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Effect of quercetin on cadmium chloride-induced impairments in sexual behaviour and steroidogenesis in male Wistar rats

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Summary

Cadmium chloride ($CdCl_2$) has been reported to cause reproductive toxicity in male rats, mainly through oxidative stress. This study examined its effect on sexual behaviour, as one of the mechanisms of reproductive dysfunction, as well as the possible ameliorative effect of quercetin (QE) on same. Thirty male Wistar rats (10 weeks old), weighing 270–300 g, were used for this study. They were either orally administered 2% DMSO, $CdCl_2$ (5 mg/kg b.w.), QE (20 mg/kg b.w.) or $CdCl_2$+QE, once daily for 4 weeks, before sexual behavioural studies. The 5th group received $CdCl_2$ for 4 weeks and allowed 4-week recovery period, before sexual behavioural test. Rats were sacrificed after sexual behavioural studies. The blood, testis and penis were collected for biochemical assays. Cadmium increased mount, intromission and ejaculatory latencies, but reduced their frequencies, compared to control. Serum nitric oxide increased, while penile cyclic guanosine monophosphate reduced in the $CdCl_2$-exposed rats, compared to control. $CdCl_2$ increased testicular cholesterol, but reduced 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-HSD activities, and testosterone concentration. QE better attenuated these negative changes compared to withdrawal of $CdCl_2$ treatment. In conclusion, $CdCl_2$ suppressed steroidogenesis, penile erection and sexual behaviour, with poor reversal following withdrawal, while QE attenuated these effects.

KEYWORDS
cadmium, heavy metals, nitric oxide, sexual behaviour, steroidogenesis

1 | INTRODUCTION

Cadmium is a toxic heavy metal that occurs extensively in nature (WHO, 1992). It is a metal of industrial importance (IARC, 1993; Martelli, Rousselet, Dycke, Bouron, & Moulis, 2006). The burning of fossil fuels, mining and agricultural activities also add to cadmium occurrence in nature (Muntau & Baudo, 1992). Cadmium is present in the air we breathe, the food and water we ingest (WHO, 1992) and is also reported to be present in cigarette smoke (Satarug & Moore, 2004). Having a prolonged half-life, cadmium eventually accumulates in the body (Matsuno, Kodama, & Tsuchiya, 1991) causing extensive damage to biological tissues. Cadmium is thus a pollutant of serious concern.

Studies have shown that cadmium increases the concentration of free radicals in various tissues of the body, thus resulting in oxidative stress (Waalkes, 2000; Stohs, Bagchi, Hassoun, & Bagchi, 2001; Kanter et al., 2016). Several studies have documented the toxic effects of cadmium on the male reproductive system to include impaired spermatogenesis (Yari et al., 2016), impaired sperm motility, decrease in testicular activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase (Manna, Sinha, & Sil, 2008), degeneration of the testes (Burukoglu & Baycu, 2008) and germ cell apoptosis (Al-Azemi et al., 2010; Kanter et al., 2016).

As economies develop, industrialisation and urbanisation increase, resulting in increased exposure to cadmium. This has thus led to interest in substances that may mitigate the toxicity due to cadmium

exposure; one of which is quercetin (QE). QE is a naturally occurring flavonoid found in fruits and vegetables (Hollman et al., 1997). It is an antioxidant (Walle, Vincent, & Walle, 2003). It scavenges free radicals and chelates transition metals (Bentz, 2009). QE has been reported to have beneficial effects in cardiovascular diseases (Bischoff, 2008) and cancer (Geleijnse, Launer, Van der Kuip, Hofman, & Witteman, 2002). QE has also been shown to boost sperm quality (Taepongsorat, Prakong, Noppadon, & Malaivijitnond, 2008). It has been found to confer protective effects against cadmium- (Kanter et al., 2016) and crude oil-induced (Ebokaiwe, Mathur, & Farombi, 2016) testicular toxicities.

In males, normal sexual function is characterised mainly by erection of the penis and ejaculation. Persistent absence of these hallmarks could be termed as male sexual dysfunction (MSD) (Guay et al., 2003). Sexual dysfunction in males could manifest as lack of desire, persistent delay in/absence of orgasm, erectile dysfunction, quick ejaculation and priapism (Yakubu & Akanji, 2011). These could be caused by a number of factors including but not limited to drug and alcohol abuse, smoking, certain disease conditions and exposure to toxic chemicals. Studies have documented the toxic effects of cadmium (Al-Azemi et al., 2010; Eleawa et al., 2014; Yari et al., 2016) and the ameliorative role of QE on some parameters of male sexual function. To date, only a few studies have reported impaired sexual behaviour in cadmium-induced reproductive impairment, and no study has reported the effect of QE on sexual behaviour of cadmium exposed rats. Therefore, our objectives were (i) to investigate the effect of cadmium exposure on male sexual behavioural parameters, (ii) to investigate the effect of QE on sexual behaviour of cadmium exposed rats and (iii) to examine the effect of withdrawal of cadmium treatment on parameters of sexual behaviour.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Cadmium chloride, QE and dimethyl sulfoxide (DMSO) were obtained from Sigma chemical Co. (St. Louis, MO, USA). All other chemicals were commercially available and were of analytical grade.

