Research Article

Chlorpyrifos Induce Asthenospermia in Descended Testis Rats

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ABSTRACT

Chlorpyrifos (CPF), an organophosphorous insecticides widely used in crop fields to reduce pest induced crop loss, has been associated with reproductive toxicity, particularly affecting male fertility. Healthy and motile sperm is essential to fertilize a definitive ovum. If the sperm unable to reach to the fertilizing zone within fallopian tube, fertilization will not occur, resulting infertility. The aim of this study was to examine the effects of chlorpyrifos exposure on sperm motility in rats with descended testes. In this study, descended (DT) male Charles Foster rats were orally given two graded doses of CPF (5.4 mg/kg bw/day and 8.1 mg/kg bw/day) for 30 days to examine the toxic effects on various semen quality parameters, including % of total motility, progressive motility (%), average path velocity (VAP), straight line velocity(VSL), distance straight line (DSL), distance average path (DAP), straightness (%STR), curve line distance (DCL), velocity curve line (VCL), normal morphology, abnormal sperm head, abnormal tail and reproductive hormone levels (LH, FSH, and testosterone). Results indicated a significant reduction of the % of total motility, progressive motility (%), average path velocity (VAP), straight line velocity (VSL), distance straight line (DSL), distance average path (DAP), straightness (STR), curve line distance (DCL), velocity curve line (VCL) and reproductive hormone levels (LH, FSH and testosterone) were observed dose dependently in CPF exposed groups of rats compared to control groups of rats. A significant increase of the % of abnormal head and tail of sperm were also observed in CPF treated groups of rats, suggesting that chlorpyrifos exposure significantly impairs the sperm motility that causes asthenospermia in descended testis rats. These findings suggest that chlorpyrifos exposure considerably impairs sperm motility in descended testis rats, primarily through oxidative stress-induced sperm damage and decreasing reproductive hormone levels. The findings highlight potential risks to male fertility associated with chlorpyrifos, underscoring the need for cautious use of this organophosphorous pesticide.

Keywords: Chlorpyrifos, sperm motility, descended testis, reproductive toxicity, male fertility.

INTRODUCTION

In modern agriculture, agrochemicals like pesticide plays an important role in food grain production. The pesticides and their residues persist in soil and water due to indiscriminate and intensive use. In developing country like India there is no strict compliance of pesticide regulation in regards to production and uses of pesticide. If the production and use of pesticide are not properly managed, they can create negative impact on human health, environment and ecosystem. Pesticide production started in India in the year of 1952 with the establishment of a plant for the production of BHC near Calcutta. The use of organochlorine pesticide was restricted in 1970. After that organophosphorous pesticide such as chlorpyrifos (CPF, [O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphor thioate]) (**Figure 1** have become widely used in agriculture, home gardens and veterinary practice. CPF have been widely used in India due to their cheap cost and effectiveness in controlling pests.

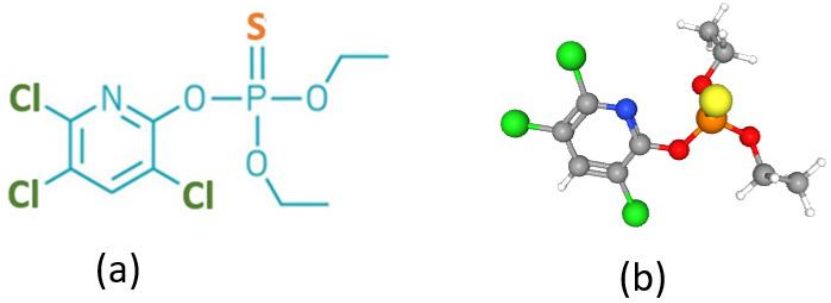

Figure 1: Chemical Structure of Chlorpyrifos (a); 3-D structure of chlorpyrifos (b)

The use of CPF to control insect pests affecting vegetable crops and ornamental plants not only exerts toxic effects on intended plants, but also exerts on other unintended living organism including human beings. Chlorpyrifos can easily enter the human body through food intake, inhalation and skin penetration [1]. CPF has been detected considerable amount in fruits, vegetables, grains, dairy products, meat, fishes, soft drinks and also detected in cervical fluid, cord blood, breast milk and meconium [2-6].

