yet considered as major antioxidants. Nevertheless, almost all these works are *in vitro* studies. Hence, *in-vivo* studies are necessary to appreciate the effects of this plant on the animal organism. The objective of this study was to evaluate the effects of the ethanol extract of *Bersama engleriana* leaves on oxidative stress and reproductive parameters in male guinea pig exposed to Cypermethrin.

MATERIALS AND METHODS

Animals, lodging, feeding, pesticide and plant material

Fifty male guinea-pigs (*Cavia porcellus*) raired at the Teaching and Research Farm of the University of Dschang were used. Their average body weight was 357.91 ± 15.18 g at the start of the assay. They were identified individually and housed in identical cages of 100 cm x 80 cm x 60 cm (length, width and height) under standard conditions with 12 h photoperiod and had free access to water and food. They were handled according to ethical guidelines of the Cameroonian National Veterinary Laboratory.

Animals were fed with elephant grass-based ration and a supplement of compounded feed. The pesticide used was cypermethrin 36% (360 g/L), commercially called Cigogne. It was obtained from Louis Dreyfus Commodities Cameroon.

Fresh leaves of *Bersama engleriana* were collected in Bagang, locality of the Bamboutos Division, West Region of Cameroon. They were dried sheltered from the sun, and then grinded at the mill. The

obtained powder was used for extractions, using 5 litres of pure ethanol for 1 kilogram of powder. The filtrate was dried in the rotary evaporator at 70 °C to obtain ethanol extract of *Bersama engleriana* leaves.

Assay

The animals were distributed into 5 groups (G1, G2, G3, G4 and G5) of 10 animals each, comparable in body weight. During 90 days, animals of G1 were given distilled water orally, while other groups received 137.50 mg/kg of body weight (bw) of cypermethrin. In addition, G3, G4 and G5 received respectively 50, 100 and 200 mg/kg bw of ethanol extract of *Bersama engleriana* leaves, dissolved in distilled water. The animal's body weight was recorded weekly and the doses of pesticide and ethanol extract adjusted accordingly.

Collection of blood and organs

Twenty four hours after the last administration of the pesticide and aqueous extract solutions, animals were anaesthetised using ether vapour. Blood was collected by cardiac puncture and used to obtain the serum. After sacrifice, organs such as the testes, epididymis, vas deferens, vesicular glands and prostate were collected.

Studied parameters and data collection

Sexual desire (libido)

The libido was expressed as the reaction time of the male in the presence of a female; one week before sacrifice, each experimental animal was housed with an adult female, and the time taken for the male to chase, sniff the ano-genital region of the female or attempt to mount was noted. The maximum observation time for any possible reaction of male in the presence of female was 5 minutes.

Sexual organs weights

The testes, epididymis, vas deferens, vesicular glands and prostate were weighed

using a scale of 160 g capacity and 10⁻³g precision.

Serum testosterone concentration

Serum content in testosterone was quantified using ELISA method, according to the instructions of Omega Diagnostics kit (Scotland, United Kingdom).

Sperm characteristics

Animal sperm mobility was evaluated by mincing epididymal tails in a petri dish containing 0.9% NaCl solution at 37 °C and the obtained preparation was observed at 400 x magnification using microscope. The sperm count was done using the Thoma haemocytometer, while sperm morphological abnormalities (small and big heads, tails winding) and the integrity of the plasma membrane were evaluated using an eosin-nigrosin solution and the hypo-osmotic test respectively.

Evaluation of the 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 (Nilsson et al., 1989), while the superoxide dismutase activity was evaluated according to Misra and Fridovich (1972). The catalase (CAT) activity was assessed using the chromic acetate method as described by Sinha (1972) and the total peroxidases (PEROX) activity was determined by the potassium iodate method (Kodjio et al., 2017).

Histopathology of the testis

The testis was fixed in Bouin's solution, and then washed, dehydrated ascending grade alcohol bath, clarified in xylene immersion, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin. The tissue sections were observed under a light microscope (400x magnifications).

Statistical analysis

Results were expressed as mean \pm standard deviation. Differences between 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.

