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Protective effects of the aqueous extract of *Bersama engleriana* leaves against cypermethrin-induced oxidative stress and reproductive toxicity in male guinea pig (*Cavia porcellus*)

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ABSTRACT: The present study aimed to evaluate the protective effects of aqueous extract of *Bersama engleriana* leaves against cypermethrin-induced oxidative stress and reproductive toxicity. Thus, 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 orally given 1 ml/kg of body weight (bw)/day of distilled water, while the other groups received 137.50 mg/kg bw of cypermethrin. In addition, G3, G4 and G5 received respectively 50, 100 and 200 mg/kg bw of aqueous extract of *Bersama engleriana* leaves. At the end of the treatment period, the testicular concentration of malondialdehyde and the activities of superoxide dismutase and catalase decreased significantly ($P<0.05$) in guinea pigs exposed to cypermethrin and aqueous extract of *B. engleriana* leaves compared to those exposed to cypermethrin only (G2), while the reverse was observed with 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 aqueous extract of *B. engleriana* leaves compared to G2 animals. The weight of testes, serum level of testosterone, sperm count, mobility and percentage of spermatozoa with entire plasma membrane increased significantly ($P<0.05$) in guinea pigs given extract compared to those submitted to cypermethrin only. Thus, the aqueous extract of *Bersama engleriana* leaves protected male guinea pigs against cypermethrin toxicity.

KEYWORDS: *Bersama engleriana*, cypermethin, oxidative stress, spermatozoa, toxicity.

1 INTRODUCTION

Pesticides are chemical products used in agriculture to eliminate harmful organisms, in order to increase agriculture yields (Merhi *et al.*, 2007). Although this important role, pesticides can provoke 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 include reduction in testosterone production and/or action (Bustos-Obregon and Gonzalez-Hormazabal, 2003), decrease in sperm count and mobility (Narayana *et al*., 2005; Ngoula *et al*., 2007 and Kenfack *et al*., 2015). One of the mechanisms by which they perform their toxicity is the generation of high production of free radicals and thus of the oxidative stress (Banerjee *et al*., 1999; Banerjee *et al*., 2001 and Amin and Hashem, 2012). It is reported that the decreased fertility in male rats exposed to cypermethrin might be due to the high production of free radicals it induced (Sharma *et al*., 2014). To attenuate the oxidative effects, antioxidant molecules such as selenium, vitamins E and C, BHT, BHA are commonly used Yousef, 2010; Djeffal, 2014 and El-Dakak, 2015). But their possible side-effects, their high cost and availability limit their utilization in developing countries. Hence, medicinal plants present in these countries 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 and Ikpeme *et al*., 2014). Among those plants is *Bersama engleriana*, found in Sub-Saharan Africa [16]. 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 (Bosch, 2008 and Lather *et al*., 2010). A study showed that the extracts from all parts (leaves, stem bark and roots) of this plant have antioxidant effects (Kuete *et al*., 2008). Mangiferin they contain has an antioxidant activity higher than that of vitamins E and C (Martinez *et al*., 2000 and Sanchez *et al*., 2000), yet considered as major antioxidants. Almost all these works are *in-vitro*, and then *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 protective effects of the aqueous extract of *Bersama engleriana* leaves on cypermethrin-induced oxidative stress and reproductive toxicity.

2 MATERIAL AND METHODS

2.1 ANIMALS, LODGING, FEEDING, PESTICIDE AND PLANT MATERIAL

Fifty male guinea-pigs (*Cavia porcellus*) raised at Dschang university teaching and research farm were used. Their mean weight was 357.91±15.18 g at the start of the assay. They were identified at the ear and housed in identical cages of 100 cm x 80cm x 60cm (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 provender diet.

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 and the powder obtained was used for extractions, using 6 litres of distilled water for 1 kilogram of powder. The filtrate was dried in the oven at 50 °C to obtain aqueous extract of *Bersama engleriana* leaves.

2.2 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 orally given distilled water, while the other groups received 137.50 mg/kg bw of cypermethrin. In addition, G3, G4 and G5 animals received respectively 50, 100 and 200 mg/kg bw of aqueous extract of *Bersama engleriana* leaves, dissolved in distilled water. The animal's body weight was recorded weekly and the doses of pesticide and aqueous extract adjusted accordingly.

2.3 COLLECTION OF BLOOD AND ORGANS

Twenty four hours after the last administration of the pesticide and aqueous extract solutions, animals were anesthetised using ether vapour and 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.

2.4 STUDIED PARAMETERS AND DATA COLLECTION

2.4.1 SEXUAL DESIRE (LIBIDO)

The libido was expressed as the reaction time of the male in the presence of a female; on the 90th day of the assay and 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.

