RESEARCH

Ameliorative potentials of diosmin and hesperidin fractions on chlorpyriphos-induced changes in reproductive hormones, sperm characteristics, and testicular glycogen in male Wistar rats

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Abstract

Reproductive deficiency is a major outcome of pesticide exposure sequel to cellular oxidative damage to sex organs. Flavonoid possess potent antioxidant capacities to mitigate pesticide related cellular injury. The present investigation examined the mitigative effect of micronized purified fractions of diosmin and hesperidin on reproductive hormones, sperm parameters, and testicular glycogen in male Wistar rats after sub-chronic Chlorpyriphos (CPF) exposure. Twenty-five male Wistar rats (120-145 g) were randomly allocated five rats per group. Group I (DW) received distilled water (2 ml/kg), Group II (S/oil) received soya oil (2 ml/kg), Group III (DAF) received Daflon at 1000 mg/kg, Group IV (CPF) received Chlorpyriphos (7.74 mg/kg), and Group V (DAF + CPF) received Daflon (1000 mg/kg) followed by CPF (7.74 mg/kg) after 30 min of Daflon. This regimen was administered daily for 60 days. After cervical venesection under light chloroform anesthesia, blood samples were examined for levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. Each rat's testicular tissue was quickly cut, collected, and glycogen evaluated. Sperm concentration, motility, morphology, and viability were measured in the right caudal epididymis. Results revealed that the untreated CPF group had significantly lower FSH, LH, testosterone, testicular glycogen, and sperm concentration. Additionally, CPF group sperm characteristics were abnormal compared to other groups. These reproductive hormones, testicular glycogen, and sperm parameters improved in the Daflon-treated groups. Hence, pre-treatment with flavonoid fractions of diosmin and hesperidin mitigated CPF-induced reproductive toxicity.

Keywords Chlorpyriphos · Oxidative stress · Reproductive hormones · Sperm characteristics · Diosmin · Hesperidin

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Introduction

Environmental chemicals have been demonstrated to impair fertility in both humans and animals (Louis et al. 2016; Pizzorno 2018). With a 50% incidence during the last few years, infertility continues to be a global health concern (Chevrier et al. 2013). There has been an emphasis on the negative reproductive consequences associated with pesticide contamination that are extensive and permanent (Umosen and Uchendu 2014). Chronic pesticide exposure may threaten the lives of non-target creatures (Zhu 2015). Previous studies have shown that pesticides contribute to reproductive impairment and consequently reduce fertility (Kenfack et al. 2015; Shittu et al. 2014). Organophosphate (OP) chemicals are well-known for their dual function as household insecticides as well as agricultural productivity boosters (Oates

et al. 2014). Chlorpyriphos (CPF) is a cheap and widely available OP pesticide that has serious health risks. CPF, like other OPs, causes toxicity by decreasing the activity of acetylcholinesterase (AChE) (Ambali et al. 2010). Other cellular pathways of CPF toxicity include increased reactive oxygen species (ROS) production and oxidative stress (OS) (Ambali and Ayo 2011; Olatunji et al. 2022). CPF exposure has been associated with oxidative damage to cellular membranes of important organs in the body, including the male reproductive system, as well as disruption of hormonal balance (Shittu et al. 2014). Pesticide-induced reproductive hormone imbalance, which reduces sperm production and quality, may lead to infertility (Joshi et al. 2007). CPF also depletes glycogen levels in the testes, impairing spermatozoa's capacity to swim and migrate efficiently enough to fertilize an egg (Umosen and Uchendu 2014). ROS causes DNA damage in spermatozoa, altering concentration, motility, morphology, and potential viability (Beam et al. 2014). The development of oxidative damage from OP exposure has been associated with decreased sperm function and poor male fertility (Leong et al. 2013).

Previous animal and human research have demonstrated the relevance of plant-derived antioxidants in the treatment of acute and chronic disorders (Heikal et al. 2013; Shittu et al. 2014; Surai and Fisinin 2014). Daflon (Daflon 500 mg®) is a micronized flavonoid derived from the plant *Rutaceae aurantiae* that includes 90% diosmin and 10% hesperidin (Rizk and Sabri 2009). Diosmin and hesperidin are powerful flavonoid antioxidants with high ROS removal capacity that can be employed to treat oxidatively induced infertility (Olatunji et al. 2022). Therefore, the purpose of this study was to assess the protective effects of flavonoid fractions of diosmin and hesperidin on alterations in reproductive hormones, testicular glycogen, and sperm characteristics in male albino rats sub-chronically exposed to Chlorpyriphos.