2.2 | Experimental animals

Sixty sexually matured rats (30 males and 30 females) aged 10 weeks old and weighing 270–300 g were obtained from the animal house of the College of Medical Sciences, University of Calabar, Nigeria, and housed in the Animal house of the department of Physiology, University of Calabar, Calabar, Nigeria. The animals were allowed 7-day habituation period before commencement of the research. The animals were exposed to 12/12 hours light/dark cycle and allowed ad libitum access to water and feed. The study protocol was approved by the animal ethics committee of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria. The rats were handled in accordance with the guidelines contained in the Animal Ethics Handbook of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria.

2.3 | Experimental design

Thirty male rats were randomly assigned into five groups ($n = 6$). Group I served as control and received 0.2 ml of 2% DMSO (vehicle). Group II received 5 mg/kg of $CdCl_2$ in 2% DMSO. Group III received QE (20 mg/kg b.w.) in 2% DMSO. Group IV received $CdCl_2$ and QE at doses as in groups II and III respectively. Administration was by oral route, once daily, for 4 weeks. Group V received $CdCl_2$ for 4 weeks at the same dose as in group II, after which they were allowed 4-week recovery period ($CdCl_2$ recovery group). Doses administered were as used in previous studies (El-Demerdash, Yousef, Kedwany, & Baghdadi, 2004; Alkhedaide et al., 2016) for $CdCl_2$ and Farombi, Adedara, Akinrinde, Ojo, and Eboh (2012) for QE. The selected dose for $CdCl_2$ was found to induce significant oxidative stress in various tissues (El-Demerdash et al., 2004; Alkhedaide et al., 2016), while the selected dose for QE was found to ameliorate chemical-induced oxidative stress in rats (Nna, Usman, Ofutet, & Owu, 2017); hence, we chose this dose to investigate its effect on sexual behaviour in male rats.

2.4 | Male rat sexual behaviour test procedure

The female rats were made receptive by sequential subcutaneous administration of 10 μg/100 g b.w. of estradiol benzoate and 0.5 mg/100 g b.w. of progesterone, 48 and 4 hr, respectively, prior to pairing as used in previous studies (Agmo, 1997; Gauthman, Adaikan, & Prasda, 2002). This treatment assures intense proceptivity and receptivity (Agmo, 1997). At the end of the 4-week administration period (8 weeks in the case of the $CdCl_2$ recovery group), each male rat was paired with a receptive female at a ratio of 1:1 after 30-minute adaptation period in a cage of dimensions 33.0 × 20.5 × 19.0 cm (length × width × height). The receptive female and male rats were observed from the cage side for sexual behavioural parameters, for 30 min. The test was carried out between 5:00 and 7:30 p.m. under a dim light. Adopting the standard procedure described in previous studies (Agmo, 1997; Gauthman et al., 2002), as used by Yakubu and Akanji (2011), the following underlisted male sexual behavioural parameters were assessed: mount latency (ML): the time from the introduction of the female until first mount by the male; mount frequency (MF): the number of times the male assumed copulatory position but failed to achieve intromission (characterised by lifting the male's forebody over the hindquarter of the female and clasping her flanks with his forepaw); intromission latency (IL): the time from the introduction of the female until the first intromission by the male; intromission frequency (IF): the number of successful vaginal penetrations made by the male (usually characterised by pelvic thrusting and springing dismount); ejaculatory latency (EL): the time from the first intromission until first ejaculation (usually characterised by longer, deeper pelvic thrusting and slow dismount, followed by a period of reduced activity); ejaculation frequency (EF): the number of times there was expulsion of semen by the males after vaginal penetration (characterised by rhythmic contraction of the posterior abdomen). As a confirmatory

test for copulation, the female rats were observed for the presence of vaginal plug using a light microscope at the end of the 30-min mating period. Briefly, 0.2 ml of normal saline was drawn into a dropper. The tip of the dropper was gently inserted into the vaginal orifice at a depth of 2–5 mm. The normal saline was flushed into the vagina and back out. A smear was made on a slide which was viewed under light microscope (Leica DM750, Leica Microsystems, Heerbrugg, Switzerland) (Cora, Kooistra, & Travlos, 2015). Sperm-positive vaginal smear confirmed copulation.

Some additional male sexual behavioural parameters computed were as follows:

$$\%\,\text{mounted} = (\,\text{number mounted/number paired}\,) \times 100$$

$$\%\,\text{intromitted} = (\,\text{number of rats that intromitted/number paired}) \times 100$$

$$\%\,\text{ejaculated} = (\,\text{number of rats that ejaculated/number paired}\,) \times 100$$

$$\text{Copulatory efficiency} = (\,\text{number of intromissions/number of mounts}\,) \times 100$$

2.5 | Sample collection

At the end of the sexual behavioural experiments, the rats were euthanised using chloroform anaesthesia. Blood samples were collected via cardiac puncture into plain sample bottles and allowed to stand for 30 min. The clotted blood was centrifuged at 2,000 *g* for 10 min to collect serum. The testis and penile tissue were carefully dissected out and separately fixed in freshly prepared phosphate-buffered saline (PBS).

