Environ Sci Pollut Res
DOI 10.1007/s11356-017-9617-x

RESEARCH ARTICLE

Low doses of chlorpyrifos interfere with spermatogenesis of rats through reduction of sex hormones

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Received: 8 February 2016 / Accepted: 23 June 2017

Abstract Use of pesticides results in indirect effects on human health. We aimed to evaluate implications of toxicological effects of subchronic chlorpyrifos exposure on reproductive function in male rats. A total of 48 adult Wistar male rats were separated into four groups (n = 12). Animals were gavaged with 2.5 mg/kg (T1), 5 mg/kg (T2), or 10 mg/kg (T3) body weight of chlorpyrifos (CPF) or distilled water (control) daily for 30 days. Organ weights, epididymal sperm parameters, DNA integrity, sex hormonal (FHS and LH) levels, and alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and creatinine concentrations were determined on day 31. Another two sets of (four groups/set; n = 10) animals were orally treated with the same doses of CPF, control animal groups were treated with distilled water only for 30 days, and fertility indices and blood plasma acetylcholine esterase (AchE) were determined on day 31. Exposure to CPF resulted in a significant ($p < 0.05$) decrease in weights of testis and epididymis. An increase in liver weight resulted in reduced sperm counts and sperm motility and an increase in sperm abnormalities. Significant reduction in serum testosterone ($p < 0.01$), luteinizing hormone ($p < 0.05$), and follicular stimulating hormone ($p < 0.05$) levels was evident in animals treated with the highest dose. A significant decrease in the number of viable implantation sites and pups was observed in female rats mated with the T3 ($p < 0.01$) and T2 ($p < 0.05$) males. The ALT, AST, GGT, and creatinine contents were significantly increased ($p < 0.05$ and $p < 0.01$, respectively) on CPF exposure. A significant ($p < 0.01$) reduction in blood plasma AchE enzyme was observed with the highest dose. Our results demonstrated that prolonged exposure of CPF induces spermatogenesis damage, possibly through interference with sex hormones and AchE enzyme resulting in reduction of fertility. Therefore, awareness programs on handling CPF (pesticides) to enhance safety warrant minimization of its hazards.

Keywords Chlorpyrifos · Organophosphate · Sperm parameters · Fertility index · AchE · FSH · LH

Introduction

Agriculture is an important sector in the economy of Sri Lanka, and therefore, use of pesticide has increased during the recent past to meet the needs of domestic food production. This situation has resulted in high imports of pesticides and fertilizers, consequently leading to many health effects such as disruption of reproductive function in wildlife (Moline et al. 2008), laboratory animals (Peiris et al. 1995; Ramasooriya et al. 1996), and humans (Sengaputta and Banergee 2014). Colborn et al. (1993) discovered that endocrine-disrupting chemicals can interfere with endocrine systems and produce a range of reproductive, developmental and metabolic dysfunctions in both humans and wildlife. Further, low-level exposures, which occur due to both occupational and environmental factors, can lead to disruption of human spermatogenesis (Ten et al. 2008).

Chlorpyrifos [*O,O*-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate], a broad-spectrum chlorinated

Responsible editor: Philippe Garrigues

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Published online: 18 July 2017

organophosphate insecticide and acaricide, accounts for nearly 50% of all the organophosphate insecticides and about 25% of all insecticides imported to Sri Lanka (Registrar of Pesticides 2007). Though the US Protection Agency has imposed a ban on sale of chlorpyrifos (CPF), farmers in Sri Lanka still continue to use CPF to control pests in rice and vegetable crops (Menike et al. 2012). A common effect of CPF is the disruption of nervous transmission by inhibition of acetylcholine esterase (AChE) at the nerve endings of insects (Yen et al. 2011), copepods, amphipods (Zafar et al. 2013), fish (Brandit et al. 2015), earthworms (Muangphra et al. 2015), and mammals (Deb and Das 2013). Similarly, in humans CPF inhibits AchE both in central and peripheral nervous systems causing neurotoxicity within hours of exposure (Lotti 1991). Chronic exposure to 5.4 and 12.8 mg/kg of CPF for 90 days decreased sperm count and motility via reduction of testosterone and increased FSH (Sai et al. 2014). Similarly, Farag et al. (2010) reported that CPF induced reproductive toxicity at 15 and 25 mg/kg CPF in male mice. However, Mandal and Das (2011) observed that CPF reproductive toxicity is obvious only at low doses of CPF (5 and 10 mg/kg) but not at higher doses.

