



Impact of Coenzyme Q10 on Hormonal Profile in Male Sprague-Dawley Rat Exposed to Sub-Chronic Concentrations of Cypermethrin

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Authors' contributions

This work was carried out in collaboration between both authors. Author EEO designed the study, supervised the experiment while author AOO carried out the experiment. Author AOO wrote the first draft of the manuscript. Authors EEO and AOO read and jointly approved the final manuscript.

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ABSTRACT

Aim: This study was aimed at evaluating the impact of Coenzyme Q10 on hormonal profile in male Sprague-Dawley rat exposed to sub-chronic concentrations of Cypermethrin.

Experimental Design: A completely randomized experimental design using standard methods for analysis. Hormonal assay was carried out by Microplate Enzyme Immunoassay using their respective test kits. Including AccuBind™ Microplate EIA Test system from Monobind Inc. Lake Forest CA 92630 USA while statistical analysis was carried out using one-way Analysis of Variance (ANOVA); where significant differences were found, Pair-wise comparisons conducted with Tukey test using SPSS 20 software.

Location and Duration of Study: This study was carried out in the Department of Biology, Ignatius Ajuru University of Education Rumuolumeni, Port Harcourt, Rivers State, Nigeria. GPS 4°48'14" N 6°59'12" E. The study lasted for 28days.

Methodology: Thirty male Sprague-Dawley rats were randomly assigned to five groups, A-E (n=6/group). Group A was given cool clean water and standard rat pellet ad libitum. Group B,C and D were administered Cypermethrin @ 10mg/kg/bw, 20mg/kg/bw, 30mg/kg/bw respectively along with

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10mg/kg/bw of coenzyme Q10 (CoQ10). Group E received 30mg/kg/bw of cypermethrin only without Coenzyme Q10. All animals were allowed access to cool clean water and standard rat pellet ad libitum. Bodyweight of the animals were taken twice a week and recorded in grams. Twenty-four hours before the animals were euthanized with ethyl ether inhalation, feed was withdrawn from the animals. Blood samples were collected by cardiac puncture between the hours of 7:00 and 9:00am into plain sample tubes according to the approved protocol of blood collection techniques. Analysis for the quantitative determination of all androgens was by Microplate Enzyme Immunoassay using the AccuBind™ Microplate EIA Test system from Monobind Inc. Lake Forest CA 92630 USA and expressed as their respective units. The animals were later dissected and the vital organs harvested and weighed. The values were subjected to statistical analysis using SPSS software 20.

Results: Results showed that there was no significant difference between the bodyweight and organ weight of animals in the treatment group compared with the control. Also, treatment with Cypermethrin only in group E, reduced the level of all the androgens considered in exposed animals. However, with concurrent administration of coenzyme Q10, the production of all androgens especially the production of Follicle Stimulating Hormone, Luteinizing Hormone and Progesterone in groups B,C,D and Estrogen in group B were significantly ($P=.05$) increased to values higher than the control.

Conclusion: This elevation indicates the role of Coenzyme Q10 as an effective antioxidant to boost hormonal level production especially the level of androgens.

Keywords: Androgens; antioxidant; COENZYMEQ10; free radicals; pyrethroids.

1. INTRODUCTION

There has been widespread concern by the general population concerning man-made chemicals released into the environment for various purposes including their use as pesticides. Increased use of these synthetic chemicals has resulted in environmental pollution and contamination. A class of these synthetic pesticides known as pyrethroids act as endocrine disruptors (ED), which are exogenous chemicals substances or mixtures thereof, that alter the structure or function of the endocrine system and cause adverse effects at the level of the organism, its progeny, populations, or subpopulations. They induce alterations in the sexual development of domestic animals, humans and wildlife [1-4]. Current conventional agricultural practices commonly rely on the use of cypermethrin and other pyrethroids introduced into the environment through air, soil and water. High concentrations of these are found in human bodies due to exposures from accumulation in fruits, vegetables, as well as, animal sources [5]. Pyrethroids are suspected to be estrogen mimics, hence, anti-androgenic in activity and have become a potential threat to fertility and development in animals and humans [6-11]. Other reports have been verified to be associated with certain male reproductive and developmental effects on human and animals such as underdevelopment, androgen-dependent tissues and testicular abnormalities, reduced sperm counts, sperm motility, sperm morphology abnormality and genotoxic effects [8,10,11,12].