Materials and methods

Laboratory animals

For this experiment, 25 male albino rats weighing 120–145 g were obtained from the National Veterinary Research Institute in Jos (Vom), Nigeria. They were confined in the animal holding facility at Ahmadu Bello University in Zaria, Nigeria. They were given commercially prepared grower pellets and had unlimited access to water. Before the experiment began, participants were allowed two weeks of acclimatization. The laboratory animal care guide was followed (CCAC 1993), and the experimental procedures were carried out in compliance with the Animal Care and Use Committee rules established at Ahmadu Bello University.

Purchase and preparation of chemicals

Soya oil and Chlorpyriphos 20% EC (Termikill®, Gujurat, India) were mixed to make a 10% solution. Before administration, 500 mg/tablet of Daflon (Daflon-500®; Les Laboratoires Servier, France) was dissolved in 5 mL of distilled water to form a 100 mg/mL daily solution.

Sub-chronic reproductive toxicity

Five groups of five rats each were randomly chosen from a total of 25 rats. Distilled water (2 ml/kg), Soya oil (2 mL/kg), Daflon (1000 mg/kg [Olatunji et al. 2022]), and CPF (7.74 mg/kg [Olatunji et al. 2022]) were given to Groups I (DW), II (S/oil), III (DAF), and IV (CPF). Group V got 1000 mg/kg of Daflon before being subjected to CPF (7.74 mg/kg) 30 min later (Olatunji et al. 2022). Each regimen was given via gavage once a day for 60 days. Following a brief chloroform anesthesia, the rats were terminated via cervical venipuncture. To analyze FSH, LH, and testosterone levels, 2 mL of blood sample was collected from the medial canthus of the eyes of each rat into sterile plain sample bottles, and were centrifuged at 1000 rpm for 10 min to obtain serum. Each rat's testicular tissue was promptly cut, collected, and measured for glycogen content. Sperm concentration, motility, morphology, and viability were assessed in the right caudal epididymis.

Evaluation of follicle-stimulating, luteinizing, and testosterone hormone levels

Following the manufacturer's instructions, serum FSH, LH, and Testosterone concentrations were measured using enzyme immunoassay (EIA) kits; K-Assay ELISA Kit, KT-15332 (Kamiya Biomedical Company, USA), E-EL-R0026 (Elabscience, USA), and Testosterone.

Evaluation of testicular glycogen levels

The gravity method of Good et al. (1993) was used to assess testicular glycogen levels using the BioVision Glycogen Colorimetric Assay Kit II (K648-100), as directed by the manufacturer. The data were given as grams of glycogen per 100 g of testicular tissue.

Evaluation of sperm characteristics

Shortly after the testes were carefully removed, the right epididymis was examined for sperm count following Yokoi et al. (2003). Sperm were extracted by slicing the right caudal epididymis into 5 ml of physiological saline, which was

then let to sit at room temperature for two minutes after being rocked for ten minutes. Following the incubation period, the supernatant fluid was diluted 1:100 using a solution that contained 25 mg of eosin stain, 1 mL of formalin (35%), and 5 g of sodium bicarbonate per 100 mL of water. The total number of sperm was counted using a hemocytometer magnified ×200 under a light microscope. Sperm motility was assessed according to Sonmez et al. (2005) guidelines. Using a pipette, remove the fluid from the cauda epididymis, and dilute it to 2 mL with Tris buffer solution. An aliquot of this solution was placed on a slide under a phase contrast microscope, and the % motility was assessed at an enlargement of ×400 using a light microscope. For every sample, estimates of motility were obtained from three distinct fields. The final motility score was calculated using the mean. Sperm cell morphology was evaluated in compliance with the guidelines provided by Atessahin et al. (2006). India ink was used to produce slides that were smeared with spermatozoa. The morphological anomalies of 500 sperm cells were examined. Spermatozoa were observed under a light microscope at a ×400 magnification and classified using phase-contrast optics. Sperm viability was assessed using the Siegel (1993) technique. To distinguish between living and dead sperm, 50 μL of liquid sperm and 50 μL of Eosin-Nigrosin stain were mixed in a microtube. One drop of the mixture was distributed on a microscope slide, and after 30 s, it was allowed to air dry. A bright field microscope was used to examine each slide with an immersion oil drop at a total magnification of 1000. Vital spermatozoa were identified by white or light pink sperm heads.