2.6 | Determination of serum nitric oxide (NO) concentration

Serum nitric oxide (NO) concentration was assessed by estimating the total nitrate/nitrite in serum using the NO_2^-/NO_3^- assay of R&D Systems, Europe (Abingdon, UK), as described by Wo et al. (2013), as NO rapidly oxidises to nitrites and nitrates. This assay involves the conversion of nitrate to nitrite by the enzyme nitrate reductase. The detection of nitrite was then determined as a coloured azo dye product of the Griess reaction that absorbed visible light at 540 nm.

2.7 | Determination of penile cGMP concentration

Quantitative assay of penile cGMP concentration was performed by radioimmunoassay, using an RIA kit (Amersham, Buckinghamshire, UK) as per the manufacturer's manual. Briefly, homogenised penile tissue sample was mixed with 2 ml dehydrated alcohol and centrifuged at 3,000 *g* for 15 min at 4°C, and the supernatant was collected. The remaining sediment was washed with 75% ethanol and centrifuged again at 3,000 *g* for 15 min at 4°C, and the ethanolic phase was kept. These two supernatants were mixed and dried at 60°C. Following redissolution, aliquots of the samples were assayed for cGMP and the results were expressed in picomoles of cGMP per microgram protein (Seidler et al., 2002).

2.8 | Testicular biochemical assays

The left testis of each rat was homogenised in 0.1 M phosphate buffer (pH 7.4) using Heidolph homogeniser and a Teflon pestle. The homogenate was thereafter centrifuged at 10,000 *g* for 30 min in a cold centrifuge (4°C). The supernatant was used to assay for testicular cholesterol concentration, 3β-HSD and 17β-HSD activities. Cholesterol was assayed in the sample by the enzymatic end-point method using a commercially available kit (Randox, UK) following the manufacturer's protocol. Testicular 3β-HSD and 17β-HSD activities were measured according to the methods of Talalay (1962) and Jarabak, Adams, Williams-Ashman, and Talalay (1962), respectively, as used by Mishra and Singh (2016). The reaction mixture in a total volume of 2 ml contained 100 μmol sodium pyrophosphate buffer (pH 8.9), 0.5 μmol NAD+ for 3β-HSD and NADP+ for 17β-HSD, 0.14 μmol dehydroepiandrosterone for 3β-HSD and 0.5 μmol testosterone for 17β-HSD, and 20 mg equivalent of microsomal protein as enzyme source. The reactions were carried out in a quartz cuvette at 25°C. The change in absorbance was measured at 340 nm for 3 min. Results were expressed as nmol/min/mg protein.

Testicular testosterone concentration was assessed in the testicular homogenate using testosterone ELISA kit (UBI MAGIWEL United Biotech Inc., Mountain View, CA, USA) following the manufacturer's protocol. The test is based on the principle of competitive solid-phase enzyme immunoassay. The test sample competes with enzyme-labelled testosterone for a fixed and limited number of antibody sites on the microtitre wells. The testosterone standard and sample were incubated with the testosterone antibody and the testosterone–horseradish peroxidase conjugate in the anti-rabbit IgG-coated well. In this solid-phase system, the antibody-bound testosterone remained on the well while unbound testosterone was removed by washing. The intensity of the colour developed when the substrate was mixed with the antibody-bound testosterone–horseradish peroxidase enzyme conjugate was read at 450 nm.

2.9 | Statistical analysis

Data are expressed as mean ± *SEM*. Data were analysed using one-way analysis of variance (ANOVA), followed by Tukey test (Post hoc test). The statistical analysis was performed using GraphPad Prism 7.0 (GraphPad Software Inc., La Jolla, CA, USA). Values of $p < .05$ were considered statistically significant.

3 | RESULTS

3.1 | Mount latency

Mount latency was significantly increased ($p < .001$) in the $CdCl_2$ group (131.00 ± 5.94 s), but significantly decreased ($p < .05$) in the QE-treated group (59.17 ± 3.13 s), compared to the control (85.50 ± 6.28 s). Although ML decreased in $CdCl_2$ + QE group (106.83 ± 8.18 s) compared to the $CdCl_2$ group, the decrease was not significant ($p > .05$). The $CdCl_2$ recovery group showed a significant reduction ($p < .001$) in ML, compared to the $CdCl_2$ group (Figure 1a).