Today, great efforts have been made to assess the adverse effects of environmental pollutants with anti-androgenic activity on mammals. Other reports on the effects of Cypermethrin on male reproduction include the works of [8,13-18].

The effect of Cypermethrin on the male reproductive system in mammals, fish and humans have been evaluated by a number of researchers [19-20]. The potential toxic effects of Cypermethrin in adult Sprague- Dawley male rats, with particular emphasis on its effect on the nervous and digestive systems has been investigated [21]. They reported that medium and high doses of Cypermethrin produced nervous signs in animals including gross lesions in the intoxicated groups, such as, bloat, congestion of lungs, heart, brain, pulmonary hemorrhage, and degenerative changes in the liver and kidneys. Microscopic effects on all organs were mild-to moderate degenerative changes at the low-dose level while medium- and high-dose intoxicated groups revealed necrotic changes, extensive hemorrhages, congestion in organs like liver, kidney, and lungs apart from the changes observed in low-dose group animals. The prevention of cypermethrin-induced reproductive toxicity in rat by resveratrol and reported weight loss of testis and epididymis, reduction in testicular sperm head counts, sperm motility and live sperm counts, as well as, increased sperm abnormalities was assessed [22]. There was also a report on the significant ($P=.05$) decrease in the levels of hormones considered such as follicle stimulating hormone (FSH), Testosterone

but a non-significant difference in the level of estradiol and luteinizing hormone in rats exposed to Cypermethrin only and those coadministered *Solanum lycopersicum* [8].

Pyrethroid toxic effects on some hormonal profile and biochemical markers among workers in pyrethroid insecticides company has also been reported [23,24]. The researchers evaluated chronic toxic effects of synthetic pyrethroids on some hormonal profile (testosterone, estrogen, progesterone and thyroid hormones) in human and concluded that chronic exposure to pyrethroid insecticides may cause endocrine disrupting effects, respiratory problems, liver function impairment, beside oxidative stress and lipid peroxidation. Other reports on the effect of Cypermethrin on hormonal profile include works on cyclicity of the ovary and the hormonal profiles of the reproductive system of Ewes following estrous synchronization with Veramix and exposure to Cypermethrin and Neocidol [20,24,25]. They concluded that neither Cypermethrin nor Neocidol had adverse effects on length of estrous cycle, estrous cyclicity and reproductive hormones in native ewes when sprayed by the proper dose (1/1000 and 1/500) of Cypermethrin and Neocidol respectively, the disruption of hormonal profile of female rats exposed to Cypermethrin [25].

Coenzyme Q10 (CoQ10) is a vitamin-like substance that has been used to enhance fertility in both males and females for several years [26]. However, the deficiency of CoQ10 has been reported in many diseases such as diabetes [27] heart failure, hypertension and others [28, 29] but paucity of information on hormone levels in individuals. Therefore, this study was designed to evaluate the impact of coenzyme q10 on hormonal profile in male sprague-dawley rat exposed to sub-chronic concentrations of cypermethrin.

2. MATERIALS AND METHODS

2.1 Location of Experiment

This investigation was carried out in the Department of Biology, Ignatius Ajuru University of Education Rumulumeñi, Port Harcourt, Rivers State, Nigeria. GPS 4°48'14" N 6°59'12" E.

2.2 Animals Care and Management

Thirty male Sprague-Dawley rats with a mean weight of 166.25±10.16g were obtained from the

Biochemistry Department of the University of Port Harcourt, Rivers State. All rats were housed individually in plastic cages with wire mesh cover in the Laboratory under standard conditions (12hL:12hD, room temperature 26 ±2°C, relative humidity 54%). The animals had access to cool clean water and standard rat pellet *ad libitum* and were acclimated for 14 days before the commencement of the experiment.

