D-Ribose-L-Cysteine Attenuated Polychlorinated Biphenyls Mediated Neuroendocrine-Transmembrane Ionic Pump ATPase Disruption and Peroxynitrite Formation in a Rat Model: A Possible Role of Testicular Antioxidant and Androgenic Enzymes

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Abstract

The very hazardous and persistent aquatic pollutants known as polychlorinated biphenyls (PCBs) are known to bioaccumulate in a range of marine mammals. Human and animal studies have shown that exposure to PCBs affects the male reproductive system. This study was conducted to investigate the therapeutic effects of D-ribose-L-cysteine (DRLC) on PCB-mediated ATPase disruption of the neuroendocrine transmembrane ion pump and peroxynitrite formation in rat models. After the first 15 days of PCB treatment, DRLC (50 mg/kg) was administered orally for 15 days, beginning on day 16 and continuing through day 30. PCB (2 mg/kg) was administered for 30 days. All tests were performed in accordance with accepted procedures. Kisspeptin (kissp), gonadotropin-releasing hormone alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glutathione peroxidase (GSH-Px), and total antioxidant capacity (TAOC) were all analyzed in blood on day 31. The testes were also removed and sent for testosterone, testicular antioxidant enzymes (L-C and PrDx-4), androgenic enzymes (3β-HSD and 17β-HSD), sulfhydryl content, and transmembrane ion pump (Na^+, K^+, Ca^{2+}, Mg^{2+}, and H^+ ions) The results of the study showed that Kisspeptin, GnRH, Testosterone, L-C, PrDx-4, 3-HSD, 17-HSD, HOST, Na^+, K^+, Ca^{2+}, Mg^{2+}, and H^+-ATPase levels were significantly increased by DRLC. In addition, DRLC therapy dramatically reduced levels of ONOO-, H2O2, 8-OHdG, ALP, and LDH, while increasing TAOC to restore GSH-Px levels. The results of the present study suggest that DRLC is a potentially effective therapeutic approach to reduce PCB-induced neuroendocrine transmembrane ion pump disruption in an experimental rat model. The primary mechanism underlying this effect may be its antioxidant activities against the formation of peroxynitrite and elevated levels of hydrogen peroxide.

Keywords: DRLC, PCBs, Ionic pump, Peroxynitrite, Sulphyldryl, HOST, Peroxiredoxin-4, Neuroendocrine

Introduction

A group of dangerous compounds known as PCBs (polychlorinated biphenyls) has been linked to a number of harmful health consequences on both people and wildlife [1,2]. PCBs continue to be released into the environment, and paints and sealants are assumed to be the main sources of current environmental discharges because they are still in "open application" [3,4]. Notably, PCB is recognized to pose a toxicological risk, which has been linked to immunological and reproductive system suppression [4-6].

Many studies [5,7] have shown associations between PCB exposure and reduced reproductive output, including decreased female fertility, greater embryonic loss, and higher calf mortality. Furthermore, both human and experimental animal studies [5,7-9] have extensively examined the effects of PCB exposure on male fertility. For instance, males' circulatory testosterone levels and sperm motility were found to be negatively correlated in human epidemiological investigations [8, 9]. Other mammals' smaller seminal vesicles, epididymis, and testicles as well as decreased sperm counts, spermatid counts, and plasma testosterone levels have all been connected to PCB exposure [10,11]. In this setting, antioxidant supplements such as D-ribose-L-cysteine (DRLC) become crucial. Furthermore, the potential of D-Ribose-L-repropharmacological cysteine's actions for the treatment of reprotoxicant-related repropathologies is gaining attention [12-16].

A ribose and cysteine derivative called D-ribose L-cysteine was created as a pro-drug to increase GSH synthesis [16]. It is primarily patented for increasing glutathione levels and as a dietary supplement to guard against illnesses linked to oxidative stress [17]. The fragile cysteine molecule is effectively delivered to the cell by D-ribose L-cysteine, which also enables glutathione to be produced quickly and spontaneously.

The DRLC molecule is also utilized by the cell as a crucial component in the creation of ATP, the body's source of energy. The study found that MDA-mediated GSH-deficient rats showed significant spermatogenesis/steroidogenesis defects and germ cell depletion, both of which are crucial in the pathophysiology of infertility [14-16, 18]. To maintain the integrity of the sperm membrane and prevent the onset of reprotoxicity in patients with infertility issues, increasing testicular GSH levels may therefore be a better approach [16]. In this respect, it was expected that D-ribose l-cysteine therapy for PCB-related reproductive problems would improve the procreative capacity of male rats.

Materials and methods

Animals

For this experiment, 20 male Wistar rats weighing 150 - 250g and aged 6 - 8 weeks were employed, all of which were purchased from the Central Animal Housing facility (CAHF) of Achievers University (AU). The animal was kept in a controlled setting with a 12:12-h cycle of light and dark. Before the trial began, the animals were acclimatized for 14 days with unlimited access to water and food. The oral route will be used for all dosing between 8 and 9 am for the next 4 weeks. The National Institutes of Health (NIH) Guideline for the Care and Use of Laboratory Animals was established, and the study techniques utilized to handle the animals were in accordance with it (Publication No. 85-23, revised). Based on the 3 Rs (3Rs: Replacement, Reduction, and Refinement), 5 animals per group were employed in this investigation by Oyovwi *et al.* [19,20].

Chemicals, drugs and their preparations

DRLC and PCBs were bought from Max International in Salt Lake City, Utah, and Sigma-Aldrich Chemical Company in St. Louis, Missouri, respectively. Just before use, DRLC was dissolved in ordinary saline and given orally. Normal saline [12], PCB [21], and DRLC [12], doses and methods were selected based on prior dose-response effects and exploratory research. To serve as normal control, however, naive rats were given normal saline (10 mL/kg, p.o.) and were divided into various groups. For 4 weeks, all administration was done orally between the hours of 8 and 9 am.