Occupational exposure of humans to pesticides, including CPF, has been recorded (Sivayoganathan et al. 1995; Van Der Hoek et al. 1998), and application of pesticides to fruit and vegetables can also contribute to pesticide exposure through diet (Marasinghe et al. 2014). In developing countries, small-scale farmers are exposed to CPF during mixing, loading, and spraying using backpack sprayers resulting in adverse health effects (Panuwet et al. 2008). Further, farmers in developing countries are at high risk to CPF exposure due to the use of backpack reservoirs for application, low knowledge on safety, and limited use of protective gear (Phung et al. 2012). Human epidemiological studies have shown that exposure to CPF resulted in decreased birth weight and reduced head circumference of babies (Tian et al. 2005) and increased risk of lung and prostate cancer (Dinham 2005).

Farmers in Sri Lanka tend to apply 30–40% higher concentrations of CPF than the recommended dose levels (Selvarajah and Hurtichelvam 2007). Though the recommended dose of CPF for field application is 4.4 kg a.i./ha, farmers in Sri Lanka apply about 5.7–6.2 kg a.i./ha (De Silva et al. 2010). The occupational exposure to CPF of the farmers in Sri Lanka ranged from 2500 to 90,000 ng/kg/day, and the total dietary CPF intake in an average diet in Sri Lanka varied from 50 to 1800 ng kg bw.day, which is about five times higher than the average intake in India (Marasinghe et al. 2014). The exposure level of CPF among the male farmers with lower education levels in Vietnam applicators is about 96 μg/kg/day during post application (Phung et al. 2012). Exposure of CPF contributes to alterations in both sperm chromatin condensation and sperm DNA integrity which could lead to infertility in humans (Salazar-Arredondo et al. 2008). Therefore, it is vital to study the effects of long-term exposure to CPF on humans. Thus for this purpose, this study was designed to investigate the effects of CPF, 2.5, 5, and 10 mg/kg, in chronic exposed mature male rats as laboratory models for humans on epididymal structure, sperm parameters, androgen profile fertilizing ability, and AchE levels.

Materials and methods

Animals and maintenance

Six-week-old male Wistar rats (135–150 g) were purchased from the Medical Research Institute, Colombo, Sri Lanka. The rats were acclimated to the laboratory environment for 7 days prior to the study. An animal house was maintained under standard hygienic conditions (temperature 25 ± 2 °C; relative humidity 45–55% ± 10) with a 12-h day-and-night cycle for 3 weeks before experiments were started. Animals were fed freely with pelleted food (Agro Industries, Seeduwa, Sri Lanka) and water. The animal experiment was performed as per committee for the purpose of control and supervision of experiments on animal norms after obtaining the institutional animal ethics committee clearance (ethical no.: 25/16).

Chemicals

All chemicals were purchased from Sigma Chemical Company Ltd. (USA) unless stated otherwise. The test material Pyrinex (CPF, 480 mg/ml; CPF is marketed under several commercial names: Terminator, Lorsban) was purchased from Lankem Ltd., Colombo, Sri Lanka.

Dose levels

Previous findings have shown that 17.5 mg/kg CPF for 30 days induced severe testicular damage (Joshi et al. 2007). The occupational exposure to daily doses of CPF in developing countries like Sri Lanka is 94,000 ng/kg/day (Phung et al. 2012), indicating that the exposure levels are lower than 17.5 mg/kg. However, in Sri Lanka for crop protection farmers use higher CPF than recommended levels (see "Introduction" section). Therefore, in the present study 2.5-, 5-, and 10-mg/kg doses were selected. Chlorpyrifos can be absorbed into the human body through different pathways, including ingestion, inhalation, and dermal absorption (Salazar-Arredondo et al. 2008). Oral ingestion was selected as the mode of exposure in this study.

Experimental design

After 1 week of acclimatization, 48 male rats were randomly divided into four groups and each group consisted of 12

animals. The first three groups treated orally were administered either with 1 ml of 2.5 mg/kg/day (treatment group 1; T1), 5 mg/kg/day (group 2; T2), and 10 mg/kg/day (group 3; T3) of CPF, respectively, or with distilled water (T4) daily for 30 days. On day 31, the animals were sacrificed for further investigations. During the treatment period, animals were observed daily between 9:00 a.m. (after the treatment) and 11:00 a.m. and between 15.00 a.m. 17.00 p.m. for any overt signs of toxicity.