RESULTS

Oxidative stress indicators

The testicular concentration of malondialdehyde, the activities of superoxide dismutase (SOD) and catalase (Table 1) decreased significantly ($P<0.05$) in guinea pigs exposed to Cypermethrin and treated with ethanol extract of *Bersama engleriana* leaves compared with those submitted only to Cypermethrin (T0+), and were comparable ($P>0.05$) with the control receiving distilled water (T0-). The activity of peroxidases increased significantly ($P<0.05$) in animals treated with Cypermethrin and ethanol extract of *B. engleriana* leaves with reference to T0+ group guinea pigs, and was comparable ($P>0.05$) with T0- animals.

Weights of genital organs

The weight of testes (Table 2) increased significantly ($P<0.05$) in guinea pigs exposed to Cypermethrin and treated with ethanol extract of *B. engleriana* leaves relatively to those submitted only to Cypermethrin (T0+), and was comparable ($P>0.05$) with the control T0- group. The weights of the epididymis, vas deferens, seminal vesicle and prostate were comparable ($P>0.05$) among treatments.

Reaction time (libido) and testosterone concentration

The time of reaction of male guinea pig in the presence of females (Figure 1) in animals treated with the insecticide and ethanol extract of *B. engleriana* leaves decreased significantly ($P<0.05$) compared with T0+ and T0- animals. The serum level of

testosterone (Figure 2) increased in guinea pigs treated with ethanol extract of *B. engleriana* leaves than in T0- and T0+ groups, but only males submitted to 100 or 200 mg/kg bw showed a significant ($P<0.05$) difference compared with T0+ animals.

Characteristics of caudal epididymal sperm

The mobility, the sperm count and the percentage of spermatozoa with entire plasma membrane generally increased significantly ($P<0.05$) in animals treated with Cypermethrin and ethanol extract of *B. engleriana* leaves with reference to those submitted only to Cypermethrin, and were comparable ($P>0.05$) with the control receiving distilled water (Table 3). Meanwhile, the percentages of spermatozoa with abnormal sizeof heads or with coiled tails decreased significantly ($P<0.05$) in animals exposed to Cypermethrin and treated with ethanol extract of *B. engleriana* leaves compared with T0+ group, and were comparable ($P>0.05$) with T0-.

Histopathology of the testis

The effects of cypermethrin and ethanol extract of *B. engleriana* leaves on the histological structure of testes in male guinea pig are illustrated in Figure 3. A typical structure of the testis was observed in T0-control, with the seminiferous epithelium containing all generations of germinal cells corresponding for the stages of seminiferous epithelium cycle with normal flagellated spermatozoa. In control group treated with cypermethrin (T0+), this arrangement was disrupted, with the lumen almost full of non-differentiated germinal cells. In animals treated simultaneously with cypermethrin and ethanol extract of *B. engleriana* leaves, no immature germinal cell was observed in the lumen. Also, no section of the seminiferous tube showed any abnormality.

Table 1: Effects of the ethanol extract of *Bersama engleriana* leaves on the oxidative stress indicators in male guinea pig exposed to cypermethrin.