2.4.2 SEXUAL ORGANS WEIGHTS

The testes, epididymis, vas deferens, vesicular glands and prostate were weighed using a scale of 160 g capacity and 10^{-3}g precision.

2.4.3 SERUM TESTOSTERONE CONCENTRATION

Testosterone was quantified in the serum using ELISA kit according to the instructions from Omega Diagnostics (Scotland, United Kingdom).

2.4.4 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 with light microscope at 400 x magnification. 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.

2.4.5 OXIDATIVE STRESS INDICATORS

A 15 % (W/V) homogenate was prepared using the right 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 dismustase activity was evaluated according to (Misra and Fridovich, 1972). The catalase (CAT) activity was assessed using the chromic acetate method as described in a previous work (Sinha, 1972) and the total peroxydases (PEROX) activity was determined by the potassium iodate method (Habbu *et al*., 2008).

2.5 STATISTICAL ANALYSIS

Results were expressed as mean ± standard deviation. Differences between groups were brought out by the use of one way ANOVA followed by the Duncan's test at 5% significance.

3 RESULTS

3.1 OXIDATIVE STRESS INDICATORS

The testicular concentration of malondialdehyde (figure 1) was significantly ($P<0.05$) lower in guinea pigs exposed to cypermethrin and treated with 100 or 200 mg/kg bw of aqueous extract of *Bersama engleriana* leaves compared to those submitted only to cypermethrin (T0+), and comparable ($P>0.05$) to the control receiving distilled water (T0-).

The activity of the superoxide dismutase (figure 2) in T0- animals and those treated with aqueous extract of *B. engleriana* leaves were comparable ($P>0.05$) to each other and significantly ($P<0.05$) lower considering T0+ animals.

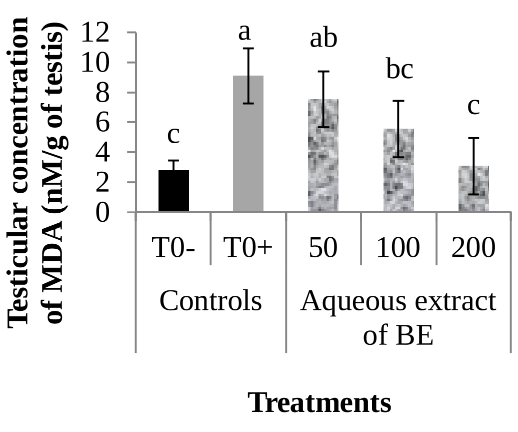

Figure 1: Effects of the aqueous extract of BE leaves on the testicular concentration of MDA in male guinea pig exposed to cypermthrin

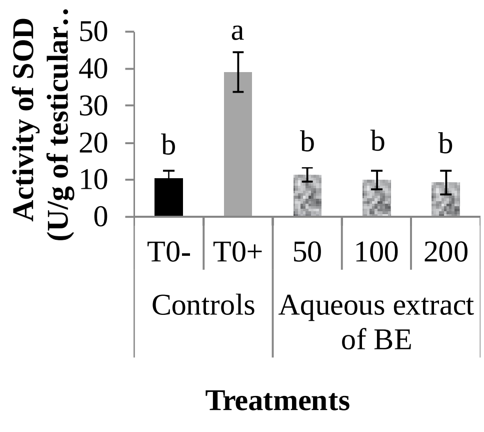

Figure 2: Effects of the aqueous extract of BE leaves on the testicular activity of SOD in male guinea pig exposed to cypermthrin

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

The testicular activity of catalase (figure 3) decreased significantly (P<0.05) in guinea pigs exposed to cypermethrin and treated with aqueous extract of *Bersama engleriana* leaves relatively to those treated only with the insecticide, but only animals receiving 200 mg/kg bw showed a value comparable (P>0.05) to that of the control receiving only distilled water.

The activity of peroxidases (figure 4) increased in T0- animals and those treated with aqueous extract of *B. engleriana* leaves with reference to T0+ animals, but the significant (P<0.05) difference was noted only with 100 mg/kg bw treated and T0- males.

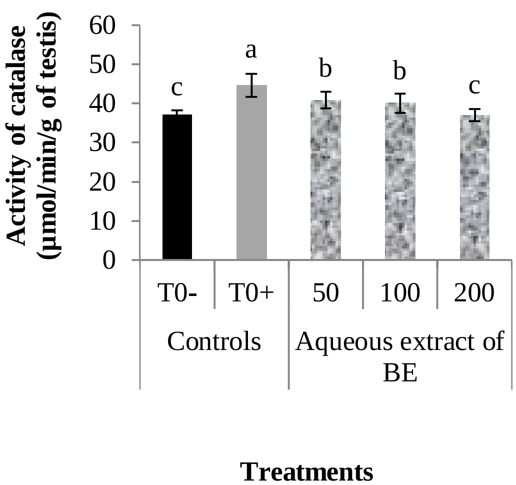

Figure 3: Effects of the aqueous extract of BE leaves on the testicular activity of CAT in male guinea pig exposed to cypermthrin