Body and organ weights

Body weights of the animals were recorded weekly during the study. Animals were sacrificed on day 31. Before sacrifice, animals were weighed again and subsequently the right testis, liver, and epididymis were excised and weights were recorded.

Sperm density and motility

Reproductive organs were used for experimental protocols as appropriate. The left cauda epididymis was used for sperm motility, and the right cauda epididymis was used for sperm count and morphology. The cauda epididymis was sectioned out, and within 5 min after excise, sperm motility was determined under a phase-contrast microscope (Nikon Eclipse, E600) at ×40 magnification. Calculated results were expressed as percentage motility (Lucio et al. 2013).

The right epididymis was diluted in 1:20 with physiological saline (0.9% NaCl) on a petri dish to disperse sperm into the medium. Sperm suspension was pipetted very gently up and down 20 times and placed in a hemocytometer and the total number of the sperm head counted under a Nikon microscope (Nikon Eclipse, E600) at ×40 magnification. Each sample was counted thrice, and the mean value was taken for calculation.

To evaluate abnormal sperm, 10 ml of sperm suspension was taken and added with 1 ml of 10% neutral buffer saline and mixed gently. Then, 2 ml of sperm suspension was mixed with two drops of 1% eosin Y, followed by 40–60 min incubation at room temperature. The sample was placed on a slide, air-dried, and mounted for permanent slide preparation (Lucio et al. 2013). The slides were evaluated by using a phase-contrast microscope (Nikon Eclipse E600) at ×100 magnification.

DNA integrity

Sperm smears from the cauda region were prepared on precleaned microscopic slides and air-dried for 5 min. Smears were fixed in Carnoy's solution for at least 3 h. Subsequently, slides were washed in distilled water and air-dried. Slides were stained with acridine orange for 5 min. Smears were evaluated using a fluorescent microscope (Olympus Corporation, Japan) with excitation of 490 nm. Two hundred sperms from each staining protocol were scored and graded. All sperms exhibiting yellow to red color were scored as denatured DNA, and sperms exhibiting green color were scored as normal DNA.

LH, FSH, and testosterone levels

Blood samples were collected from scarified animals through heart puncture and allowed to clot at room temperature for 1 h. Samples were centrifuged at 3000×g for 10 min to obtain serum. The serum samples were used or stored at −80 °C for subsequent assays. Serum samples were analyzed for luteinizing hormone (LH), follicular stimulating hormone (FSH), and testosterone concentrations which were assessed by FSH and LH enzyme-linked immunosorbent kit (Randox Laboratories Ltd., UK) and testosterone enzyme-linked immunosorbent assay using a commercial kit was (Randox Laboratories Ltd., UK).

Renotoxicity and hepatotoxicity

Blood was collected from heart puncture to tubes containing heparin. Plasma was separated (as above), and alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and creatinine concentrations were determined (Chang et al. 2013).

Fertility test

Four groups of animals (n = 10 per group) were gavaged either with 2.5, 5, and 10 mg/kg of CPF or with distilled water for 30 consecutive days. On day 31 of treatment, males were paired overnight with a proestrus females (at 16:30–17:00 p.m.). The following morning (08:00 a.m.–08:30 a.m.), successful mating was confirmed by the presence of sperm in the vaginal smear. If spermatozoa were present, their number was estimated (in duplicate) using an improved Neubauer hemocytometer and gross morphology was noted by microscopic examination (×200).

On day 14 of gestation, the female rats were laparatomized under mild anesthesia (isoflurane: 0.1 ml/l) and aseptic conditions. Upon laparotomy, the number of dead and live uterine implants and the number of corpora lutea in both uterine horns were recorded. Further, the width and the length of implants were recorded. At the end of the gestation period, the number of live and dead pups was recorded. Once surgery was completed, animals were kept under heat lamps for 30 min to prevent hypothermia and transferred to clean cages until recovery. To manage pain, buprenorphine

Table 1 Changes in body weight and testicular and epididymal weights of rats treated with chlorpyrifos or distilled water