Statistical analysis

Data were given as mean ± SEM and statistically evaluated using one-way ANOVA and Tukey's post-hoc multiple comparison test. All analyses were conducted using Graphpad Prism version 4.0, San Diego, California, USA (www.graphpad.com). Microsoft excel (2012) was used to plot charts of data obtained in this study. p-values < 0.05 were considered significant.

Results

Follicle stimulating concentration

The concentration of FSH was significantly ($P < 0.05$) lower in the CPF group (10.81 ± 0.43 ng/mL) when compared to S/oil (14.03 ± 0.63 ng/mL), DAF (15.65 ± 0.56 ng/mL) and DAF + CPF (14.29 ± 0.64 ng/mL) groups (Fig. 1).
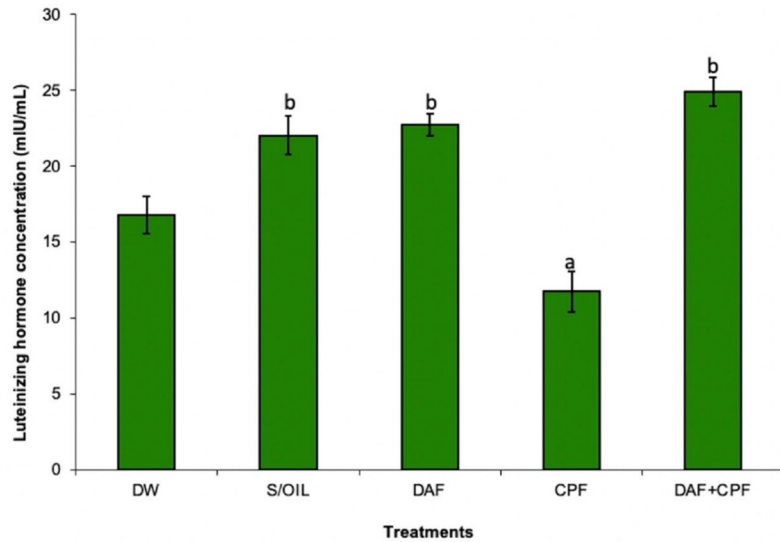
Luteinizing hormone concentration

LH concentration in the CPF group (11.77 ± 1.34 mIU/mL) was significantly ($P < 0.05$) lower than those recorded in S/oil, DAF, and DAF + CPF groups with values of 22.05 ± 1.28, 22.74 ± 0.74 and 24.94 ± 0.92 mIU/mL, respectively (Fig. 2).
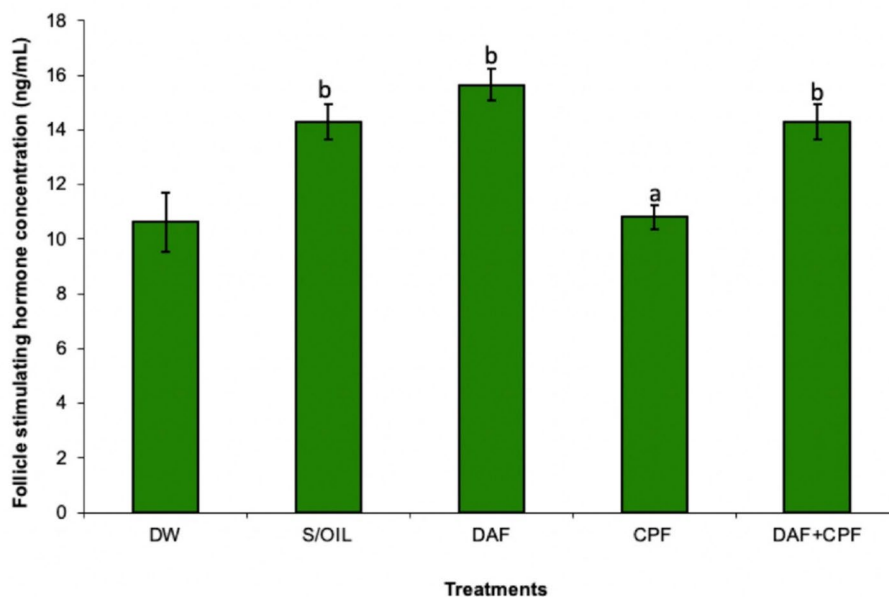
Fig. 1 Sub-chronic effect of Daflon and CPF exposure on follicle-stimulating hormone concentration in adult male rats. [a] Significantly (P < 0.05) lower when compared to S/oil, DAF and DAF + CPF groups [b] DW: Distilled water, S/oil: Soya oil, DAF: Daflon, CPF: Chlorpyriphos