Experimental protocol

Twenty-four rats were used and was divided into 4 treatment groups with 6 animals ($n = 6$) each. Rats in group 1 served as the control group and received normal saline (10 mL/kg, p.o.), while group 2 was given D-ribose-L-cysteine (50 mg/kg, p.o.) daily for 15 days, and group 3 was given PCB (Aroclor 1254) at a dose of 2 mg/kg, p.o., for 30 days without interruption. However, DRLC therapy was administered to rats in group 4 from days 16 through 30 in addition to PCBs for 30 days.

Blood and tissues preparation

Twenty-four h following the final dose, the treated rats were euthanized, and heparinized tubes were used to collect blood from the retro-orbital venous plexus. Plasma samples were obtained by centrifuging the blood samples at 3,000 rpm for 10 min. The plasma samples were then kept frozen at −20°C until tests for total antioxidant capacity (TAOC), lactate dehydrogenase (LDH), glutathione peroxidase (GSH-Px), gonadotropin-releasing hormone, Kisspeptin and alkaline phosphatase (ALP) were run using an ELISA strip reader (Robonik India Private Limited, Mumbai, India). The testes were also removed and sent for testosterone, testicular antioxidant enzymes (L-C and PrDx-4), androgenic enzymes, (3β-HSD and 17β-HSD) sulfhydryl content and transmembrane ionic pump (Na^+, K^+, Ca^{2+}, Mg^{2+}, $H+$ ions) measurement.

Estimation of reproductive hormones

The levels of kisspeptin (kissp), gonadotropin-releasing hormone (GnRH), and testosterone were measured in serum and testicular samples, respectively using ELISA kits purchased from Diagnostic Systems Laboratories, Inc. (Monobind Inc., USA). The manufacturer's instructions as well as Oyovwi *et al.* [22], procedures were followed.

Assessment of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH)

Following the manufacturer's instructions, Sigma Aldrich Co. provided kits for measuring the activity of ALP and LDH.

Assessment of serum oxidative stress markers

The Oyovwi *et al.* [20], approach was used to measure the serum glutathione peroxidase (GPx) activity by dismutating $H2O2$ at 340 nm via the glutathione/NADPH/glutathione reductase system. According to the manufacturer's instructions, serum total antioxidant capacity (TAOC) was evaluated using assay kits from Elabscience, USA.

Determination of the activities of testicular proton pumps ATPase activities

Oyovwi *et al.* [20], procedure was utilized to assay tissue homogenate to evaluate testicular $Na+/K+$ ATPase activity. In a reaction mixture consisting of 0.8 mL of ice-cold 10% (w/v) trichloroacetic acid (TCA), 1 mL of ammonium molybdate, and 1 mL of 9 % ascorbic acid. A spectrophotometer was used to measure the absorbance at 725 nm [23]. A reaction combination of 0.1 mL supernatant, 1 mL 1.25 % ammonium molybdate, and 1 mL 9 % ascorbic acid was used to evaluate the activity of the testicular $Ca2+$ ATPase. The absorbance at 725 nm was then measured using a spectrophotometer. Based on a modified procedure that was previously reported [20], $H+$ activity was estimated. As previously reported [20], a reaction mixture containing tissue homogenate, 375 mM Tris HCl buffer (pH 7.6), 25 mM $MgCl2$, distilled water, and 10 mM ATP was used to evaluate the activity of $Mg2+−ATPase$.

Sperm membrane and oxidative sperm DNA integrity determination

The Hypo-osmotic Swelling Test (HOST) was performed in accordance with accepted procedures [24]. In 100 mL of distilled water, 0.735 g of sodium citrate dehydrate and 1.351 g of fructose were combined to create the hypo-osmotic solution. One milliliter of the hypo-osmotic solution was mixed well with 0.1 milliliter of epididymal sperm suspension. After mixing, the sperm suspension reaction mixture was incubated at 37°C for 30 to 60 min. A drop of the mixture was incubated before being placed on a glass slide and covered with a fresh cover slip. To see the enlargement of the sperm tail, the slide was viewed via a ×40 lens. Spermatozoa without swelling tails (abnormal spermatozoa) were thought to be HOS Negative, while sperm with swollen tails (regular spermatozoa) was thought to be HOS Positive/reactive. The proportion of HOS-positive cells was calculated from a total of 100 sperm cells. Accordingly, 8-hydroxy-2′-deoxyguanosine (8OHdG), an index of oxidative sperm DNA damage, was determined using a standard ELISA kit (Elabscience Biotechnology Co., Ltd, USA) following the manufacturer's manual.

Testicular non-enzymatic (L-Carnitine and sulfhydryl) and enzymatic (Peroxiredoxin-4) antioxidant assays

Epididymal L-Carnitine (L-C) was estimated as μmol/mg protein before deproteinization [25]. Moreover, membrane testicular content of sulfhydryl was measured, also, using sodium dodecyl sulfhate (10%) together with DTNB and plasma membrane suspension at 405 nm and later, expressed at nm SH/mg protein [26]. Peroxiredoxin-4 in testes was assayed based on peroxiredoxin antibody-peroxiredoxin antigen interactions (immunosorbent) using an Enzyme linked-immunosorbent assay

(ELISA) kit purchased from R&D Systems, Inc., United State of America-USA), with sensitivity and intra- and inter-assay variation coefficients of 0.094 ng/mL, < 8 % and < 10 %. The assay was performed in line with the manufacturer's instructions.