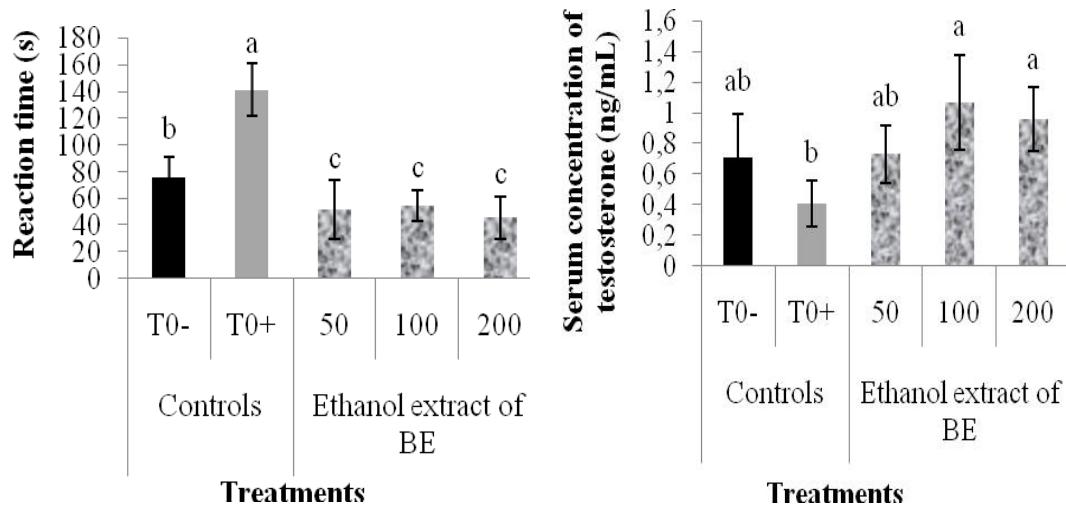
Indicators of the oxidative stress	Treatments					P	
	Controls		Ethanol extract of <i>B. engleriana</i> leaves (mg/kg bw)				
	T0- (n = 5)	T0+ (n = 5)	50 (n = 5)	100 (n = 5)	200 (n = 5)		
Malondialdehyde (nM/g of testis)	2.77±0.66 ^b	9.09±1.83 ^a	3.17±1.74 ^b	3.50±1.63 ^b	4.82±0.66 ^b	0.00	
SOD (U/g of testicular proteins)	10.52±1.89 ^b	39.09±5.43 ^a	6.29±1.44 ^b	10.49±2.61 ^b	6.62±0.95 ^b	0.00	
Catalase (μmol/min/g of testis)	37.17±1.14 ^b	44.68±6.00 ^a	38.44±2.57 ^b	37.18±0.99 ^b	37.81±1.16 ^b	0.00	
Peroxidases (μmol/min/g of testis)	1.59±0.77 ^a	0.62±0.28 ^b	1.78±0.52 ^a	1.60±0.60 ^a	2.32±0.52 ^a	0.00	

a, b: within the same line, values with the same letters are not significantly ($P>0.05$) different. n: Number of observations.
 bw: body weight. T0-: group receiving 1 mL/kg bw of distilled water. T0+: group receiving 137.50 mg/kg bw of cypermethrin.

Table 2: Effects of the ethanol extract of *Bersama engleriana* leaves on the relative weight of genital organs in male guinea pig exposed to cypermethrin.

Relative weight of genital organs (g/100 g bw)	Treatments					P	
	Controls		Ethanol extract of <i>B. engleriana</i> leaves (mg/kg bw)				
	T0- (n = 8)	T0+ (n = 8)	50 (n = 8)	100 (n = 8)	200 (n = 8)		
Testes	0.49±0.06 ^a	0.37±0.09 ^b	0.49±0.06 ^a	0.49±0.06 ^a	0.51±0.05 ^a	0.04	
Epididymis	0.10±0.01	0.08±0.02	0.10±0.02	0.09±0.02	0.10±0.01	0.62	
Vas deferens	0.04±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.85	
Seminal vesicle and prostate	0.57±0.09	0.58±0.04	0.71±0.04	0.69±0.09	0.64±0.06	0.31	

a, b: within the same line, values with the same letters are not significantly ($P>0.05$) different. n: Number of observations.
 bw: body weight. T0-: control group receiving 1 mL/kg bw of distilled water. T0+: control group receiving 137.50 mg/kg bw of cypermethrin.



a, b, c: histograms values with the same letters are not significantly ($P>0.05$) different. BE: *Bersama engleriana*.