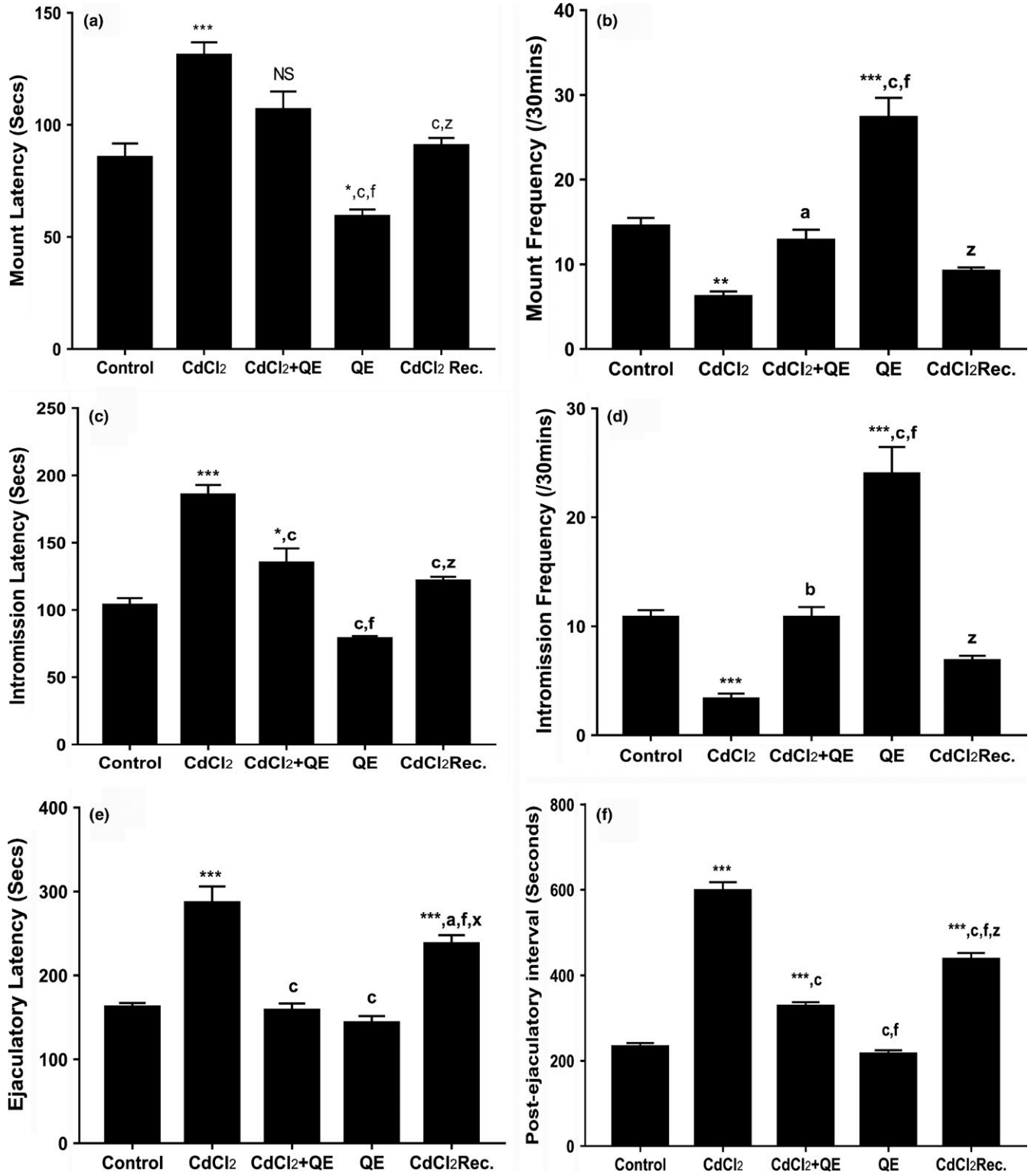

FIGURE 1 Comparison of (a) mount latency, (b) mount frequency, (c) intromission latency, (d) intromission frequency, (e) ejaculatory latency and (f) post-ejaculatory interval (PEI) between the different experimental groups. Values are mean ± *SEM*, $n = 6$. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ versus control; a = $p < .05$, b = $p < .01$, c = $p < .001$ versus $CdCl_2$; f = $p < .001$ versus $CdCl_2$+QE; x = $p < .05$, z = $p < .01$ versus QE

3.2 | Mount frequency

Mount frequency decreased significantly ($p < .01$) in $CdCl_2$ group (6.00 ± 0.65 per 30 min), but increased significantly ($p < .001$) in the QE group (29.00 ± 2.94 per 30 min) compared to the control (14.00 ± 0.99 per 30 min). Mount frequency was significantly increased ($p < .05$) in $CdCl_2$ + QE group (13.00 ± 1.28 per 30 min), compared to the $CdCl_2$ group. Following withdrawal of cadmium treatment

TABLE 1 Computed male rat sexual behavioural parameters in the different groups

Parameter	Control	$CdCl_2$	$CdCl_2$ + QE	QE	$CdCl_2$ Rec.
Presence of pre-coital exploratory behaviour (%)	100.00	100.00	100.00	100.00	100.00
% Mounted	100.00	100.00	100.00	100.00	100.00
% Intromitted	100.00	66.67	100.00	100.00	100.00
% Ejaculated	100.00	50.00	83.33	100.00	66.67
Presence of more than one ejaculation (%)	50.00	16.67	33.33	50.00	16.67
Copulatory efficiency (%)	74.98	53.75	84.91	87.38	75.20

(9.00 ± 0.48 per 30 min), MF increased, although the increase was not significantly different ($p > .05$) from the $CdCl_2$ group (Figure 1b).

3.3 | Intromission latency

Intromission latency was significantly increased ($p < .001$) in $CdCl_2$ group (185.50 ± 7.37 s) compared to the control (103.67 ± 5.17 s). Intromission latency significantly decreased ($p < .001$) in $CdCl_2$+QE group (134.83 ± 10.99 s) compared to the $CdCl_2$ group, but was significantly high ($p < .05$) compared to control. The $CdCl_2$ recovery group (121.50 ± 3.18 s) had a significantly reduced ($p < .001$) IL, compared to the $CdCl_2$ group, but was high ($p > .05$) compared to the control (Figure 1c).