Determination of peroxynitrite and hydrogen peroxide in rat testicular tissues

Peroxynitrite was identified using a spectrophotometric absorbance of 412 nm from phenol induce nitration in a reaction with sodium phosphate buffer pH 7.4 and 0.1M NaOH [27]. In a reaction mixture containing homogenate [28], alkaline copper sulfhate solution, and Folin-phenol Ciocalteau's reagent (25 °C), the protein content of the testes was measured at 750 nm and expressed as mg/mL. The H_2O_2 was assayed via an incubation reaction with phosphate buffer (50 mM, pH7.6), horse radish peroxidase (8.5 units/mL) 0.28 nM phenol red, 5.5 nM dextrose in the presence of an enzyme source (35°C). Generated level of H_2O_2 was quantified as μmol/min/mg protein. Furthermore, nitrite level was also measured as an index of nitrergic stress using the Griess reagent [29].

Determination of testicular 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) activities

The Oyovwi*et al.* [20], method was used to measure the activities of 3ß-hydroxysteroid dehydrogenase (3ß-HSD) and 17ß-hydroxysteroid dehydrogenase (17ß-HSD). Sodium pyrophosphate buffer (pH 9.0), cofactors NAD for 3ß-HSD and NADPH for 17ß-HSD, a substrate (dehydroepiandrosterone for 3ß-HSD and androstenedione for 17ß-HSD), and 100 L of testicular protein were all included in the reaction mixture. The reactions were conducted at 23 °C in a quartz cuvette with a 1.0 cm route length. In a UV-Vis spectrophotometer, the absorbance at 340 nm was measured every 20 s for 3 min (UV-1700 Shimadzu, Japan). The enzyme activities were measured in terms of the amount of NAD that was converted to NADH or NADP per minute per milligram of protein (3ß-HSD) or 17ß-HSD, respectively.

Statistical data analysis

The use of Graph-Pad Prism 8 was required for the statistical analysis. Mean ± standard error of the mean was used to express the values of the measured parameters (S.E.M). One-way analysis of variance (ANOVA), followed by Bonferroni multiple comparison tests, was employed for comparisons between the various groups. When the *p*-value was less than 0.05, the findings were deemed statistically significant.

Results and discussion

DRLC abates PCBs-induced alteration in neuro-endocrine-related factors in rats

As presented in **Figures1(a) - 1(c)**, a one-way analysis of variance (ANOVA) followed by a posthoc test revealed that PCBs significantly ($p < 0.05$) decreased Kisspeptin (**Figure 1(a)**) and GnRH (**Figure 1(b)**) activities, as well as decreased testicular testosterone level when compared to normal control groups. In comparison to PCB-treated rats, DRLC treatment dramatically restored PCB-induced changes in serum kisspeptin, serum GnRH and testicular testosterone levels (**Figures 1(a) - 1(c)**).
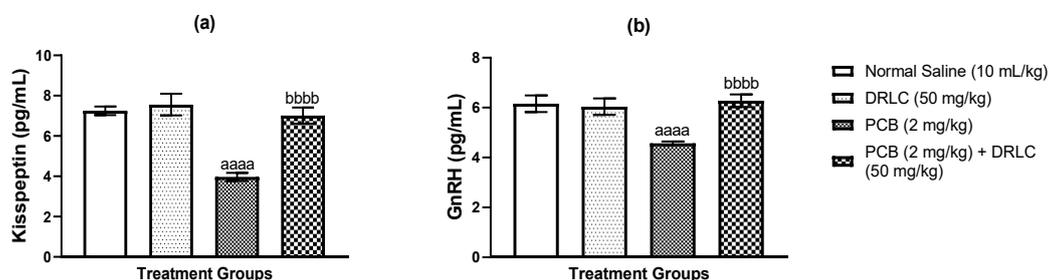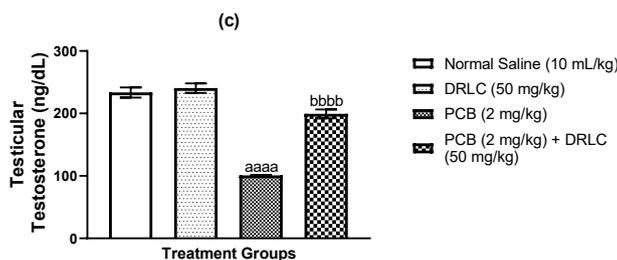
Figure 1 (a-c). In male Wistar rats, DRLC reduce PCB-induced changes in the following neuroendocrine-related factors: Kisspectin (Kissp), Gonadotropin-releasing hormone (GnRH), and testosterone, TS (c). The mean and S.E.M. are shown as bars (n = 6). After the one-way ANOVA, the Bonferroni posthoc test was performed. aaaa $p < 0.0001$ as compared to control group and bbbb $p < 0.0001$ as compared to DRLC group, respectively.

DRLC PCBs-induced alteration in testicular androgenic enzymes activities in rats'testes