	Control	T1	T2	T3
Testes weight (mg)	1.05 ± 0.003	0. 989 ± 0.023	0.994 ± 0.026	0.8133 ± 0.01*
Epididymis weight (mg)	0.472 ± 0.01	0.3708 ± 0.01	0.3717 ± 0.01	0.2214 ± 0.11*
Liver weight (mg)	11.02 ± 0.024 10.80 ± 0.502 10.56 ± 0.475	10.80 ± 0.502	11.56 ± 0.475	15.14 ± 0.273*
Initial body weight (g)	184.0 ± 9.72	185.68 ± 3.62	183.75 ± 10.82	182.62 ± 4.85
Final body weight (g)	241.54 ± 8.31	243.24 ± 7.59	234.7 ± 8.82	221.61 ± 6.62
Weight change (%)	30	31	28	20**

T1, 2.5 mg/kg; T2, 5 mg/kg; T3, 10 mg/kg of chlorpyrifos. Percentages were calculated as testicular weight/body weight × 100. Data were analyzed using ANOVA, and data are expressed as the mean ± SEM

*$p < 0.05$

(0.1 mg/kg) was given every 12 h subcutaneously for 3 days.

Blood acetylcholine esterase activity

Another set of animals (10 animals/group) were treated either with CPF or the control for 30 days, and on post-treatment day 31, blood samples were collected from the tail tips and the rapid field method for acetylcholine esterase (AchE) assay was used to determine the activity of blood AchE level. In the presence of AchE, hydrolysis of Ach and production of acetic acid subsequently decrease the pH of the reaction mixture. Cholinesterase activity was expressed as (ΔpH/incubation time) = (pH 1 − pH 2) − ΔpH of the blank. pH was measured using a pH meter (Model: pH3110., Weilheim, Germany).

Statistical analysis

All statistical analysis was performed using SPSS statistical package (version 16.0). Body weight, testes weights, epididymal weights, sperm concentrations, and sperm motility were analyzed using ANOVA and least significant test. Other data log transformations were carried out before using ANOVA and least significant test. At all times, a p value of ≤ 0.05 was considered to be significant.

Results

Behavior and external features

No deaths were observed among any of the groups. Further, rats did not exhibit any apparent signs of toxicity such as lacrimation and tremor. However, following high and mid doses of CPF treatment, rats exhibited drowsiness and longer sleeping hours.

Body, epididymal, and testicular weights

Absolute body weight gains and percentage body weight change of the male rats on days 1 and 31 are shown in Table 1. Except in the highest group, no change in body weight gain was observed in the male rats treated with CPF. The percentage body weight gain exhibited a decreasing tendency with increasing dose. A significant change ($p < 0.01$) and a significant reduction ($p < 0.05$) in both testes and epididymal weights were observed in the rats treated with the highest dose. Similarly, the liver weights were significantly ($p < 0.05$) increased in the animals treated with the highest dose.

Epididymal sperm count and motility

The epididymal sperm count in the T3 group was significantly ($p < 0.01$) reduced compared to the control. Further, there was a significant decrease in the sperm motility in both mid ($p < 0.05$) and high ($p < 0.01$) doses of CPF (Table 2).

Sperm abnormalities

Sperm abnormalities significantly increased in animals treated with T2 ($p < 0.05$) and T3 ($p < 0.01$). In the highest treated group, total abnormal sperms were at least doubled when compared to the control (Table 2).

Evaluation of DNA integrity of rats

No significant differences were observed in DNA integrity of sperm from both caput and cauda epididymis regions of T1, T2, and T3 males (Fig. 1). However, in T3 and T2 animals, caput sperm showed red to yellow color sperm heads compared to the control.

Table 2 Epididymal sperm count and motility of rats treated either with chlorpyrifos or distilled water

	Control	T1	T2	T3
Sperm characteristic				
Sperm density ($\times 10^6$/ml)	120.23 ± 3.42	110.85 ± 2.57	106.41 ± 4.23	65.93 ± 3.41**
Sperm motility (%)	95.71 ± 8.63	90.73 ± 7.29	63.36 ± 4.29*	59.47 ± 0.88**
Sperm abnormalities (%)	15.48 ± 2.13	14.60 ± 3.54	20.31 ± 2.59*	41.00 ± 9.32**
Serum hormonal levels (ng/ml)				
Testosterone	4.25 ± 0.74	4.88. ± 0.90	3.94 ± 0.43	2.93 ± 0.54**
FSH level	2.98 ± 0.94	3.0 ± 0.64	2.76 ± 0.29	1.86 ± 0.08
LH level	3.56 ± 0.27	3.41 ± 0.94	2.98 ± 0.74	2.88 ± 0.21*
Fertility indices				
No. of pregnant females	19/20	18/20	17/20	16/10
No. of implantation sites	9.96 ± 1.83	9.19 ± 1.05	7.96 ± 0.56*	6.17 ± 0.60 *
No. of viable fetus	9.85 ± 1.67	8.73 ± 1.12	6.98 ± 1.42*	6.23 ± 1.05
No. of resorption sites	3	3	5	6