Fig. 2 Sub-chronic effect of Daflon and CPF exposure on luteinizing hormone concentration in adult male rats. [a] Significantly ($P < 0.05$) lower when compared to S/oil, DAF and DAF+CPF groups [(b)]. DW: Distilled water, S/oil: Soya oil, DAF: Daflon, CPF: Chlorpyriphos
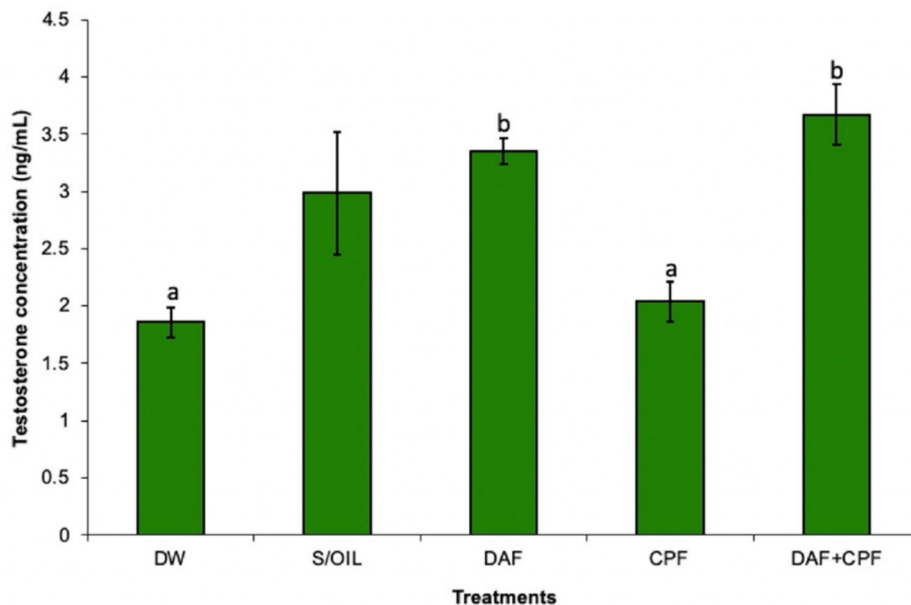
Testosterone hormone concentration

In Fig. 3, a significant ($P < 0.05$) decrease in testosterone concentration was recorded in the CPF group (2.03 ± 0.18 ng/mL), when compared to DAF + CPF (3.67 ± 0.26 ng/mL). Also, a significant increase in testosterone concentration in the DAF (3.35 ± 0.11 ng/mL) and DAF+CPF (3.67 ± 0.26 ng/mL) groups was recorded when compared to the DW (1.85 ± 0.12 ng/mL) group.

Testicular glycogen concentrations

Testicular glycogen concentration was significantly ($P < 0.05$) lower in the CPF group (1.38 ± 0.46 ng/mL) when compared to that of DAF, S/oil, and DAF+CPF groups with values of 3.18 ± 0.27, 2.52 ± 0.24 and 2.17 ± 0.04 ng/mL, respectively (Fig. 4).

Fig. 3 Sub-chronic effect of Daflon and CPF exposure on testosterone concentration in adult male rats. [a] Significantly ($P < 0.05$) lower when compared to DAF and DAF+CPF [(b)] DW: Distilled water, S/oil: Soya oil, DAF: Daflon, CPF: Chlorpyriphos