3.4 | Intromission frequency

Cadmium chloride (3.00 ± 0.49 per 30 min) significantly decreased ($p < .001$) the IF, while QE (24.00 ± 2.48 per 30 min) significantly increased ($p < .001$) IF when compared to the control (11.00 ± 0.65 per 30 min) (Figure 1d). Intromission frequency significantly increased ($p < .01$) in $CdCl_2$+QE group (11.00 ± 0.95 per 30 min) compared with the $CdCl_2$ group. Although IF in the $CdCl_2$ recovery group (7.00 ± 0.48 per 30 min) was higher than that of the $CdCl_2$ group, the difference was not significant ($p > .05$) (Figure 1d).

3.5 | Ejaculatory latency

Ejaculatory latency significantly increased ($p < .001$) in the $CdCl_2$ group (286.83 ± 19.45 s), compared to the control (162.17 ± 5.12 s) (Figure 1e). Ejaculatory latency significantly decreased ($p < .001$) in $CdCl_2$+QE group (158.50 ± 8.11 s), compared to the $CdCl_2$ group. EL significantly decreased ($p < .05$) in $CdCl_2$ recovery group (237.67 ± 10.39 s) compared to the $CdCl_2$ group, but significantly increased ($p < .001$) compared to the control and $CdCl_2$+QE groups (Figure 1e).

3.6 | Post-ejaculatory interval

Cadmium chloride (598.17 ± 19.95 s) significantly increased ($p < .001$) the PEI, compared to the control (232.50 ± 8.97 s) (Figure 1f). The PEI in $CdCl_2$+QE group (327.33 ± 9.90 s) was significantly high ($p < .001$) compared to the control, but significantly low ($p < .001$) compared to the $CdCl_2$ group. PEI decreased significantly ($p < .001$) in the $CdCl_2$ recovery group (436.83 ± 15.70 s) compared to the $CdCl_2$ group,

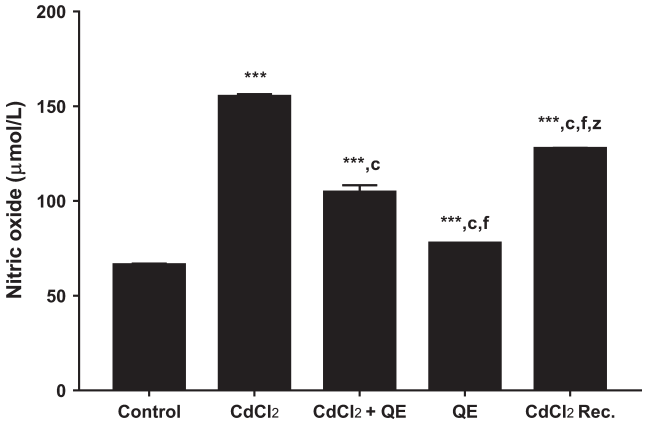

FIGURE 2 Comparison of serum concentration of nitric oxide between the different experimental groups. Values are mean ± *SEM*, $n = 6$. ***$p < .001$ versus control; c = $p < .001$ versus $CdCl_2$; f = $p < .001$ versus $CdCl_2$ + QE; z = $p < .001$ versus QE

but was significantly high ($p < .001$) compared to the control and $CdCl_2$+QE groups (Figure 1f).

3.7 | Computed sexual behaviour parameters

The result of the computed sexual behaviour parameters as presented in Table 1 shows that all male rats in all the groups exhibited pre-coital exploratory behaviour and also mounted. Apart from the $CdCl_2$ group where only 66.67% of the animals intromitted, all rats in the other groups intromitted. All rats in the control and QE groups ejaculated as against 50%, 83.33% and 66.67% for $CdCl_2$, $CdCl_2$+QE and $CdCl_2$ recovery groups respectively. For presence of more than one ejaculation, results show that half the male rats each in control and QE groups had more than one ejaculation, while only 16.67%, 33.33% and 16.67% for $CdCl_2$, $CdCl_2$+QE and $CdCl_2$ recovery groups, respectively, had more than one ejaculation. The copulatory efficiency arranged in order of magnitude was QE (87.38%) > $CdCl_2$+QE (84.91%) > control (74.98%) > $CdCl_2$ recovery (75.20%) > $CdCl_2$ group (53.75%). This implies that copulatory efficiency was highest in the QE group and lowest in the $CdCl_2$ group (Table 1).

3.8 | Serum nitric oxide (NO) concentration

Serum NO concentration increased significantly ($p < .001$) in $CdCl_2$ group (155.23 ± 0.47 µmol/L), compared to the control (66.37 ± 0.26 µmol/L) (Figure 2). Serum NO concentration decreased