The impact of DRLC on the levels of testicular androgenic enzyme activities in rats treated with PCBs is depicted in **Figures 2(a)** - **2(b)**. In the reversal protocols, PCBs reduced 3ß-HSD (**Figure 2(a)**) and 17ß-HSD (**Figure 2(b)**) in comparison to control groups. DRLC reversed the decreased in 3ß-HSD (**Figure 2(a)**), and 17ß-HSD (**Figure 2(b)**) induced by PCBs. However, as compared to normal control groups, the effects of DRLC therapy alone on 3ß-hydroxy steroid dehydrogenase (3ß-HSD) and 17ß-hydroxy steroid dehydrogenase (17ß-HSD) levels were insignificant.
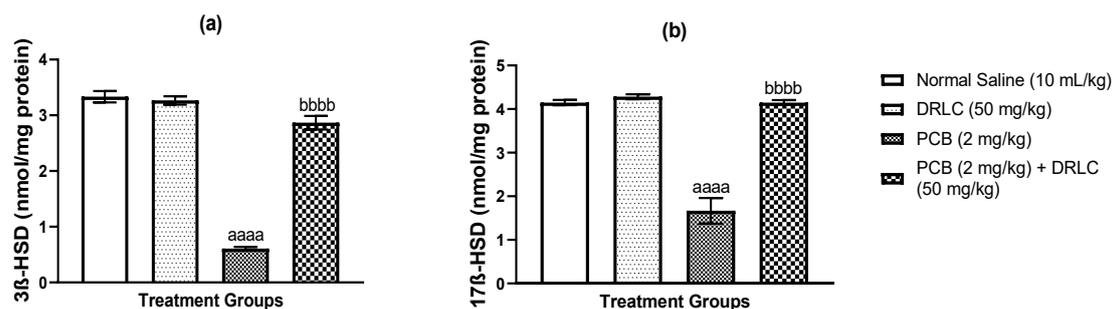

Figure 2 (a-b). In rats, DRLC reduces PCB-induced changes in testicular 3ß-hydroxy steroid dehydrogenase (3ß-HSD) (a) and 17ß-steroid dehydrogenase (17ß-HSD), (b) activity. Bars represent the mean ± S.E.M (n = 6). After the one-way ANOVA, the Bonferroni posthoc test was performed. In comparison to the control group, aaaa $p < 0.0001$; in comparison to the DRLC group, bbbb $p < 0.0001$.

DRLC increases ionic-pumps ATPases activities in rat's testes exposed to PCBs

PCBs lowered Na+/K+ ATPase (**Figure 3(a)**), Ca2+ ATPase (**Figure 3(b)**), Mg2+ ATPase (**Figure 3(c)**), and H+ ATPase (d) activity in rats when compared to normal control groups, as demonstrated in **Figures 3(a)** - **3(d)**. DRLC therapy significantly reversed PCBs-mediated Na+/K+, Ca2+ Mg2+, and H+ ATPase alterations in rats treated with PCBs (**Figures 3(a)** - **3(d)**).
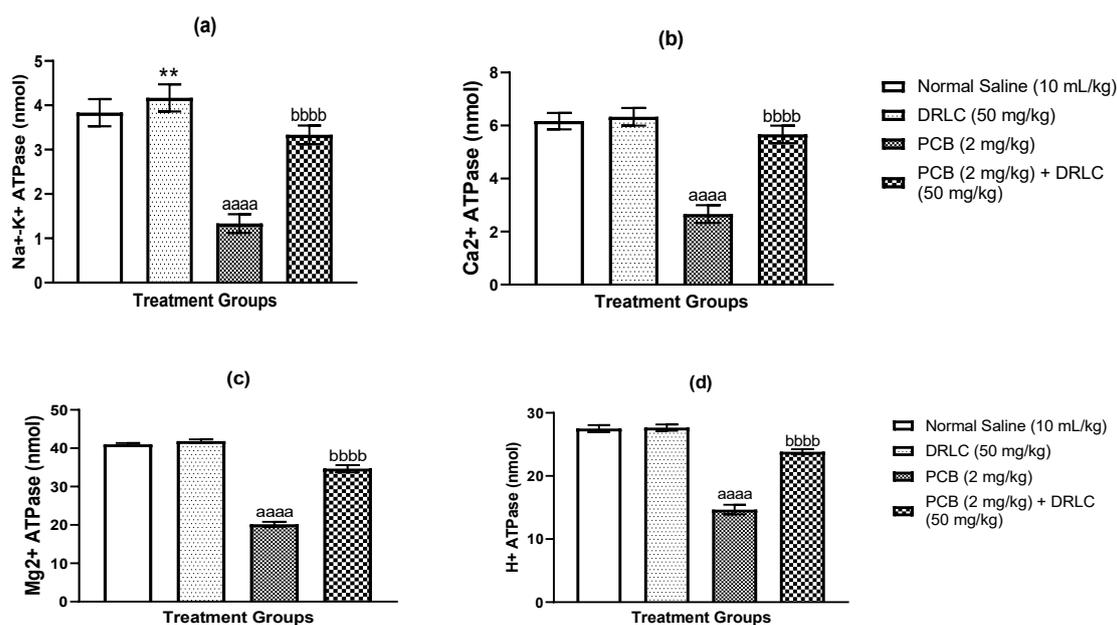
Figure 3 (a-d). DRLC increases ionic-pumps activities in rat testes submitted to PCBs: Na⁺/K⁺ ATPase (a), Ca^{2+} ATPase (b), Mg^{2+} ATPase (c), and H⁺ ATPase (d) activities. The mean ± S.E.M. are shown as bars (n = 6). After the one-way ANOVA, the Bonferroni posthoc test was performed. **$p < 0.01$, aaaa $p < 0.0001$, and bbbb $p < 0.0001$ when compared to the control group and the DRLC group, respectively.