2.3 Experimental Design

The thirty male rats were randomly assigned to the control group (Group A) and four treatment groups, B,C,D,E (n=6/group). Group A were administered cool clean water and standard rat pellet only *ad libitum*. Groups B,C,D received respectively, daily oral gavage of 10mg/kg bw, 20mg/kg bw, 30mg/kg bw of Cypermethrin along with 10mg/kg bw of Coenzyme Q10, while Group E were gavaged 30mg/kg bw/day of Cypermethrin without Coenzyme Q10. All animals has access to clean cool clean water and standard rat pellet only *ad libitum*.

2.4 Analysis of Bodyweight and other Vital Organs

From the commencement of the experiment, the weights of the animals were taken twice a week using a digital weighing balance (Denver instrument: Model BT 210) and recorded to the nearest 0.01 grams. At the expiration of the experiment the male rats were euthanized with ethyl ether inhalation. The heart, liver, kidney, testes, epididymides and spleen of each rat was removed, freed from adhering tissues and weighed to the nearest 0.01grams.

2.5 Blood Collection

Twenty-four hours before the animals were euthanized with ethyl ether inhalation, feed was withdrawn from the animals. Blood samples were collected between 7am to 9am by cardiac puncture according to the approved protocol of blood collection techniques. Blood was collected into plain tubes without anti-coagulant and allowed to clot; thereafter the samples were centrifuged at 3000g for 10 minutes.

The quantitative determination of the concentration of Testosterone, Progesterone, Follicle Stimulating Hormone and Luteinizing Hormone in the serum was carried out by Microplate Enzyme Immunoassay using their

respective AccuBind™ Microplate EIA Test system from Monobind Inc. Lake Forest CA 92630 USA [30].

2.6 Statistical Analysis

Data from the following end points: Body weight, organ weight, reproductive organ weight and Hormones were subjected to one-way Analysis of Variance (ANOVA); where significant differences were found, Pair-wise comparisons conducted with Tukey test using SPSS 20 software.

3. RESULTS

3.1 Effect on Bodyweight and Organ Weight

The effect of sub-chronic administration of Cypermethrin and Coenzyme Q10 on body weight and reproductive organ weight is shown in Table 1.

The mean body weight of Sprague-Dawley rats exposed to Cypermethrin and coenzyme Q10 is shown in Table 1. There was no significant increase in the body weight of all animals coadministered Coq10 in group B,C,D with the control group and the group without Coq10. All reproductive organ and vital organ weights did not show any significant difference in the groups although the group E, administered Cypermethrin only had the lowest reproductive organ weight.

3.2 Effect of oral Administration of Cypermethrin and Coenzyme Q10 on Hormonal Profile

The effect of oral administration of Cypermethrin and Coenzyme Q10 on Hormonal profile of male Sprague-Dawley rats is shown in Fig. 1(A-E). The concentration of Follicle Stimulating Hormone (FSH) was 0.4 ± 0.02 U/L in the control group, this remained steady in group B coadministered 10mg/kg/bw of Cypermethrin and 10mg/kg/bw of Coq10. There was a significant increase ($P=.05$) in the level of FSH to 1.19 ± 0.08 U/L in group C, with concomitant administration of 20mg/kg/bw of cypermethrin along with 10mg/kg/bw of Coenzyme Q10 with 35% increase. This level decreased non-significantly to 0.83 ± 0.04 U/L in group D but with the administration of 30mg/kg/bw of Cypermethrin only without CoQ10, the concentration of FSH decreased significantly

($P=.05$) to 0.23 ± 0.10 U/L in group E, compared to the control group (Fig 1A).

The effect of oral administration of Cypermethrin (CYP) and Coenzyme Q10 on Luteinizing Hormone (LH) production in male Sprague-Dawley rats is shown in Fig. 1B. The concentration of LH was 8.83 ± 0.11 IU/L for control (0mg of CYP). There was an increase in the concentration of LH to 14.35 ± 2.28 IU/L in group B with the administration of 10mg/kg/bw of Cypermethrin and coadministration of 10mg/kg/bw CoQ10. At 20mg/kg/bw of Cypermethrin in group C there was a decrease in this concentration to 10.7 ± 1.51 IU/L which later increased to 12.45 ± 0.68 IU/L with administration of CoQ10. With the administration of 30mg/kg/bw of CYP without the addition of CoQ10 in group E, the level of LH decreased significantly to 3.88 ± 0.36 IU/L when compared with group A.