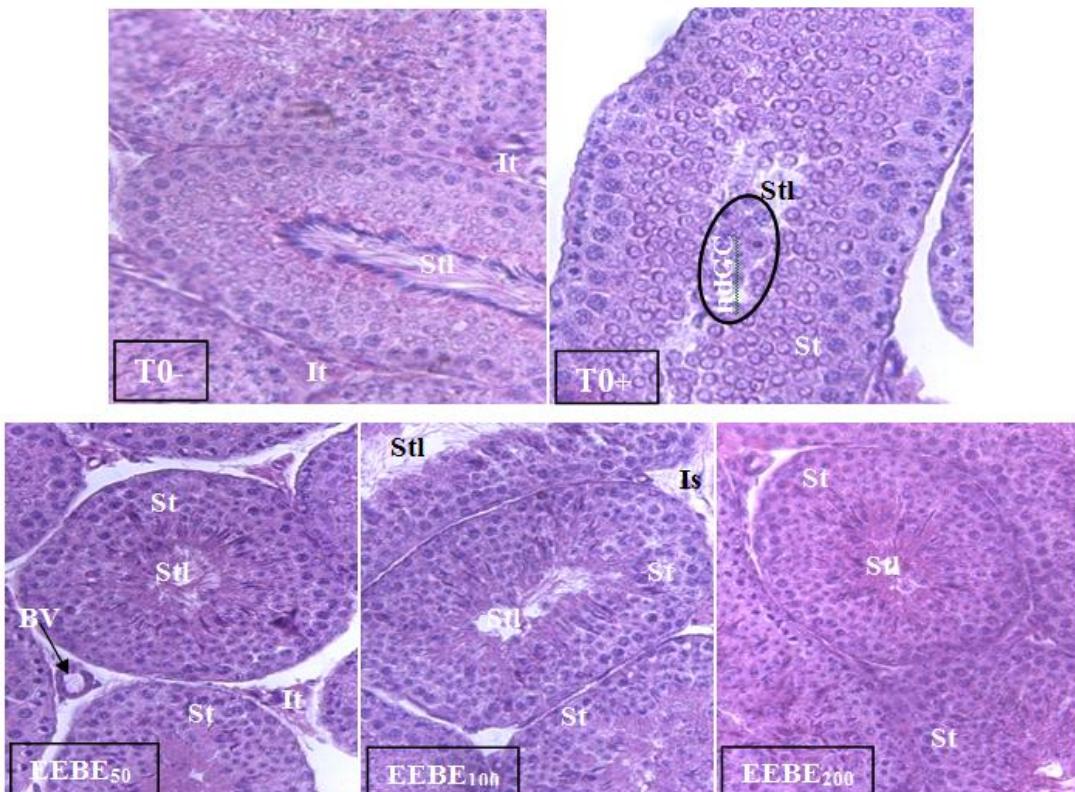
Figure 1: Effects of the ethanol extract of BE leaves on the reaction time (libido) in male guinea pig exposed to cypermethrin.

Figure 2: Effects of the ethanol extract of BE leaves on the serum content testosterone in male guinea pig exposed to cypermethrin.

Table 3: Effects of the ethanol extract of *Bersama engleriana* leaves on the caudal epididymal sperm characteristics in male guinea pig exposed to cypermethrin.

Caudal epididymal sperm characteristics	Treatments					P	
	Controls		Ethanol extract of <i>B. engleriana</i> leaves (mg/kg bw)				
	T0- (n = 6)	T0+ (n = 6)	50 (n = 6)	100 (n = 6)	200 (n = 6)		
Mobility (%)	75.00±12.25 ^{ab}	65.00±14.14 ^b	88.33±7.53 ^a	81.88±12.52 ^a	82.86±9.16 ^a	0.01	
Number/tails of epididymis (x 10 ⁶)	21.67±3.84 ^{ab}	16.41±4.88 ^b	23.08±9.74 ^{ab}	26.92±9.47 ^a	26.41±5.88 ^a	0.04	
Number/g of epididymal tails (x 10 ⁶)	52.63±12.57 ^{ab}	39.15±12.34 ^b	51.92±11.12 ^{ab}	51.84±9.52 ^{ab}	53.67±6.48 ^a	0.04	
Spermatozoa with IPM (%)	81.00±6.54 ^a	68.13±6.94 ^b	86.17±3.71 ^a	87.00±6.03 ^a	86.33±6.47 ^a	0.00	
Spermatozoa with small and big heads (%)	17.17±5.60 ^b	26.00±6.14 ^a	19.67±6.74 ^b	13.50±1.87 ^b	15.33±3.83 ^b	0.00	
Spermatozoa with coiled tails (%)	4.25±1.08 ^b	7.38±1.69 ^a	2.75±0.99 ^b	4.50±1.64 ^b	4.00±1.78 ^b	0.00	

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. IPM: integrated plasma membrane. T0-: group receiving 1 mL/kg bw of distilled water. T0+: group receiving 137.50 mg/kg bw of cypermethrin.



ndGC: non differentiated germinal cells; **Is:** interstitial space; **Stl:** seminiferous tubule lumen; **It:** interstitial tissue; **St:** seminiferous tubule; **BV:** blood vessel. **T0-**: control 1 (distilled water); **T0+**: control 2 (cypermethrin only); **EEBE₅₀**, **EEBE₁₀₀** and **EEBE₂₀₀**: cypermethrin + 50, 100 or 200 mg/kg bw of ethanol extract of *Bersama engleriana* leaves respectively.