DRLC increases PrDx-4 (Peroxiredoxin-4) and L-C (L-Carnitine) concentration in rats testes submitted to PCBs

Figures 4(a) - **4(b)** shows the impact of DRLC treatment on PCBs-mediated decreases in testicular L-C (**Figure 4(a)**) and PrDx-4 (**Figure 4(b)**) concentration in rats. PCBs administration mediated a marked decrease in PrDx-4 and L-C levels in rat testes, but DRLC significantly ($p < 0.05$) reversed this deficiency (**Figure 4**).
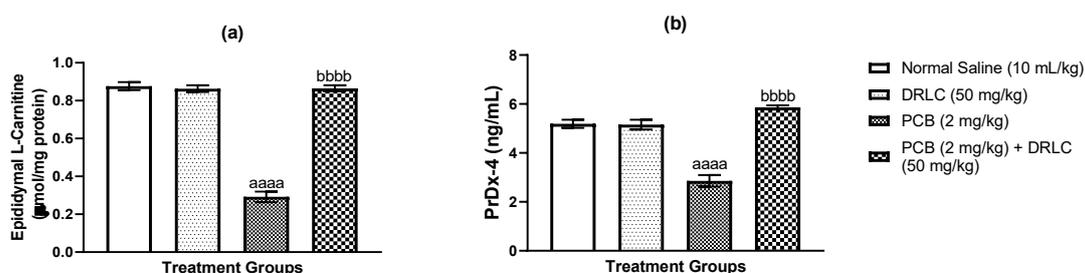

Figure 4 (a-d). DRLC increases PrDx-4 (Peroxiredoxin-4) and L-C (L-Carnitine)activities in rat testes submitted to PCBs L-C (L-Carnitine),(a) and PrDx-4 (Peroxiredoxin-4)(b). The mean ± S.E.M. are shown as bars (n = 6). After the one-way ANOVA, the Bonferroni posthoc test was performed. bbbb $p < 0.0001$ compared to the DRLC group; aaaa $p < 0.0001$ compared to the control group.

DRLC abates PCBs-induced peroxynitrite (ONOO⁻) formation and sulfhydryl (SH) depletion in rats'testes

As presented in **Figure 5**, PCBs treatment exhibited a significantly depleted sulfhydryl *(SH) content* (**Figure 5(a)**) and an increase in *peroxynitrite*ONOO⁻ (**Figure 5(b)**) when compared with control animals. Interestingly, DRLC also significantly reversed the elevated *peroxynitrite* and reduced sulfhydryl levels as compared to the PCBs group (**Figures 5(a)-5(b)**).
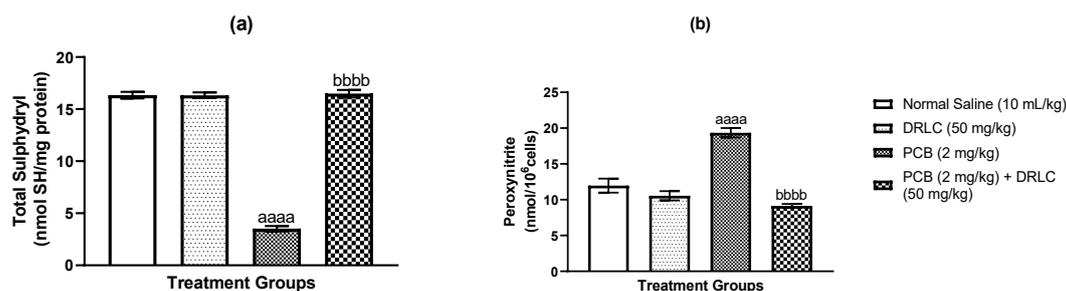
Figure 5 (a-b). DRLC abates *PCBs-induced peroxynitrite (ONOO⁻) formation and sulphydryl (SH) depletion in rat's testes*. The mean ± S.E.M. are shown as bars (n = 6). After the one-way ANOVA, the Bonferroni posthoc test was performed. bbbb $p < 0.0001$ compared to the DRLC group; aaaa $p < 0.0001$ compared to the control group.

DRLC enhances sperm membrane and sperm DNA integrity in rats treated with PCBs as determined by the hypo-osmotic swelling test (HOST) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) analysis

The reversal impacts of DRLC on PCBs-mediated changes in sperm membrane and sperm DNA integrity as HOST and 8-OHdG investigations are shown in **Figures 6(a) - 6(b)**. PCBs altered sperm membrane and sperm DNA integrity as indicated by reduced HOST (**Figure 6(a)**) and increased 8-OHdG (**Figure 6(b)**) values of rat's cauda epididymal spermatozoa respectively. However, DRLC therapy significantly reduced PCB-induced changes in HOST and 8-OHdG in comparison to rats treated with PCBs (**Figures 6(a)-6(b)**). Furthermore, sperm membrane integrity was much higher in DRLC than in the control group, as indicated by higher HOST levels, whereas sperm DNA integrity was lower, as indicated by a decline in 8-OHdG. (**Figures 6(a)-6(b)**).
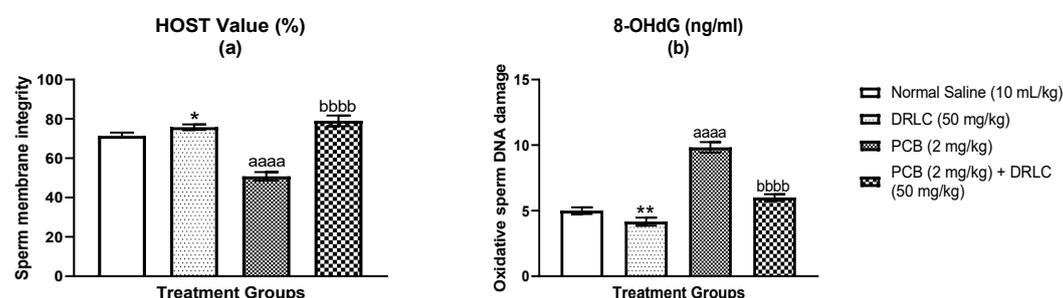

Figure 6 (a-b). DRLC enhances sperm membrane (a), as well as sperm DNA integrity (b) in rats treated with PCBs. The mean and S.E.M. are shown as bars (n = 6). After the one-way ANOVA, the Bonferroni posthoc test was performed. $^{*}p < 0.05$, $^{**}p < 0.01$ aaaap and bbbbp are significantly different from the control group and the DRLC group, respectively, by < 0.0001. HOST: 8-OHdG (8-hydroxy-2′-deoxyguanosine), hypoosmotic swelling test.