The effect of oral administration of Cypermethrin and Coenzyme Q10 on Testosterone production in male Sprague-Dawley rats is shown in Fig. 1C. The concentration of testosterone was 2.4 ± 0.36 µg/ml in the control group. With the addition of 10mg of CYP and 10mg/kg/bw of Coq10, the concentration decreased to 0.78 ± 0.23 µg/ml. A significant increase of 1.50 ± 0.04 µg/ml which was twice that in group C was recorded in group B with coadministration of 10mg/kg/bw of Coq10. The concentration decreased to 0.82 ± 0.04 µg/ml in group D and further decreased significantly to 0.23 ± 0.13 µg/ml in group E when compared with group A.

The effect of oral administration of cypermethrin and Coenzyme Q10 on Estrogen production in male Sprague-Dawley rats is shown in Fig. 1D. The level of E2 was 561.25pg/ml in the control group. Upon the addition of 10mg/kg/bw of CoQ10 there was an increase to 862.5pg/ml in group B, but latter decreased to 523pg/ml resulting in a 20% decrease. The estrogen level decreased to 500pg/ml with the administration of 20mg of Cyp in group C but increased to 783pg/ml and remained constant even in group D. At 30mg/kg/bw of CYP without coadministration of CoQ10, the level of E2 decreased to 500pg/ml lower than the control.

The effect of oral administration of cypermethrin and Coenzyme Q10 on Progesterone production in male Sprague-Dawley rats is shown in Fig. 1E. The level of progesterone was 15.9 ± 0.51 µg/ml in the control group. Upon the coadministration of 10mg/kg/bw of CoQ10 in group B, the

concentration increased to $19.65 \pm 4.30 \mu\text{g/ml}$ and remained almost steady in group C with $19.33 \pm 0.59 \mu\text{g/ml}$. At 20mg/kg/bw of Cypermethrin and 10mg/kg/bw of CoQ10 in group D, the values decreased non-significantly

to $15.3 \pm 2.98 \mu\text{g/ml}$. The coadministration of 30mg of Cypermethrin and CoQ10 produced the same level of progesterone as in the control which later declined to $10.32 \pm 0.34 \mu\text{g/ml}$ at 30mg/kg/bw of Cypermethrin without CoQ10.