Fig. 4 Sub-chronic effect of Daflon and CPF exposure on glycogen concentration in adult male rats. [a] Significantly ($P < 0.05$) lower when compared to DAF and DAF+CPF [b] DW: Distilled water, S/oil: Soya oil, DAF: Daflon, CPF: Chlorpyriphos
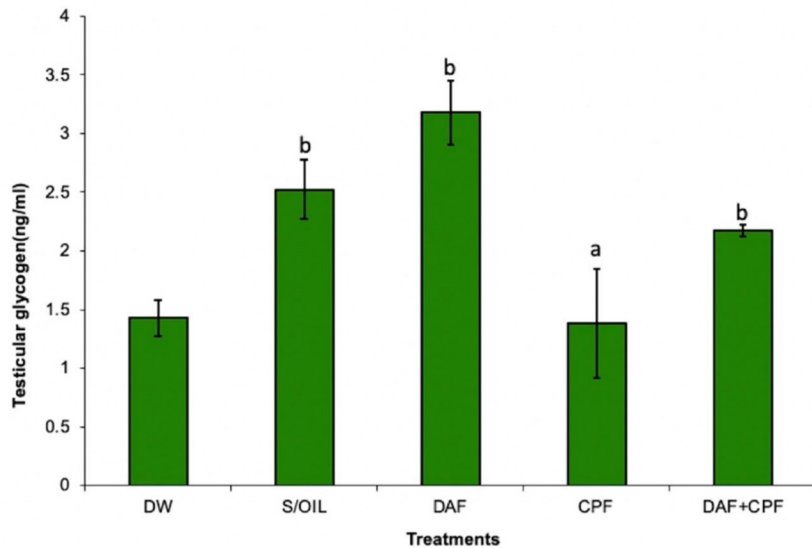
Sperm concentration

A significant ($P < 0.05$) decrease in sperm concentration in the CPF group (40.43 ± 0.26 count $\times 10^6$ cells/mL) was recorded when compared to S/oil (67.63 ± 2.27 count $\times 10^6$ cells/mL) and DAF+CPF (62.43 ± 1.36 count $\times 10^6$ cells/mL). Sperm concentration in the DAF+CPF was significantly higher when compared to the DW (48.20 ± 1.95 count $\times 10^6$ cells/mL) group (Table 1).

Sperm motility

Progressive sperm motility

The progressive motility of spermatozoa significantly ($P < 0.05$) decreased in the CPF group ($31.33 \pm 1.76\%$) compared to those of DW and DAF with values of $55.67 \pm 6.22\%$ and $61.00 \pm 4.04\%$ respectively. Also, a significant ($P < 0.05$) increase in progressive sperm motility in the DAF group ($61.00 \pm 4.04\%$), was observed when compared to S/oil

Table 1 Sub-chronic effect of Daflon and CPF exposure on sperm characteristics in adult male rats

GRP/Parameters	DW (GRP I)	S/Oil (GRP II)	DAF (GRP III)	CPF (GRP IV)	DAF+CPF (GRP V)
SC ($\times 10^6$ cells/mL)	48.20 ± 1.95^a	67.63 ± 2.27^b	50.93 ± 1.56	40.43 ± 1.09^a	62.43 ± 1.36^b
SPM (%)	55.67 ± 6.22	48.67 ± 2.33	61.00 ± 4.04^b	31.33 ± 1.76^a	43.00 ± 2.08
SNPM (%)	35.33 ± 1.85	23.67 ± 2.33	15.00 ± 1.52^a	34.33 ± 2.33^b	32.67 ± 1.76
NMS (%)	20.00 ± 0.57	34.33 ± 3.71	28.33 ± 1.20	34.33 ± 0.88^b	27.33 ± 1.76
HA (%)	18.00 ± 1.15	21.67 ± 2.60	18.33 ± 1.20	37.33 ± 5.45^b	21.67 ± 0.88
HMPA (%)	3.66 ± 0.33	4.33 ± 1.20	2.33 ± 0.66	4.33 ± 0.88	3.33 ± 0.33
TA (%)	4.00 ± 0.57	3.66 ± 1.20	8.33 ± 0.88	13.33 ± 1.45^b	8.00 ± 0.57
NS (%)	73.00 ± 1.52	68.67 ± 1.45	70.00 ± 0.57	52.67 ± 2.40^a	68.00 ± 1.15
SVL (%)	70.67 ± 0.88	62.00 ± 2.64	63.67 ± 2.33	53.33 ± 0.88^a	61.00 ± 3.05
SVD (%)	26.33 ± 2.18	38.00 ± 2.64	36.33 ± 2.33	51.33 ± 3.84^b	39.00 ± 3.05

Key: SC (Sperm count), SPM (Sperm progressive motility), SNPM (Sperm non-progressive motility), NMS (Non-motile spermatozoa), HA (Head abnormality), HMPA (Head and mid-piece abnormality), TA (Tail abnormality), NS (Normal spermatozoa), SVL (Sperm viability live), SVD (Sperm viability dead)

DW Distilled water, *S/oil* Soya oil, *DAF* Daflon, *CPF* Chlorpyriphos

[a] Significantly ($P < 0.05$) lower

[b] Significantly ($P < 0.05$) higher

(48.67 ± 2.33) and DAF + CPF $(43.00 \pm 2.08\%)$ groups (Table 1).