DRLC attenuates PCBs-induced increased alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in rats' testes

In comparison to the control group, PCBs caused a substantial rise (p-value < 0.0001) in serum ALP. Compared to rats treated with PCBs alone, the administration of the DRLC combination resulted in a considerable reduction in the levels of ALP (**Figure 7(a)**). Additionally, as compared to the control group, the use of PCBs resulted in a substantial increase (p-value < 0.0001) in the level of LDH, while the use of the DRLC combination resulted in a decrease in the level of LDH that was almost identical to the level produced by the control group (**Figures 7(a) -7(b)**).
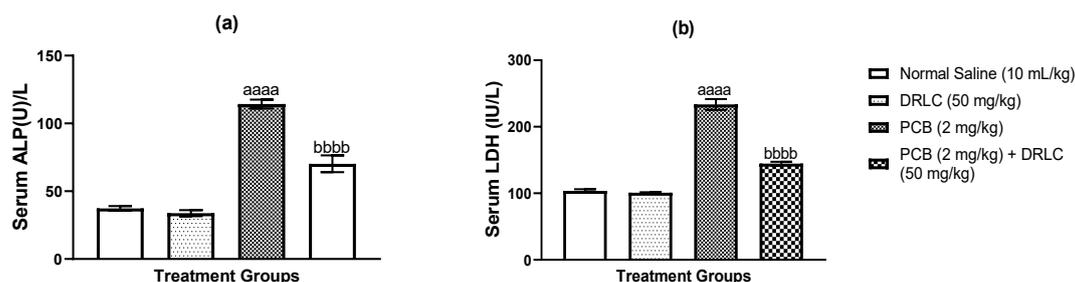
Figure 7(a-b). DRLC attenuates *PCBs-induced increased alkaline phosphatase (ALP) (a) and lactate dehydrogenase (LDH) (b) in rat testes*. The bars display the mean ± S.E.M. for n = 6. The one-way ANOVA was followed by the Bonferroni posthoc test. When compared to the control group, aaaa *p* < 0.0001, and bbbb *p* < 0.0001 when compared to the DRLC group.

DRLC mitigates against PCBs-mediated changes in oxidative stress markers in rats'testes

In comparison to the control group, PCBs caused a statistically significant drop (*p*-value < 0.0001) in the level of GPx. However, using DRLC along with PCBs nearly brought the level of GPx back to normal (**Figure 8(a)**). In addition, PCBs raised H2O2 concentration (**Figure 8(c)**) and considerably lowered (*p*-value 0.0001) the level of TAOC (**Figure 8(b)**) in comparison to the control group. It's interesting to note that the application of DRLC therapy had effects comparable to those of the control group in relation to the PCBs group alone.
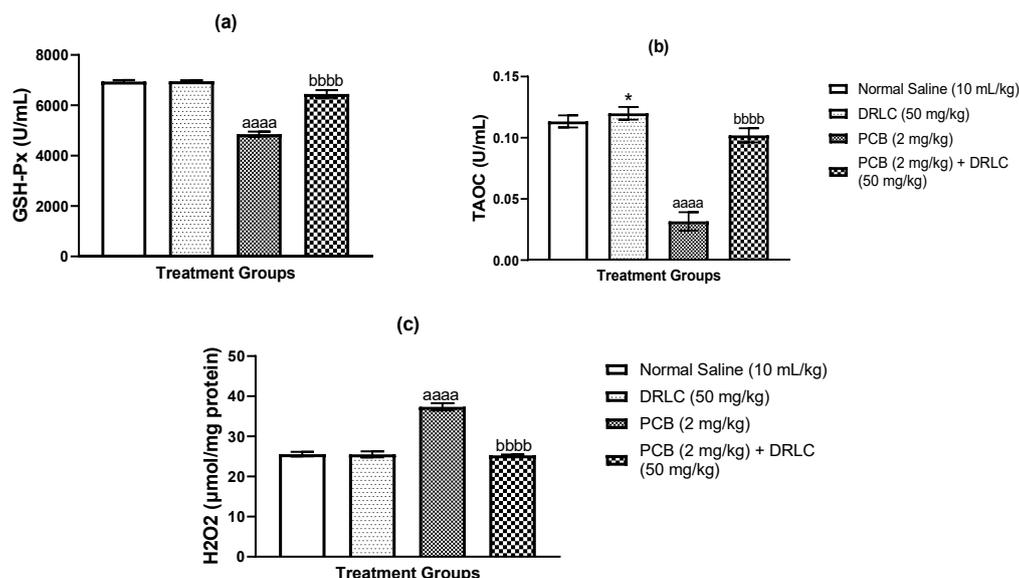

Figure 8(a-c). DRLC*mitigates against PCBs-mediated changes in oxidative stress markers in rat' testes*. Bars represent the mean ± S.E.M (n = 6). Following the one-way ANOVA, the Bonferroni posthoc test was used. aaaa *p* < 0.0001 when compared to the control group; bbbb *p* < 0.0001 when compared to the DRLC group GSH-Px stands for glutathione peroxidase, TAOC is for total antioxidant capacity, and H2O2 stands for hydrogen peroxide.

The main goal of this study was to look into the potential therapeutic effects of DRLC against the development of PCB-mediated disruption of neuro-endocrine-transmembrane ionic pump ATPases and peroxynitrite formation in an experimental rat model because this supplement has previously been shown to have better effects when used for human consumption in the management of a variety of health problems by improving antioxidant status [18,13-16,30].