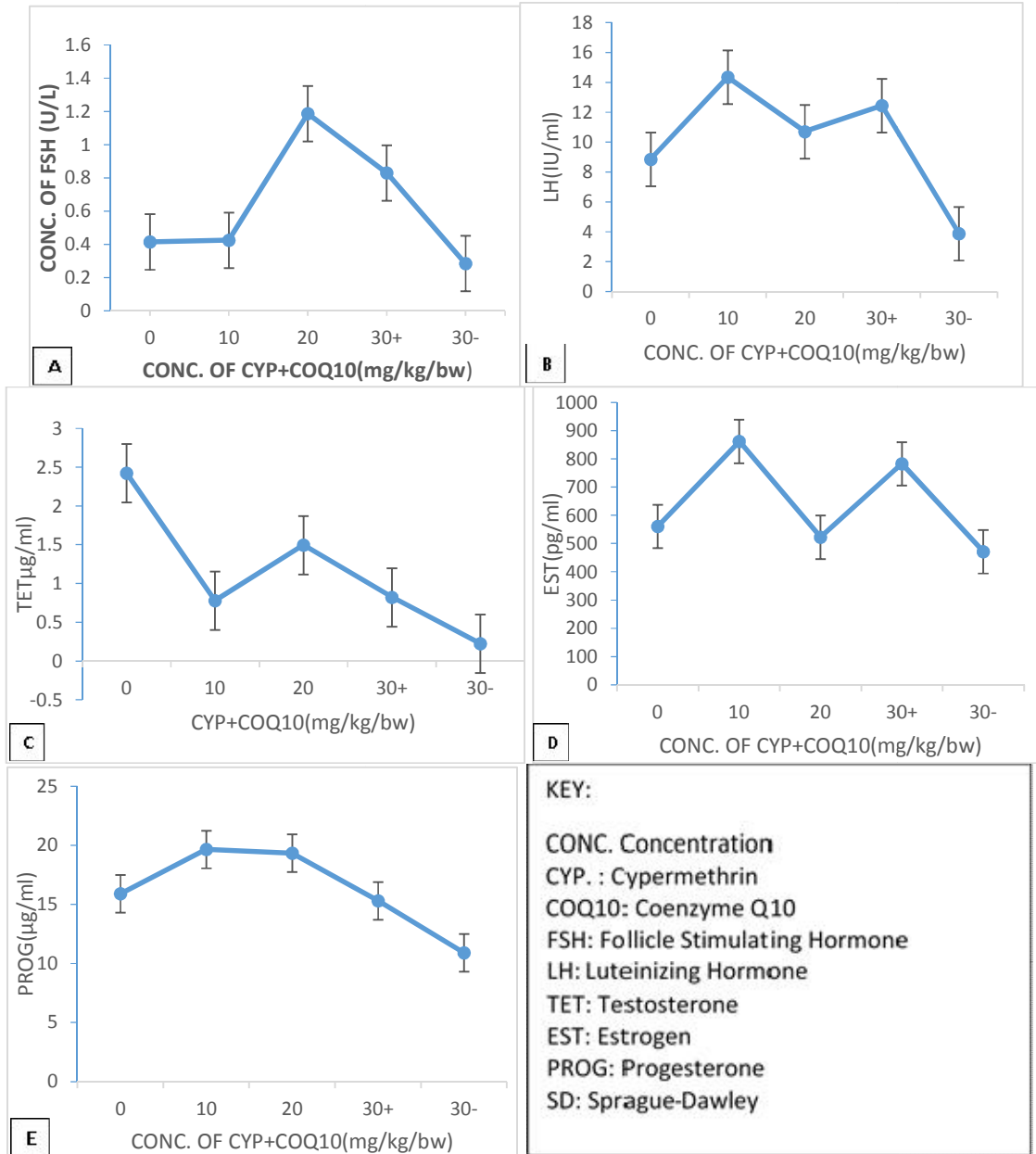


Fig. 1A. Conc. of CYP and COQ10 on FSH levels in SD rats. **Fig.1B:** Conc. Of CYP and COQ10 on LH levels in SD rats. **Fig 1C:** Conc. Of CYP and COQ10 on TET levels in SD rats. **Fig1D.** Conc. Of CYP and COQ10 on EST levels in SD rats. **Fig. 1E.** Conc. Of CYP and COQ10 on PROG levels in SD rats

Table 1. Effect of Cypermethrin (CYP) and co-administration of Coq10 on Reproductive organ weight in male Sprague-Dawley rat

Concentration (mg/kg ^{-bw})	Body Weight (g)	P TW (g)	LT (g)	R T (g)	P EW (g)	L EW (g)	REW (g)
0	166.67±27.13	2.13±0.56	1.00±0.26	1.12±0.32	0.76±0.13	0.40±0.09	0.35±0.08
10	158.22±11.16	1.92±0.35	0.92±0.17	1.02±0.18	0.61±0.41	0.32±0.26	0.29±0.17
20	168.61±28.97	2.05±0.54	1.01±0.25	1.04±0.28	1.39±0.63	0.72±0.23	0.84±0.22
30+	163.17±22.7	1.93±0.58	1.04±0.26	0.89±0.33	0.80±0.47	0.44±0.20	0.43±0.24
30-	165.52±27.9	1.68±0.65	0.86±0.35	0.82±0.31	0.40±0.16	0.27±0.07	0.25±0.06

*Values are Mean ± SD, PTW=Paired testes weight, LT=Left testis, RT=Right tests, PEW=Paired epididymal weight, LEW=Left epididymal weight, REW=Right epididymal weight ; 30⁺ =30mg CYP +Coq10; 30⁻ =30mg CYP without Coq10

Table 2. Effect of Cypermethrin(CYP) and co-administration of Coq10 on Vital organ weight in male Sprague-Dawley rat