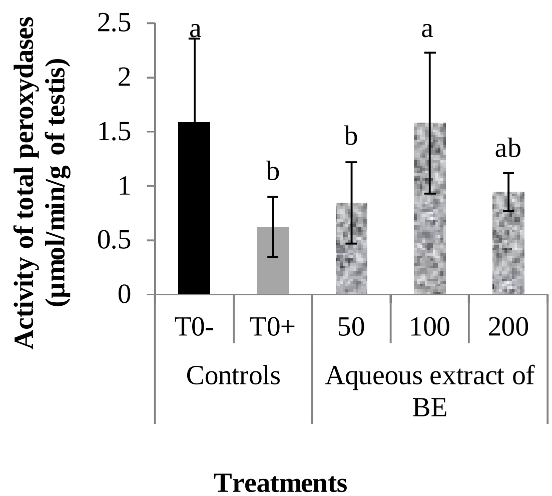

Figure 4: Effects of the aqueous extract of BE leaves on the testicular activity of peroxidases in male guinea pig exposed to cypermthrin

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

3.2 Reaction time of males in the presence of female (libido) and testosterone concentration

The time of reaction (figure 5) in T0- guinea pigs and those treated with aqueous extract of BE leaves were comparable (P>0.05) to each other and significantly (P<0.05) low with respect to T0+ animals. The serum level of testosterone (figure 6) increased in guinea pigs treated with aqueous extract of BE leaves than in T0- and T0+ groups, but only males submitted to 50 or 200 mg/kg bw showed a significant (P<0.05) difference relatively to T0+ animals.

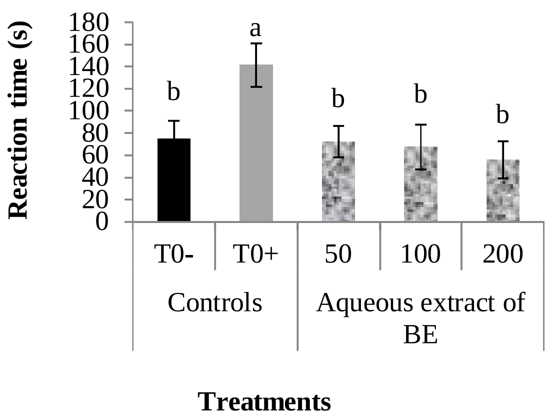

Figure 5: Effects of the aqueous extract of BE leaves on the time of reaction (libido) in male guinea pig exposed to cypermthrin

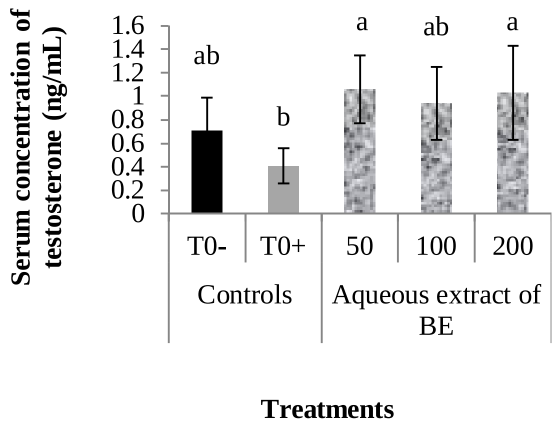

Figure 6: Effects of the aqueous extract of BE leaves on the serum testosterone concentration in male guinea pig exposed to cypermthrin

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

3.3 Weight of genital organs

The effects of the aqueous extract of BE leaves on the weight of sexual organs in guinea pig exposed to cypermthrin are presented in table 1. The weight of testes increased significantly (P<0.05) in guinea pigs exposed to cypermethrin and treated with 100 or 200 mg/kg bw of aqueous extract of *B. engleriana* leaves in relation to those submitted only to cypermethrin (T0+), and was comparable (P>0.05) to the control receiving distilled water (T0-). The weights of the epididymis, vas deferens, seminal vesicle and prostate were comparable (P>0.05) among treatments.

Table 1: Effects of the aqueous extract of Bersama engleriana leaves on the weight of genital organs in male guinea pig exposed to cypermthrin

weight of genital organs (g/100 g bw)	Treatments					P
	Controls		Aqueous extract of *B. engleriana* 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}	0.44±0.05^{ab}	0.46±0.03^{a}	0.47±0.07^{a}	0.04
Epididymis	0.10±0.01	0.08±0.02	0.09±0.01	0.10±0.01	0.10±0.02	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	0.65±0.13	0.61±0.13	0.58±0.13	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-: group receiving 1 mL/kg bw of distilled water. T0+: group receiving 137.50 mg/kg bw of cypermethrin.