Figure 3: Histological sections of testis in male guinea pig exposed to cypermethrin and treated with ethanol extract of *Bersama engleriana* leaves (HE x 400).

DISCUSSION

The decrease in testicular concentration of malondialdehyde and superoxide dismutase and catalase activities in guinea pigs exposed to Cypermethrin and ethanol extract of *B. engleriana* leaves in the present study is similar to the observations of Madkour (2012), in rats submitted to 0.6 mg/kg bw of lambda-cyhalothrine and 200 mg/kg bw of curcumin and the findings of Mossa et al. (2015) in mice exposed to 13.8 mg/kg bw of cypermethrin and 150 or 300 mg/kg bw of *Cedrellopsis grevei*. This result might be explained by the action of antioxidant compounds contained in the

extract. Indeed, phytochemical tests of this extract revealed the presence of compounds such as phenols, flavonoids, xanthons, terpenoids and anthaquinons. These antioxidant molecules could have neutralised free radicals by transferring protons (Hodek et al., 2002) or inhibiting enzymes responsible of their production, like aldose reductase, xanthinoxidase, lipoxygenase, phospholipase and cyclooxygenas (Benavente-Garcia et al., 1997) and then protecting cells against Cypermethrin-induced oxidative stress. Such actions could have reduced the lipids peroxidation of the plasma membrane and then the concentration of malondialdehyde

and the activity of antioxidant enzymes, superoxide dismutase and catalase. The MDA being an excellent substrate for peroxidases (Anita and Suresh, 2009; Golamreza et al., 2010; Saramma and Padmaja, 2013), the decrease of its concentration might explain the increase of peroxidases level. The increase in the testosterone concentration and libido in animals treated with the insecticide and ethanol extract of *B. engleriana* leaves in this study could be due not only to the antioxidant compounds it contains, but also to the androgenic properties of molecules such as steroids, terpenoids and saponins (Ahangarpour et al., 2013). The increase in sperm count, mobility and plasma membrane integrity and decrease of the percentage of abnormal spermatozoa in this study could be due to the increase in testosterone level and the antioxidant effects of the ethanol extract of *B. engleriana* leaves. In fact, the plasma membrane of spermatozoa is rich in polyunsaturated fatty acids; rendering them susceptible to lipid peroxidation. Then the antioxidant compounds could protect spermatozoa DNA against free radicals and improve sperm characteristics (Jedlinska et al., 2006). The presence of immature germinal cells in the seminiferous tubule lumen of guinea pigs exposed to Cypermethrin might be the consequence of an exfoliation of non-differentiated germinal cells, which could have then affected the weight of testes. According to Oguntibeju et al. (2010), antioxidant molecules have positive effects on the genital organs weights, since they protect tissues against oxidation and degeneration of free radicals. Hence, antioxidant compounds contained in the ethanol extract of *B. engleriana* leaves could have prevent the oxidation of the testes tissues in animals treated with Cypermethrin, favouring in such a way a better spermatogenesis and steroidogenesis.

Conclusion

The ethanol extract of *Bersama engleriana* leaves has protected efficiently the reproductive parameters of male guinea pigs against the Cypermethrin-induced oxidative stress. Hence, it can be used as alternative to synthetic antioxidants, on conditions of the toxicological study of this plant extract.

COMPETING INTERESTS

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. BNV, AK, NF, EAN and AT contributed substantially to the conception and the design of the study, data analysis and interpretation. NK contributed to the evaluation of oxidative stress indicators. BNV, AJNG and AMMT contributed in other data acquisition. BNV, AK AJNG and AMMT contributed in drafting the article or revising it critically for important intellectual content. All authors read and approved the final manuscript.

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