Non-progressive sperm motility

A significant ($P < 0.05$) increase in non-progressive sperm motility in the CPF group $(34.33 \pm 2.33\%)$ was recorded when compared to S/oil and DAF groups, with 23.67 ± 2.33 and $15.00 \pm 1.52\%$ values respectively. The non-progressive sperm motility in the DAF group $(15.00 \pm 1.52\%)$ decreased when compared to that of DAF + CPF $(32.67 \pm 1.76\%)$ (Table 1).

Non-motile spermatozoa

There was a significant ($P < 0.05$) increase in non-motile sperms in the CPF group $(34.33 \pm 0.88\%)$ was recorded, when compared to DW $(20.00 \pm 0.57\%)$, DAF $(28.33 \pm 1.20\%)$ and DAF + CPF $(27.33 \pm 1.76\%)$ groups (Table 1).

Sperm morphology

Sperm head abnormality

Head abnormality of spermatozoa significantly ($P < 0.05$) increased in the CPF group $(37.33 \pm 5.45\%)$ than in rats given DW, S/oil, DAF, and DAF + CPF groups with values of 18.00 ± 1.15, 21.67 ± 2.60, 18.33 ± 1.20 and $21.67 \pm 0.88\%$, respectively (Table 1).

Sperm head and mid-piece abnormality

There was no significant ($P > 0.05$) difference in head and mid-piece abnormality in all the groups, but DAF $(2.33 \pm 0.66\%)$ group had the lowest percentage of head and mid-piece abnormality when compared to the DW $(3.66 \pm 0.33\%)$, S/oil $(4.33 \pm 1.20\%)$, CPF $(4.33 \pm 0.88\%)$ and DAF + CPF $(3.33 \pm 0.33\%)$ groups respectively (Table 1).

Sperm tail abnormality

The number of spermatozoa with tail abnormalities was significantly ($P < 0.05$) higher in the CPF group $(13.33 \pm 1.45\%)$, compared to DW, S/oil, DAF, and DAF + CPF groups with values of 4.00 ± 0.57, 3.66 ± 1.20, 8.33 ± 0.88 and $8.00 \pm 0.57\%$, respectively (Table 1).

Normal spermatozoa

The number of normal sperm cells significantly ($P < 0.05$) decreased in the CPF group $(52.67 \pm 2.40\%)$, when compared

to DW, S/oil, DAF, and DAF + CPF groups, with values of 73.00 ± 1.52, 68.67 ± 1.45, 70.00 ± 0.57 and $68.00 \pm 1.15\%$, respectively (Table 1).

Sperm viability

Live spermatozoa

A significant ($P < 0.05$) decrease in live sperm cells in the CPF group $(53.33 \pm 0.88\%)$ was recorded, when compared to DW, DAF, and DAF + CPF groups, with values of 70.67 ± 0.88, and 63.67 ± 2.33 and $61.00 \pm 3.05\%$, respectively (Table 1).

Dead spermatozoa

Table 1 depicts a significant increase in dead sperm cells in the CPF group $(51.33 \pm 3.84\%)$ when compared to DW, DAF and DAF + CPF groups, with values of 26.33 ± 2.18, 36.33 ± 2.33 and $39.00 \pm 3.05\%$, respectively.

Discussion

The lower level of reproductive hormones found in this study is in line with research by Shittu et al. (2013) and Umosen and Uchendu (2014), who discovered that rats exposed to chlorpyriphos had decreased serum concentrations of testosterone, luteinizing hormone, and follicle-stimulating hormone. Research has demonstrated that pesticides impact endocrine functions across the entire spectrum of hormonal regulation, encompassing hormone production, release, storage, transit, clearance, receptor identification, and binding (Mathur et al. 2009). Other pathways of CPF toxicity include anti-gonadal activities in the pituitary gland, which may impair gonadotropin levels and function in human and experimental animals (Faraga et al. 2010; Heikal et al. 2014). FSH, LH, and testosterone are the primary regulators of germ cell growth. FSH enhances spermatogenesis by binding to Sertoli cell receptors, whereas LH stimulates testosterone production in Leydig cells, which then stimulates spermatogenesis in the Sertoli and peritubular cells of the seminiferous tubules (O'Donnel et al. 1994). Thus, a decrease in LH, as seen in this study, leads to a decrease in testosterone levels. The failure of the pituitary gland to release FSH and LH clearly reveals CPF's inhibitory action on the pituitary gland, hence reducing gonadotrophin production and influencing steroidogenesis (Heikal et al. 2013; Shittu et al. 2014). Pre-treatment with Daflon reversed the hormonal imbalance observed in the CPF group, indicating that the flavonoids diosmin and hesperidin can increase gonadotropin output from the pituitary gland. This conclusion