Gonadal injury is a common adverse effect of numerous toxicants and chemotherapeutic medications, affecting both the endocrine and exocrine processes of the testis [31,32]. Normal pulsatile

GnRH levels are connected with the cyclic release of LH and FSH, and Kisspeptin is hypothesized to play a role in GnRH release. It's also important for adult fertility control and sex hormone-mediated feedback control regulation. Reduced kisspeptin levels indicate an issue with the kiss1 gene and hypothalamus function, which could result in lower levels of the male reproductive hormone. It's also worth mentioning that GnRH blocking has been linked to lower LH and FSH levels. Kisspeptin modulates the HPG axis by acting on GnRH neurons, which regulate testicular function by boosting testosterone production through LH and FSH release [32]. In this study, PCBs affected the neuro-endocrine function when compared to a control group; they appeared to lower Kisspectin, GnRH, and testosterone levels considerably. This could be due to a change in the nuclei that control the substance's release in the hypothalamus. This could be the principal method through which they disturb the neuroendocrine system. Nonetheless, it has been found that the spermatogenic process is testosterone-dependent [22], and that testosterone and other testicular androgens can initiate and maintain spermatogenesis [22]. As a result, low testosterone levels may cause germ cell degeneration and spermatogenic arrest. Other studies have found that PCBs cause a considerable decrease/inhibition in testicular androgenesis, which will have an impact on spermatogenesis as well as the structural architecture of the seminiferous tubules [33,34].

Surprisingly, as compared to the group treated alone with PCBs, the therapeutic usage of the DRLC resulted in a considerable increase in Kisspectin, GnRH, and testosterone levels. The spike in serum levels of ALP and LDH generated by PCBs, which was corroborated by testicular antioxidant enzymes and androgenic enzyme measurements, was another sign of gonadotoxicity. Adenosine nucleotides are converted to adenosine, a potent vasodilator and anti-inflammatory mediator that can shield tissues from physical harm, in part due to the activity of ALP on the surface of endothelial cells [35,36]. LDH, on the other hand, plays a part in the metabolism of the testicles and spermatogenesis [20].

Testicular injury may be caused by changes in the levels of these enzymes. ALP and LDH levels were practically normalized when DRLC was used, which could be ascribed to DRLC's antioxidant and anti-inflammatory characteristics. DRLC has been shown to reduce the harmful effects of PCBs on reproductive potential in the past [13-16,18,30]. The potential of DPP-4 to counteract mitochondrial dysfunction, boost energy generation, and decrease DNA damage caused by PCBs [37], could be the hypothesized mechanism. Additionally, treatment with DPP-4 inhibitors has been reported to interfere with sperm forward motility. Other research suggests that the DPP-4 enzyme is involved in sperm mobility control [38].

An increasing body of research has shown that testicular androgenic enzymes like (3-HSD and 17-HSD) have a regulatory androgenic function and are involved in oxidative phosphorylation in the testes [20]. Androstenedione is known to be converted into testosterone in the seminiferous tubes of the testes by 3-HSD and 17-HSD, which enhances sexual function and gamete fertility. In this study, it was discovered that PCBs significantly decreased the amounts of 3-HSD and 17-HSD in the testicles, which corresponded to reduced levels of testosterone and decreased sperm maturation, acrosomal response, and fertilization. Better testicular function and sperm maturation, as well as reproductive advantages, are suggested by the protective effects of DRLC against PCB-induced biogenic depletion of 3-HSD and 17-HSD [20,39].

Regarding the impact of PCBs on oxidative profile in this study, PCBs caused a substantial decrease in GSH-Px and TAOC levels, as well as an increase in H_2O_2. It has been found that oxidative stress is a key factor in PCB-induced gonadal damage. The capacity of PCBs to interact with cellular macromolecules is thought to be the fundamental mechanism of their involvement in oxidative damage [18,40-42]. Furthermore, PCBs can raise the ratio of superoxide dismutase to GPx and/or catalase, increasing H_2O_2 buildup and oxidative DNA damage [43]. A high concentration of H_2O_2 may produce the hydroxyl radical (OH•), one of the deadliest free radicals due to its high reactivity and brief half-life, which swiftly initiates DNA cross-linking through the Fenton reaction and lipid peroxidation. The antioxidant enzyme activity and antioxidant capacity were both increased by DRLC in the current investigation. This could be due to a reduction in lipid peroxidation. This result was in line with Cocco *et al.* [37], and Kabel *et al.* [38], who claimed that greater GLP-1 levels, which could be avoided by a GLP-1 receptor antagonist, were responsible for the antioxidant advantages of DPP-4 inhibitors. Additionally, it has been demonstrated that GLP-1 stimulates AMP-activated protein kinase, which raises the activity of antioxidant enzymes and significantly reduces oxidative stress. Our findings revealed that DRLC might also restore GSH peroxidase activity and increase antioxidant capacity. DRLC, a natural flavonoid, is well-known for its cytoprotective properties, which include lowering lipid peroxidation and enhancing the antioxidant system [40,44,16].

Peroxynitrite production, for example, is known to cause negative effects such as protein nitrosation, which alters membrane functioning. Indeed, elevated NO peroxynitrite levels in the sperm are

closely linked to sperm capacitation, sperm oocyte binding, and infertility [45]. PCBs have been shown to cause up-regulation and production of pro-oxidant radicals in the hypothalamus arcuate nucleus, as well as nitration of redox-sensitive tyrosine hydroxylase, the key enzyme required for dopamine synthesis. Francis *et al.* [46], has linked this effect to a lack of sexual satisfaction and infertility. As a result, decreased TAOC and peroxynitrite production have been recognized as key factors in testicular inflammation, cell membrane structural derangement, and testicular performance alterations [46]. However, the fact that DRLC dramatically reduced the impacts of PCB-induced decreased TAOC activities, as well as hydrogen peroxide and peroxynitrite levels, suggests that it has antioxidant activity in the testes.