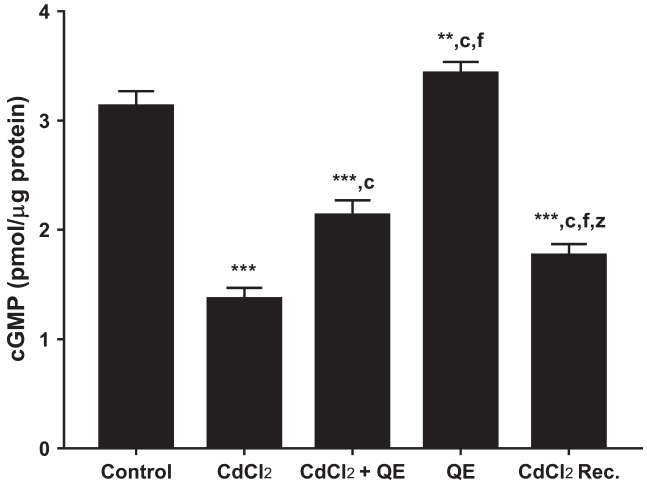

FIGURE 3 Comparison of penile cGMP concentration between the different experimental groups. Values are mean ± *SEM*, *n* = 6. $^{**}p < .01$, $^{***}p < .001$ versus control; c = $p < .001$ versus $CdCl_2$; f = $p < .001$ versus $CdCl_2$ + QE; z = $p < .001$ versus QE

significantly ($p < .001$) in the $CdCl_2$+QE group (104.63 ± 1.50 µmol/L) compared to the $CdCl_2$ group, but was significantly higher ($p < .001$) than the control. Serum NO concentration decreased significantly ($p < .001$) in the $CdCl_2$ recovery group (127.73 ± 0.13 µmol/L) compared to the $CdCl_2$ group, but was significantly higher ($p < .001$) than the control and $CdCl_2$+QE groups (Figure 2).

3.9 | Penile cGMP concentration

Penile cGMP concentration decreased significantly ($p < .001$) in the $CdCl_2$ group (1.37 ± 0.04 pmol/µg protein), but increased significantly ($p < .01$) in the QE group (3.43 ± 0.04 pmol/µg protein) compared to the control (3.13 ± 0.06 pmol/µg protein) (Figure 3). Penile cGMP concentration increased significantly ($p < .001$) in $CdCl_2$+QE (3.13 ± 0.06 pmol/µg protein) and $CdCl_2$ recovery (1.77 ± 0.04 pmol/µg protein) groups compared to the $CdCl_2$ group. However, penile cGMP was significantly low ($p < .001$) in the $CdCl_2$+QE and $CdCl_2$ recovery groups, compared to the control and $CdCl_2$+QE groups (Figure 3).