is consistent with previous findings where the efficiency of antioxidant-containing flavonoids is documented in restoring hypothalamic-pituitary function by reducing oxidative damage within its axis (Shittu et al. 2013). Olatunji et al. (2022) reported a CPF-induced increase in pituitary and testicular MDA concentrations, as well as decreased antioxidant enzyme activity (superoxide dismutase and catalase), indicating that ROS-induced damage to the pituitary and testes is mediated by oxidative stress induction. As a result, DAF's antioxidant ability to counteract ROS created by CPF's harmful effects may have contributed to the increased levels of FSH, LH, and testosterone.

The CPF group exhibited a notable reduction in testicular glycogen concentration, while the DAF group showed an increase, in comparison to the control group (DW). These results align with previous studies conducted by Shittu et al. (2013) and Al-Amoudi (2015), which also reported a decrease in testicular glycogen levels due to exposure to CPF. Glycogen is an important source of energy for overall body metabolism (Rajawat et al. 2014). Protein synthesis in spermatogenic cells is glucose-dependent, therefore a decrease in glycogen concentration impacts protein synthesis and, as a result, inhibits spermatogenesis (Bhushan et al. 2013; Gawish 2014). In this study, exposure to CPF resulted in substantial glycogen depletion in the testes, indicating decreased glucose storage. The reduction in total glycogen concentration could be attributed to its use to detoxify the pesticide or its metabolites via the process of glucuronidation, which involves harmful metabolites combined with glucose phosphate and being expelled from the liver via bile. Furthermore, the decrease in glycogen content could be related to an increase in enzyme activity (phosphorylase-A and phosphorylase-B), which are involved in glycogenolysis for the glucuronidation process. The increased amounts of testicular glycogen in the groups treated with DAF alone and with DAF + CPF demonstrate that this antioxidant can protect the liver and, as a result, the testes from CPF-induced damage.

Low sperm count, serum gonadotropins, and testosterone commonly accompany abnormal spermatogenesis (Babu et al. 2004). Sperm count is one of the most sensitive assays for spermatogenesis, which is linked to fertility (El-bendary et al. 2014). This study found that the CPF group's caudal epididymal sperm concentration was significantly lower than other groups. Other researchers found that herbicides like CPF reduced sperm count in animals (Shittu et al. 2013; Umosen and Uchendu 2014). Narayana et al. (2006) found that methyl parathion, an organophosphate, decreased adult male rat sperm concentration. In this study, gonadotrophin suppression may have decreased testicular sperm density, altering the caudal epididymis biochemical environment. Sertoli cell activity is directly affected by toxicants, which can cause epithelial

disorganization, decreased spermatogenesis, and tubular atrophy (Bedwal et al. 1994; Olorunshola et al. 2011), lowering sperm count.

In their study, Pallav and Rajdeb (2014) discovered that pesticide exposure has a detrimental impact on spermatozoa, disrupts the functioning of Sertoli or Leydig cells, and reduces the quality of semen. DAF + CPF treatment may have increased FSH, LH, and testosterone, which may explain the considerable increase in sperm count. This shows the new spermatogenetic property of this antioxidant agent in protecting and restoring the testes from CPF damage. This work shows for the first time that DAF can increase sperm concentration after sub-chronic CPF exposure. Other sperm properties including motility, morphology, and viability govern fertilizing capacity, hence unfavorable changes affect conception (W.H.O. 2010).

Based on movement patterns, WHO guidelines classify sperm motility as sperm progressive motility (SPM), sperm non-progressive motility (SNPM), or non-motile spermatozoa (NMS). In this study, the CPF group had significantly lower SPM and higher SNPM and NMS than the treated groups, thus agreeing with previous work (El-Kashoury and Tag El-Din 2010; Kenfack et al. 2007). Negative sperm motility impairs fertilization. An optimum ATP pool is needed for spermatozoa movement, therefore even a little shortage lowers motility and may cause infertility. The decreased glycogen concentration in the CPF group may explain the considerable inhibition of sperm motility in this study. Because glycogen stores glucose, it produces ATP, which is needed for sperm motility (Gawish 2014). Thus, greater glycogen levels in DAF and DAF + CPF groups may explain their increased sperm motility.