T1, 2.5 mg/kg; T2, 5 mg/kg; T3. 10 mg/kg of chlorpyrifos. Data are expressed as the mean ± SEM

Measurement of FSH, LH, and testosterone

FSH, LH, and testosterone levels in the serum were observed to be significantly ($p < 0.05$, $p < 0.01$) reduced in group T3 respectively by 37.5, 19, and 31% (Table 2). However, rats from mid- and low-dose levels did not exhibit a significant effect on serum hormone levels.

Fertility

Fertility data are summarized in Table 2. The females mated with T2- and T3-treated males showed a significant ($p < 0.05$ and $p < 0.01$, respectively) reduction in number of implantation sites and live fetus when compared to the control group.

Hepatotoxicity and renotoxicity

Animals exposed to the highest dose of CPF had significantly increased ALT, AST ($p < 0.05$), and GGT ($p < 0.01$) levels. In contrast, significantly increased creatinine levels were observed both in mid ($p < 0.5$) and high ($p < 0.01$) doses (Table 3).

Blood AchE level

Effects of CPF on AchE level are summarized in Fig. 2. Animals treated with the highest dose showed a significant reduction ($p < 0.01$) in blood AchE levels by 50% compared to the control.

Discussion

Organophosphorus pesticides are among the most widely used pesticides, and extensive uses have profound impact on the environment and are hazardous to organisms including humans (Karanth et al. 2004). The present study demonstrates that CPF did not exhibit general toxicity signs, no significant

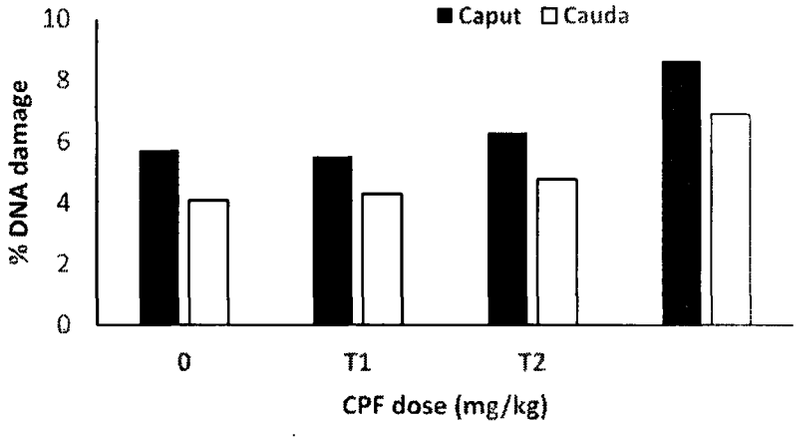

Fig. 1 DNA integrity of sperm epididymal spermatozoa. Spermatozoa were obtained from caput and cauda regions of the epididymis from the rats ($n = 12$) treated either with chlorpyrifos (T1 2.5 mg/kg, T2 5 mg/kg; T3 10 mg/kg) or the control (distilled water). Data are expressed as the mean ± SEM

Table 3 Serum levels of serum creatinine, ALT, and AST in animals treated with 2.5 mg/kg (T1), 5 mg/kg (T2), and 10 mg/kg (T3) CPF and the control

Serum parameters	Control	T1	T2	T3
Hepatotoxicity				
ALT (U/l)	23.53 ± 2.64	24.20 ± 2.20	24.20 ± 2.20	25.16 ± 3.0*
AST (U/l)	22.86 ± 8.13	22.69 ± 6.69	22.69 ± 6.69	24.41 ± 5.28*
GGT (U/l)	8.34 ± 1.54	8.63 ± 0.74	8.63 ± 0.74	14.63 ± 1.43**
Renotoxicity				
Creatinine (mg/dl)	0.46 ± 0.09	0.49 ± 0.06	1.24 ± 0.04*	2.23 ± 0.03**