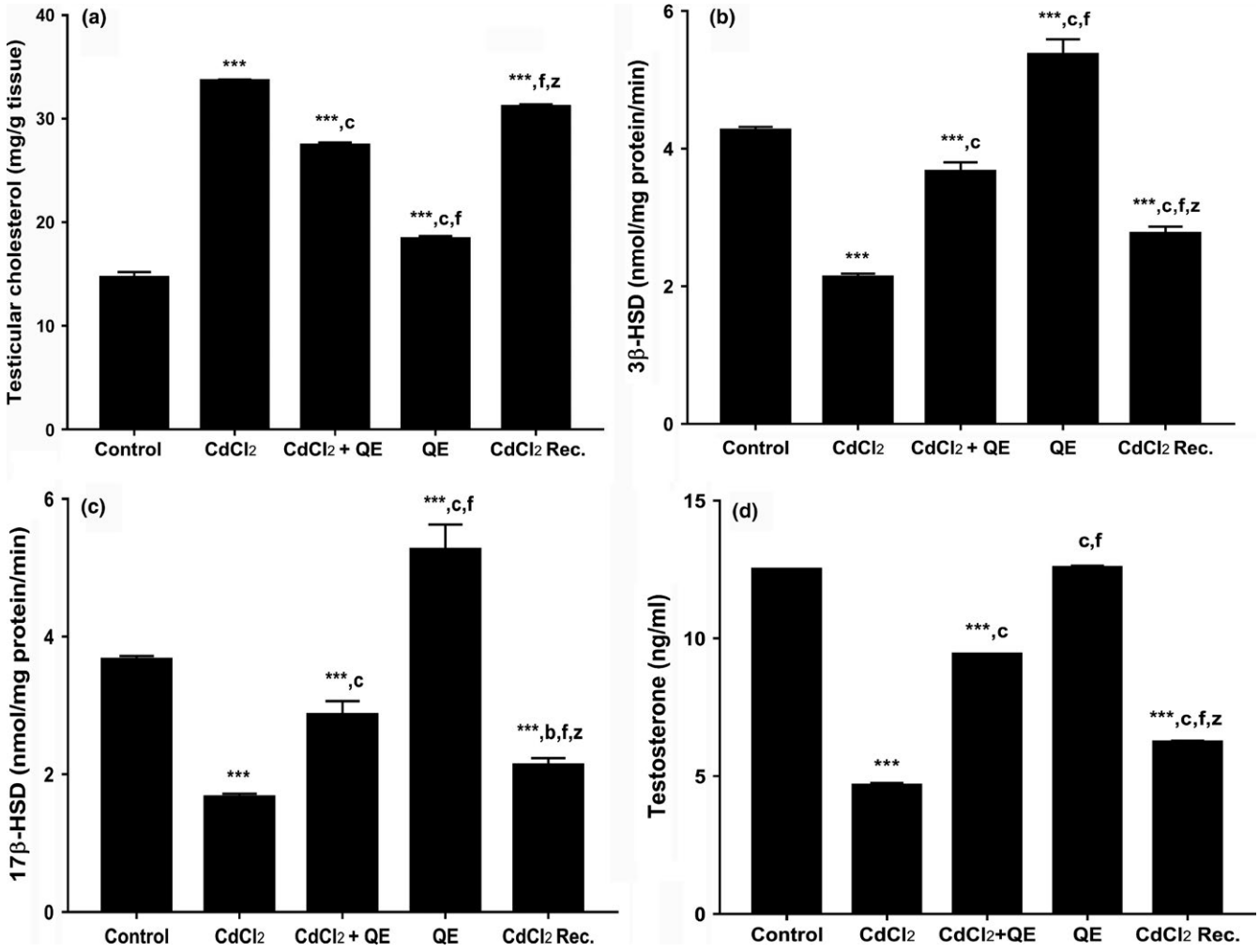

FIGURE 4 Comparison of testicular (a) cholesterol concentration, (b) 3β-HSD activity, (c) 17β-HSD activity and (d) testicular testosterone concentration between the different experimental groups. Values are mean ± *SEM*, *n* = 6. $^{***}p < .001$ versus control; b = $p < .01$, c = $p < .001$ versus $CdCl_2$; f = $p < .001$ versus $CdCl_2$+QE; z = $p < .001$ versus QE

3.10 | Testicular cholesterol concentration

Figure 4a shows that testicular cholesterol concentration increased significantly ($p < .001$) in the $CdCl_2$ (33.67 ± 0.04 mg/g tissue) and QE (18.40 ± 0.11 mg/g tissue) groups compared to the control (14.67 ± 0.21 mg/g tissue). Testicular cholesterol concentration decreased significantly ($p < .001$) in the $CdCl_2$+QE group (27.43 ± 0.11 mg/g tissue), compared to the $CdCl_2$ group, but was significantly high ($p < .001$) compared to the control. The $CdCl_2$ recovery group had a significantly higher ($p < .001$) cholesterol concentration (31.17 ± 0.09 mg/g tissue) compared to the control and $CdCl_2$+QE groups (Figure 4a).

3.11 | Testicular 3β-hydroxysteroid dehydrogenase activity

Figure 4b shows that $CdCl_2$ significantly reduced ($p < .001$) testicular 3β-HSD activity (2.13 ± 0.02 nmol/mg protein/min), while QE significantly increased ($p < .001$) testicular 3β-HSD activity (5.37 ± 0.09 nmol/mg protein/min), compared to the control (4.27 ± 0.02 nmol/mg protein/min). The activity of 3β-HSD increased significantly ($p < .001$) in the $CdCl_2$+QE group (3.67 ± 0.06 nmol/mg protein/min), compared to the $CdCl_2$ group, but decreased significantly ($p < .001$) compared to the control. The activity of 3β-HSD increased significantly ($p < .001$) in the $CdCl_2$ recovery group (2.77 ± 0.04 nmol/mg protein/min) compared to the $CdCl_2$ group, but decreased significantly ($p < .001$) compared to the control and $CdCl_2$+QE groups (Figure 4b).

3.12 | Testicular 17β-hydroxysteroid dehydrogenase activity

The activity of 17β-HSD decreased significantly ($p < .001$) in the $CdCl_2$ group (1.67 ± 0.02 nmol/mg protein/min), but increased significantly ($p < .001$) in the QE group (5.27 ± 0.15 nmol/mg protein/min), compared to the control (3.67 ± 0.02 nmol/mg protein/min) (Figure 4c). The activity of 17β-HSD was significantly increased ($p < .001$) in $CdCl_2$+QE group (2.87 ± 0.08 nmol/mg protein/min) compared to the $CdCl_2$ group, but significantly decreased ($p < .001$) compared to the control. The $CdCl_2$ recovery group (2.13 ± 0.04 nmol/mg protein/min) showed a significant increase ($p < .01$) in 17β-HSD activity, compared to the $CdCl_2$ group, but showed a significant decrease ($p < .001$) compared to the control and $CdCl_2$+QE groups (Figure 4c).

3.13 | Testicular testosterone concentration

Testicular testosterone concentration was significantly decreased ($p < .001$) in the $CdCl_2$ group (4.67 ± 0.09 ng/ml) relative to the control (12.50 ± 0.00 ng/ml) (Figure 4d). QE did not significantly alter testicular testosterone concentration (12.57 ± 0.08 ng/ml) relative to the control ($p > .05$). Testosterone was significantly high ($p < .001$) in the $CdCl_2$+QE group (9.42 ± 0.01 ng/ml) compared to the $CdCl_2$ group, but significantly low ($p < .001$) compared to the control. Testicular testosterone concentration was significantly high ($p < .001$) in the $CdCl_2$ recovery group (6.23 ± 0.06 ng/ml) relative to the $CdCl_2$ group, but significantly low relative to the control and $CdCl_2$+QE groups (Figure 4d).

4 | DISCUSSION

Several studies have reported the toxic effects of cadmium on different facets of the male reproductive system. However, the effect of cadmium on sexual behaviour, an important aspect of the male reproductive function, is scarcely reported. Additionally, there are no reports showing whether QE can ameliorate the likely effects of cadmium on sexual behaviour and reversal of negative effects following withdrawal of cadmium exposure.