Pesticide-induced testicular toxicity causes teratospermia (Baykalir et al. 2016; Sai et al. 2014). Other researchers (El-bendary et al. 2014; Kenfack et al. 2015) found similar abnormalities in the CPF group, including a large increase in head and tail sperm and less normal spermatozoa. Along with the parent substance, OP oxon metabolites like CPF were discovered to cause organophosphorus sperm genotoxicity (Salazar-Arrendo et al. 2008). In groups exposed to CPF, Faraga et al. (2010) found a decrease in morphologically normal spermatozoa and sperm count. This study's CPF group showed pesticide-induced free radical formation in spermatozoa, which may cause teratospermia (Baykalir et al. 2016). Due to their high membrane polyunsaturated fatty acid content, spermatozoa may be predisposed to problems due to increased testicular oxidant indicators (Olatunji et al. 2022). Sperm lipid matrix peroxidation causes rapid ATP loss, axonemal damage, reduced sperm viability, and increased morphological abnormalities, and in extreme cases, spermatogenesis is completely prevented (Sanocka and Kurpisz 2004). These morphological abnormalities may explain the CPF group's lower live spermatozoa rate.

Golec et al. (2003) found that agricultural workers exposed to OPs have a higher incidence of sperm morphological abnormalities. Observations in post-CPF exposure showed spermatozoa morphological abnormalities. These aberrations distinguish fertile and infertile males (Abdelaziz et al. 2010; Golec et al. 2003). According to Dutta and Sahu (2013) and El-bendary et al. (2014), CPF can modify testicular DNA and sperm chromatin structure, creating morphological defects in mitotic and meiotic chromosomes. Pesticide-induced sperm toxicity may cause OP mutagenicity and spermatotoxicity (Topham 1980). Oxidative stress and a decrease in local antioxidant defense might damage sperm DNA, notably during epididymal development, causing morphological defects (Dutta and Sahu 2013). Dysmature cells, defined by excess residual cytoplasm, persistent nuclear histones, inadequate zona binding, and altered chaperone content, were found in CPF-exposed sperm cells by Abdelaziz et al. (2010). The vaso-protective components (hesperidin and diosmin) of Daflon reduce cell membrane hyperpermeability, protecting blood vessels from supplying the testes and reducing toxicant passage through the blood-testes barrier, minimizing sperm abnormalities. As an antioxidant, Daflon may have protected the shield surrounding the sperm cell plasma membrane and lipid layer structure, enhancing the sperm's ability to tolerate ROS attack. This may explain the CPF + DAF group's significant increase in normal spermatozoa.

This study similarly found a substantial drop in sperm live ratio and an increase in sperm dead ratio in the CPF group, supporting Golec et al. (2003) and Dutta and Sahu (2013). Sperm viability is a key indicator of fertilizing potential (Joshi et al. 2007; Dutta and Sahu 2013). Therefore, any detrimental effect on motility and morphology will severely reduce sperm viability (Ben et al. 2012; El-bendary et al. 2014). Pesticide-induced lipid peroxidation destroys the sperm membrane's lipid matrix, causing fast ATP loss, axonemal damage, and reduced sperm viability (Baykalir et al. 2016). Direct spermicidal effects of CPF may reduce sperm viability (Dutta and Sahu 2013; El-bendary et al. 2014). Micronization of daflon (Simsek et al. 2007; Osman et al. 2013) made it more likely to act intracellularly and at the membrane level, protecting the cell from ROS and toxic metabolites, and this may have allowed the antioxidant to cross the blood-testes barrier, hence protecting the testes from CPF-induced damage as reported in the histology of our earlier published work (Olatunji et al. 2022).

Conclusion

Inference from this research demonstrated that Daflon treatment improved the level of reproductive hormones, glycogen content, sperm count, motility, morphology, and viability in CPF-exposed rats. Therefore, pretreatment with Daflon or ingestion of food rich in diosmin and hesperidin may be beneficial to mitigate CPF-induced reproductive deficiency in farmers and household users.

Limitations

Our investigation was limited by the lack of more sophisticated technology to measure sperm parameters coupled with the high cost of hormonal assay kits which limited the number of hormones that could have been assayed. Further studies should be performed on the molecular interplay involved in the combination therapy between chlorpyriphos and the flavonoid compounds, diosmin and hesperidin.

Declarations

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