Data expressed as mean ± SE

*$p < 0.05$, **$p < 0.01$

change in general behavior, and mortality. The longer sleeping hours (lethargy) observed in the rats could be due to alteration on cholinergic synaptic functions in the brain stem (Timofeeva and Gordon 2001). Reduction in AchE enzymes confirms the observation. Yet, exposure of rats to CPF resulted in a significant reduction in body weight gain and elevation in liver weights, especially at the highest concentration group (T3 group). Oxidative stress could be a contributing factor for the decreased body weight (Mossa et al. 2015). Significant increase in liver weight observed with the high dose can be attributed to toxicological effect or due to reduction of body weight gain (Mossa and Abbassy 2012). The results of this study indicate that exposure to rats for 10 mg/kg CPF triggers slight increase in ALT and AST levels and significant increase in GGT level, which are marker enzymes in serum used in appraisal of hepatic damage (Mansour and Mossa 2010). Therefore, elevated levels of ALT, AST, and GGT indicate liver damage as a result of CPF exposure (Kim et al. 2008). Similarly, serum creatinine concentration is one of the traditional screening indices for kidney function and renal structural integrity. The rise in creatinine level might be due to damage produced in kidney tubules by CPF (Shekish et al. 2013).

A range of contaminants, chemicals, and gases can affect the reproduction process in humans and animals, leaving behind damage that, in some cases, cannot be compensated (Fattahi et al. 2012). The results of our current study indicate that CPF causes reduction in sperm concentration, sperm motility, and testes weights. Reproductive organ weights are indicators of reproductive toxicity of a chemical (Zidan 2009). The results are comparable to results obtained by reduction of testicular weights observed due to CPF exposure which is in agreement with results reported by Joshi et al. (2007). Reduction of testicular weights could be used as a sensitive parameter to interpret male gonadal toxicity (Dutta and Sahu 2013). The reduced FSH, LH, and testosterone concentrations in the CPF-treated group agreed with the result obtained by previous workers (Sai et al. 2014; Shittu et al. 2013; Zidan 2009). Previous study reported to cause profound effects of CPF on hypothalamic gonadotropic releasing hormone gene expression and growth hormones (Cole et al. 2011). Reduced levels of LH and FSH observed in the present study can be attributed to the ability of CPF to suppress the gene expression of gonadotropin synthesis or its inference with steroidogenesis (Zidan 2009). Similar to other organophosphorus pesticides, the present study confirms that CPF is capable of inhibiting

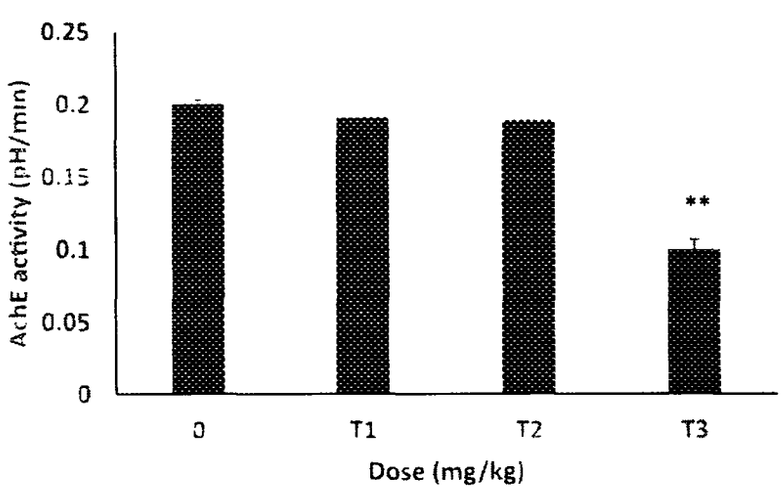

Fig. 2 Effects of Judo 40 on AchE (acetylcholine esterase) activity of rats. The *bars* represent rats ($n = 10$) treated either with chlorpyrifos (T1 2.5 mg/kg, T2 5 mg/kg, T3 10 mg/kg) or the control (distilled water) for 30 days. Results are expressed as the mean ± SEM. **$p < 0.01$