3.4 Characteristics of caudal epididymal sperm

Table 2 summarises the effects of the aqueous extract of *Bersama engleriana* leaves on the caudal epididymal sperm characteristics in male guinea pig exposed to cypermthrin. The sperm mobility, the sperm count and the rate of spermatozoa with entire plasma membrane generally increased significantly (P<0.05) in animals exposed to cypermethrin and treated with 100 or 200 mg/kg bw of aqueous extract of BE leaves compared to those exposed only to cypermethrin, and were not different (P>0.05) to the control receiving distilled water. Meanwhile, the percentages of spermatozoa having small and big heads, coiled tails decreased significantly (P<0.05) in animals exposed to cypermethrin and given aqueous extract of *B. engleriana* leaves with respect to T0+ group.

Table 2: Effects of the aqueous extract of Bersama engleriana leaves on the caudal epididymal sperm characteristics in male guinea pig exposed to cypermthrin

Caudal epididymal sperm characteristics	**Treatments**					**P**
	Controls		Aqueous extract of B. engleriana leaves (mg/kg bw)			
	T0- (n = 6)	T0+ (n = 6)	50 (n = 6)	100 (n = 6)	200 (n = 6)	
Mobility (%)	75.00± 12.25^{bc}	65.00± 14.14^{c}	75.71± 16.18^{bc}	85.00± 7.56^{a}	82.86± 7.56^{ab}	0.00
Number/tails of epididymis (x 10^6)	21.67± 3.84^{ab}	16.41± 4.88^{c}	14.71± 2.72^{c}	21.29± 4.62^{ab}	22.92± 6.13^{a}	0.01
Number/g of epididymal tails (x 10^6)	52.63± 12.57^{ab}	39.15± 12.34^{bc}	41.44± 7.29^{abc}	49.79± 7.69^{ab}	54.01± 14.43^{a}	0.01
Spermatozoa with EPM (%)	81.00± 6.54^{ab}	68.13± 6.94^{c}	81.14± 5.40^{ab}	85.75± 4.06^{ab}	86.00± 4.90^{a}	0.00
Spermatozoa with small and big heads (%)	17.17± 5.60^{b}	26.00± 6.14^{a}	18.50± 6.98^{b}	16.14± 3.93^{b}	13.67± 2.73^{b}	0.00
Spermatozoa with coiled tails (%)	4.25± 1.08^{b}	7.38± 1.69^{a}	3.40± 0.89^{b}	2.75± 0.96^{b}	3.00± 1.41^{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. EPM: entire plasma membrane. T0-: group receiving 1 mL/kg bw of distilled water. T0+: group receiving 137.50 mg/kg bw of cypermethrin.

4 Discussion

The decrease in testicular concentration of malondialdehyde and activities of superoxide dismutase and catalase in guinea pigs exposed to cypermethrin and aqueous extract of *Bersama engleriana* leaves in the current study is similar to the observations done in rats submitted to 0.6 mg/kg bw of lambda-cyalothrine and 200 mg/kg bw of curcumin (Madkour, 2012) in mice exposed to 13.8 mg/kg bw of cypermethrin and 150 or 300 mg/kg bw of *Cedrelopsis grevei* (Mossa *et al.*, 2015). This observation might be explained by the action of antioxidant compounds such as phenols, flavonoids, xanthons, terpenoids and anthaquinons, revealed in this extract following the phytochemical tests. These molecules could have neutralised free radicals by transferring protons, capturing them (Hodek *et al*., 2002) or inhibiting enzymes responsible of their production, like aldose reductase, xanthin oxydase, lipoxygenase, phospholipase and cyclooxygenas (Van Acker *et al*., 1996 and Benavente-Garcia *et al*., 1997) and then protecting cells against cypermethrin-induced oxidative stress. Such actions could have reduced the peroxidation of the plasma membrane lipids and thus the concentration of malondialdehyde and the activity of antioxidant enzymes, superoxide dismutase and catalase. Malondialdehyde is an excellent substrate for peroxidases (Anita and Suresh, 2009 and Golamreza *et al*., 2010), therefore, the decrease of its concentration might explain the increase of peroxidases level. The increase in testosterone concentration and libido in animals treated with the insecticide and aqueous extract of *Bersama 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 the decrease of the percentage of abnormal spermatozoa in this study might be due to the increase in testosterone level on one hand and to the antioxidant effects of the aqueous extract of *Bersama engleriana* leaves on the other hand. In fact, antioxidant compounds could protect spermatozoa DNA against free radicals and improve sperm characteristics (Jedlinska *et al*., 2006).

5 Conclusion

The aqueous 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.

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