In the present study, all aspects of sexual behaviour assessed were impacted negatively by $CdCl_2$. Mount and intromission frequencies which are both indices reflecting libido and sexual potency (Yakubu & Akanji, 2011) were both decreased following $CdCl_2$ exposure. Sexual motivation is indicated by mount and intromission latencies, the relationship being an inverse one (Yakubu & Akanji, 2011); that is, the higher the mount and intromission latencies, the lower the sexual motivation and vice versa. The present study showed that both mount and intromission latencies were increased following cadmium exposure. Similarly, ejaculatory latency and post-ejaculatory interval were both increased after $CdCl_2$ exposure. Although EL and PEI were decreased in the $CdCl_2$ recovery group compared to $CdCl_2$ group, the levels were still above those of the control group. Prolonged ejaculatory latency has been reported to be indicative of improved sexual function (Ahmad, Latif, & Qasmi, 2004; Yakubu & Akanji, 2011). However, in the context of this study, the prolonged ejaculatory latency caused by $CdCl_2$ was accompanied by decreased mount and intromission frequencies and increased ML, IL and PEI (an index of libido and recovery after first ejaculation). Thus, the prolonged EL observed in the $CdCl_2$-treated rats may be indicative of suppressed stimulation of the ejaculatory reflexes, and so an impairment of sexual function (Malviya, Jain, Gupta, & Vyas, 2011). Consistent with our findings, Clark et al. (1994) previously reported decreased mount and intromission frequencies, and decreased erectile function 48 and 72 hr, respectively, after exposure to cadmium. In the present study, co-administration with QE increased these indices to near control levels, while withdrawal of $CdCl_2$ did not seem to improve both mount and intromission frequencies, as no significant differences were seen when compared to the $CdCl_2$ treated group.

Sexual behaviour parameters, especially intromission, are indicative of erection efficiency and the ease with which ejaculatory reflexes are activated (Agmo, 1997). Erection is the result of actions of NO and its second messenger, cGMP (Boolell et al., 1996). In the signalling pathway, NO stimulates guanyl cyclase which in turn leads to cGMP accumulation by acting on GTP (Frielse, Schultz, & Koesling, 1996). Soluble guanyl cyclase (sGC) is thought to be the key enzyme for

NO-dependent cGMP accumulation in the penis (Kuthe et al., 2003). In the present study, $CdCl_2$ increased NO without a corresponding increase in penile cGMP concentration. It is likely that the sGC was not responsive to the $CdCl_2$-induced increase in serum NO concentration. The increase in NO following $CdCl_2$ administration may be to reduce oxidative damage and improve tolerance to cadmium (Xu, Wang, Yin, Sun, & Mi, 2010). When co-administered with $CdCl_2$, QE decreased NO concentration. As an antioxidant, it has been reported that QE reduced $CdCl_2$-induced oxidative stress (Stohs et al., 2001; Amara et al., 2008; Nna et al., 2017), causing a decreased NO concentration.

Cholesterol is the main substrate for the biosynthesis of testosterone. This process requires the enzymes 3β-HSD and 17β-HSD (Abarikwu & Farombi, 2014) among others. In the present study, while $CdCl_2$ increased testicular cholesterol concentration, it decreased the activities of 3β-HSD and 17β-HSD, consistent with previous reports (Gupta, Kim et al., 2004; Sadik, 2008), leading to a corresponding decrease in testicular testosterone concentration. This decrease in testicular testosterone amidst high cholesterol may be partly attributed to the decreased activities of 3β-HSD and 17β-HSD, which are important enzymes required for steroidogenesis. The suppressed activities of 3β-HSD and 17β-HSD decreased biosynthesis of testosterone by the testes, leading to accumulation of the substrate, cholesterol. Gupta, Gupta, et al., (2004) previously reported a decrease in gene expression of steroidogenic acute regulatory protein (StAR), which is responsible for cholesterol transport into the Leydig cell for steroidogenesis. In the light of the report of Gupta, Gupta, et al., (2004), it is likely that a greater percentage of the cholesterol in the $CdCl_2$ group may not have entered the Leydig cell, thus reducing the substrate's availability for testosterone biosynthesis. Furthermore, reports have shown that hydrogen peroxide, a powerful oxidant, impedes steroidogenesis in Leydig cells (Stocco, Wells, & Clark, 1993; Diemer, Allen, Hales, & Hales, 2003). As cadmium is known to increase oxidative stress in tissues, including the reproductive tissues (Stohs et al., 2001; Amara et al., 2008), consistent with our observation (data not shown), the Leydig cell count and viability may have been adversely affected by exposure to cadmium, thus contributing to the observed decrease in steroidogenesis. Decreased testosterone biosynthesis may be responsible, in part, for the poor sexual behaviour of rats in this study, because indices of sexual behaviour are known to show androgen dependence. Withdrawal of $CdCl_2$ treatment did not reverse the negative impact on steroidogenesis. This maybe attributable to the fact that cadmium accumulates in biological systems (Matsuno et al., 1991) and sustains its negative effects for a long time after exposure. Simultaneous administration of $CdCl_2$ and QE preserved steroidogenesis in this study. This may have been achieved through QE's antioxidant effect.

5 | CONCLUSION

This study shows that $CdCl_2$ adversely affects sexual behaviour. This effect can be most likely linked to the suppressed steroidogenesis and cGMP pathway for penile erection, with poor reversal following withdrawal of $CdCl_2$ treatment. Also, the present study has shown that QE attenuates $CdCl_2$-induced impairment in sexual behaviour by improving steroidogenesis and penile cGMP levels.

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CONFLICT OF INTEREST

None declared.

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