Concentration (mg/kg ^{-bw})	Body Weight (g)	Liver (g)	Kidney (g)	Heart (g)	Spleen (g)
0	166.67±27.13	4.12±1.29	0.62±0.10	0.653±0.10	0.737±0.19
10	158.22±11.16	5.545±0.74	0.57±0.34	0.653±0.15	0.695±0.21
20	168.61±28.97	5.440±0.74	0.94±0.19	0.820±0.17	0.803±0.20
30+	163.17±22.70	5.571±0.73	0.723±0.15	0.803±0.17	0.803±0.20
30-	165.52±27.90	5.087±1.84	0.657±0.15	0.653±0.15	0.863±0.14

*Values are Mean ± SD. *30⁺ =30mg CYP +Coq10; 30⁻ =30mg CYP without Coq10

4. DISCUSSION

Changes in the body weight and relative weights are important criteria for evaluation of organ toxicity and can be used as a valuable tool or index of pesticides related organ damage [8].

In this present study there was no significant difference between the bodyweight and organ weight of the control animals and the treatment group. This may be indicative that Cypermethrin is not a systemic toxin and so may not have obvious effect on the bodyweight or organ weight of the animals but silently destroy various target cells of the body.

Recently, great efforts have been directed toward the study of the adverse effects of environmental contaminants with anti-androgenic activity [8,16,17,18,31,32]. Pollutants as well as pesticides that exhibit anti-androgenic activity could be responsible for the increased incidence in various male infertility and other sexual disorders including low sperm counts and quality, azoospermia and hypogonadism [17,20, 32]. Many chemicals especially, Endocrine disruptors act as antagonists by binding to Androgen receptors and preventing the transcription of androgen-dependent genes such that they inhibit sexual development and maturation as well as, impairment of optimal reproductive organs functions during spermatogenesis. This observation is in agreement with previous reports on several pyrethroid pesticides [9,31]. Administration of Cypermethrin concurrently with Coenzyme Q10 resulted in a decrease in the concentration of Testosterone, the main androgen responsible for the initiation and maintenance of spermatogenesis in mammals. The mechanism of action mimicking the action of natural pyrethrums which are anti-androgenic and disrupts the transcription of androgen-dependent genes reflected in the reduction of the production of testosterone. CoQ10 is a component of the electron transport chain responsible for generating energy in the cells. Levels of CoQ10 tend to diminish as you age, resulting in reduced cellular energy capacity, which has obvious fertility implications. Particularly relevant for use in enhancing fertility, CoQ10 also functions as an antioxidant, decreasing the damaging effects of free radicals on the reproductive system. [29].

However, in this study, the concentration of all other androgens including FSH, LH,

Progesterone and Estrogens were significantly elevated with concomitant administration of Coenzyme Q10 when compared with groups without CoenzymeQ10. This elevation indicates the role of Coenzyme Q10 as an antioxidant in ameliorating the oxidative stress produced by increased release of free radicals by Cypermethrin. The result of this study agrees with the works of [8,20] who reported the effect of Cypermethrin on sperm morphology, sperm reserves, testicular architecture and hormonal profile of Sprague-Dawley rats. Based on these findings, the use of Cypermethrin especially as indoor intervention insecticides should be reduced to the barest minimum. Moreover, the fact that CoQ10 decreases with age should encourage aging persons and those occupationally exposed to Cypermethrin or other pyrethroids to include the use of Coenzyme Q10 antioxidant as a lifestyle.

5. CONCLUSION

Results showed that there was no significant difference between the bodyweight and organ weight of animals in the treatment group compared with the control. Also, treatment with Cypermethrin only in group E, reduced the level of all the androgens considered in exposed animals. However, with concurrent administration of coenzyme Q10, the production of all androgens especially the production of Follicle Stimulating Hormone, Luteinizing Hormone and Progesterone in groups B,C,D and Estrogen in group B were significantly ($P=.05$) increased to values higher than the control. This elevation indicates the role of Coenzyme Q10 as an effective antioxidant to boost hormonal level production especially the level of androgens.

ETHICAL APPROVAL

The experiment was conducted according to the institutional animal care protocols at the Ignatius Ajuru University of Education Rumulumeni, Port Harcourt, Nigeria and followed approved guidelines for the ethical treatment of experimental animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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