AChE activity. Prolonged brain AChE inhibition by CPF, resulting in interference with neuron transmission, may have suppressed synthesis and/or release of gonadotropins (LH and FSH), in the brain due to inhibition of their releasing hormone (Khokhar and Tyndale 2012). Luteinizing hormones (LH) target Leydig cells to produce testosterone, and exposure to CPF may cause oxidative damage to the Leydig cells thus reducing testosterone levels (Silmen et al. 2014). The low circulating LH can also exert a pervasive effect on the Leydig cells, leading to reduced testosterone levels. Chloropyrifos irreversibly inhibits CYP3A4 thus inhibiting the formation of 3-hydroxycarbofuran in human liver microsomes resulting in activation of testosterone metabolism (Usmani et al. 2004). The reduction in epididymal sperm count in rats treated with the highest dose of chlorpyrifos is in agreement to results obtained by previous workers (Shittu et al. 2013). Since normal FSH concentrations are essential for normal spermatogenesis, the low FSH levels observed in the highest dose of CPF may have contributed to low sperm count observed in the treated group (Plant and Marshall 2001). The reduction of sperm count in the CPF group is in agreement with the results obtained by previous workers (Shittu et al. 2013). The reduction in sperm count can be partly attributed to oxidative stress induced in the testes (Shittu et al. 2012; Elsharkawy et al. 2014).

Similarly, LH is important for functional epididymal maturation of spermatozoa including acquisition of progressive motility via Ca^{2+} uptake by epidermal specific β-defensin secreted by the principal cells (Lehrer and Wuyuan 2012). Thus, reduction of the LH level may cause reduced motility of spermatozoa observed in the present study.

In the present study, we observed that at least 10% of sperms from the epididymis of the treated groups were decapitated sperms. Similar observations have been recorded in previous findings in the epididymal sperms (Arena et al. 2008). Sperm abnormalities in the epididymis were related to epididymal functional capability mainly because epididymal functions are hormone dependent (Raj et al. 2014). The low level of testosterone may be the major factor inducing sperm abnormalities (Guido et al. 2014). It has been shown that functional restoration is possible with testosterone replacement (Pakarainen et al. 2005).

Another finding of the present study is the inhibitory effects of CPF on fertilization. The pregnancy indices reflect the ability of male to fertilize female (Gupta et al. 2001). It has been reported that it is essential for spermatozoa to exhibit proper movement in the fluids of the female reproductive system to reach and fertilize an egg, and therefore, motility is a prime factor in successful fertilization (Rahman et al. 2013). Since fertilization is a highly specific and an extremely subtle process, for a successful fertilization, tail movement of the sperm and integrity of the sperm head are extremely important. Increased head abnormalities indicate the possible cause for the reduced fertility of sperm (Peiris and Moore 2001a). The plasma membrane of the sperm is involved with fertilization process, mainly during the hyperactivation process, acrosome reaction, and binding to the egg plasma membrane (Gadella 2014). Further abnormalities of the head can have detrimental effects on fertilization since a normal head is important for the sperm to penetrate the egg. Abnormalities in the tail structure could contribute to impaired function and can have detrimental effects on fertilization (Peiris and Moore 2001b). Tail abnormalities are mainly due to aberration which occurs in the formation of disulfide bonds during epididymal maturation of spermatozoa (Ijiri et al. 2014). These factors collectively may possibly result in reduction of implantation sites and viable pups. On the contrary, possible loss of cauda epididymal functions can lead to loss of successful storage, which can contribute to reduction in sperm fertilization ability. Therefore, both increased sperm morphology and reduced sperm density of CPF exposure may result in reduction in pregnancy indices (Heikal et al. 2014).

Studies conducted with farmers showed impaired fertility due to a reduction in semen quality and possibly lower testosterone levels in exposed males (Peiris-John and Wickremasinghe 2008). Many pregnant women and young children continue to be exposed to high levels of CPF (Rauh et al. 2012) resulting in impairment of fetal growth and development brought about by prenatal exposure to organophosphates (Peiris-John and Wickremasinghe 2008). The results of the present study also confirm that continuous exposure to CPF is a major concern for human health.

Conclusion

In conclusion, our results demonstrate that even after 30 days of repeated exposure to CPF induces toxic insult to the male reproductive system and results in reduction in sperm count, sperm motility, and normal morphology eventually affecting the fertilizing ability of the sperm. The findings have important implications for reproductive and neurological risk assessments. It is important to take necessary action to ban chlorpyrifos to protect the farmer communities from exposure to this drift-prone pesticide, which is found in significant quantities as residue on fruits and vegetables, in air, water, dust, and people's bodies.

Compliance with ethical standards The animal experiment was performed as per committee for the purpose of control and supervision of experiments on animal norms after obtaining the institutional animal ethics committee clearance (ethical no: 25/16).

Funding This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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