Ion-activated adenosine triphosphatases (ATPase) are trans-membrane ionic pumps that play a key role in metabolite exchange between Sertoli and developing germ cells and are used to assess the germinal epithelium's metabolic health [47]. As a result, changes in these enzyme pumps have an impact on the testis' physiological function, potentially reducing sperm motility. Although the mechanisms behind changes in ATPase activity are complex, oxidative stress has been suggested as a role in testicular ATPase dysfunction [48,49]. Furthermore, ATPases are sulphydryl (SH)-containing enzymes, and their thiol groups may be a target for nitrite and its derivatives, such as peroxynitrite [50]. One of the main causes of sperm failure is the generation of peroxynitrite, which depletes the free thiol content of the sperm membrane, which is required for sperm motility and metabolism [50]. It is important to note that ionic pumps such as Na^+, K^+, Ca^{2+}, and Mg^{2+} ATPase are markers of the metabolic state of the germinal epithelium, i.e. spermatogenesis and testicular metabolism, and play a critical role in the exchange of metabolites between Sertoli and developing germ cells. An essential biological mechanism for sperm maturation and storage is the acidification of the epididymis and vas deferens segments, which is carried out by cells rich in H^+−ATPase [51]. Because of ATPase disruption, it has been shown that impaired acidity of these segments slows sperm maturation [20]. PCBs exposure induces lower testicular Na^+K^+−, Ca^{2+}−, Mg^{2+}−, and H^+−ATPase activity, implying a change in testicular ionic pump ATPase balance, according to our findings. Treatments with DRLC, on the other hand, dramatically reduced the effects of PCBs on Na^+/K^+ ATPase, Ca^{2+} ATPase, and H^+ ATPase activities, suggesting improved membrane function and sperm motility cellular components, and hence improved spermatogenesis and sperm function.

HOST is a useful endpoint for evaluating spermatozoa's capacity and potential to influence fertilization [52]. It measures osmotic stress, sperm folding, and sperm chromatin integrity. The structural integrity of both the sperm membrane and the acrosomal membrane is dependent on HOST value and PrDx-4 concentration, and its low concentration may harm the structural integrity of both the cell membrane and the acrosome, resulting in changes in sperm motility and fertilizing ability [53]. Meanwhile, in the present study, HOST values were altered in PCBs-treated rats. However, the significantly higher HOST value reported in this investigation with DRLC-treated mice is compelling evidence that DRLC has the potential to minimize spermatozoa friction. It could also indicate an increase in structural sperm membrane integrity (as measured by an increase in HOST value), which could stimulate spermatozoa metabolism, motility, and fertilizing capacity [53,10], demonstrating its potential protective mechanism against testicular injury caused by PCB-induced peroxynitrite formation and oxidative sperm DNA damage (Yakubu *et al.*, 2007; Ahmed *et al.*2019) Regardless, the study's originality indicated that DRLC delivered in this way prevents peroxynitrite production caused by PCBs and reduces oxidative sperm DNA damage (8-OHdG).

The significance of altered L-Carnitine (L-C) in the pathophysiology of reproductive dysfunction has been widely established, relation to the interaction of oxidative stress and membrane phospholipids [54]. As a result, determining L-Carnitine biochemical changes as a function of epididymal activity and spermatozoa metabolism becomes critical. Indeed, L-C is abundant in male reproductive organs, including the epididymis, and it plays a critical role in sperm metabolism and maturation, particularly in terms of energy disbursement for the spermatogenic process, sperm maturation, and motility [55]. L-Carnitines have also been shown to defend against ROS by acting as an antioxidant, eliminating harmful intracellular levels of acetyl-coenzyme A (acetyl-CoA) (one of the enzymes implicated in disease state energy production), and replacing membrane phospholipids [55]. Reduced epididymal L-C levels have been linked to sperm abnormalities in various studies [55]. PCBs were shown to drastically lower testicular L-carnitines and sulphydryl levels in this investigation.

Treatments with DRLC, on the other hand, reduced the effect of PCBs on L-carnitines and sulphydryl levels in the testis. Furthermore, peroxiredoxin-4 is an antioxidant enzyme that is only found in rats' and humans' acrosomal regions [56]. Peroxiredoxin-4 is known to catalyze the breakdown of peroxynitrite and H_2O_2 in the testes, implying a preventive response against ROS-induced spermatozoa

damage. Peroxiredoxin-4 is also involved in the development of acrosomes during sperm production in rodents [56]. The PCB-induced reduction in the peroxiredoxin-4 expression found in this study could indicate that peroxynitrite production is causing infertility by suppressing peroxiredoxin-4 expression and so reducing the odds of effective sperm capacitation and acrosome response [56]. The capacity of DRLC to reduce the deleterious effects of PCBs on peroxiredoxin-4 expression in the testes, on the other hand, suggests that it could be useful in circumstances like male reproductive failure.

Conclusions

The current findings suggest that DRLC could be a promising therapeutic modality for reducing PCB-induced neuroendocrine-transmembrane ionic pump disruption in an experimental rat model, with the main mechanism being its antioxidant activities as established by Oyovwi*et al*. [57], against peroxynitrite formation and increased hydrogen peroxidation. Overall, our findings suggest that testicular antioxidant enzymes (L-C and PrDx-4), androgenic enzymes, sulphydryl content (3-HSD and 17-HSD), and transmembrane ionic pump (Na+, K+, Ca2+, Mg2+, H+ ions) ATPase play a major role in testicular and sperm membrane function required for spermatogenesis. As a result, DRLC could be a novel therapeutic option for male reproductive dysfunction caused by chemo-toxicity.

Acknowledgments

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