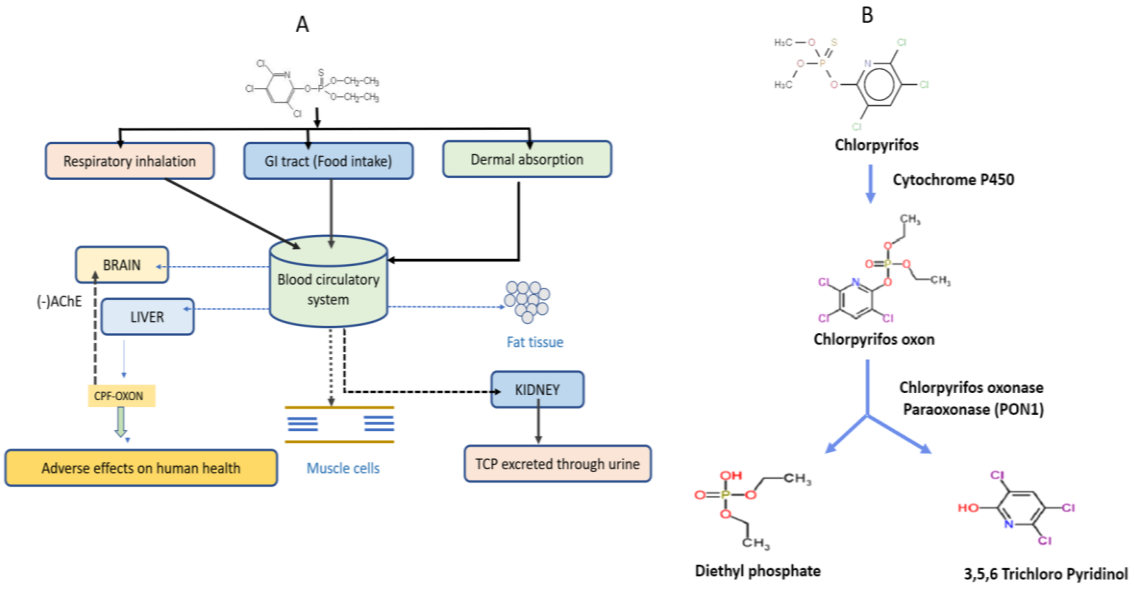

Figure 2.: A. Schematic representation showing the pharmacokinetic of CPF. B. Metabolism of of chlorpyrifos in liver by cyto P450 and PON1 (Adoppted from Timchalk et al., 2007)

After entering into the human body CPF spread to the various organ systems through blood and metabolized in the liver. In liver, the cytochrome P450 enzyme system (CYP450) converts CPF to chlorpyrifos oxon and then to 3,5,6-trichloro-2-pyridinol (TCP). TCP is the metabolite of CPF and excreted through urine **(Figure 2)**. The oxon is formed when CYP450 replaces the sulphur in the P=S group with oxygen in a process known as oxidative desulfuration[10]. The CPF-oxon is more potent acetylcholinesterase inhibitor than chlorpyrifos itself [7]. Paraoxonase (PON1) is a calcium-activated enzyme present in different tissues such as liver, brain, and blood; hydrolyzes the oxon, plays a pivotal role in detoxification of chlorpyrifos oxon. CPF oxon hydrolyzed by A-esterase to produce diethylphosphate and 3,5,6-trichloro-2-pyridinol (TCPY), which is the main biological metabolite . The active metabolite of chlorpyrifos is chlorpyrifos oxon which is further metabolised by A- and B-esterase to form diethyl phosphate and TCPY, which may undergo phase II sulfation and glucoronidation [8].Chlorpyrifos oxon mediates CPF toxicity by binding irreversibly to acetylcholinesterse (AChE) and inducing cholinergic hyperstimulation in the nervous system and neuromuscular junctions .CPF detoxification is considered to be facilitated by a-esterase (chlorpyrifos oxonase and paraoxonase) and carboxylesterase. CPF-oxon metabolite which in turn binds with the serine hydroxyl groups of acetyl cholinesterase (AChE). Thus, AChE in the post synaptic membrane of neuromuscular junction is permanently inhibited. As a result, ACh acts on respiratory or wing muscle of insects for a long time and thus the muscle becomes paralyzed. So, the insects cannot fly and the insects become die due to arrest of respiration (Mileson BE, 1998). Moreover, it has been reported that CPF-oxon exerts toxicity on the muscle by binding directly to muscarinic acetylcholine receptor and inhibiting cAMP accumulation in rat striatum (Huff RA, 1994). It has been reported that CPF induces oxidative stress, inhibits the activity of AChE, promotes neurotoxicity and degeneration of dopaminergic and cholinergic neurons [9].CPF also exerts reproductive and developmental neurotoxicity in animals due to its endocrine disrupting function [10]. The reproductive effects of CPF in mammalian animals have been reported discretely. It has also been reported that CPF causes disruption of the of breast cell cycle through production of reactive oxygen species (oxidative stress) [11].

The male reproductive system is notably vulnerable to persistent environmental pollutant, including pesticides, such as CPF. The susceptibility of the reproductive system to such toxicants can lead to impaired fertility, which is a growing concern worldwide. Among the various aspects of male reproductive health, sperm quality—including parameters such as motility, morphology, and concentration—plays a critical role in fertility potential. Impaired sperm motility is one of the leading causes of male infertility and has been associated with multiple environmental exposures. Studies have shown that chlorpyrifos can disrupt the hypothalamic-pituitary-gonadal (HPG) axis, alter testosterone levels, and impact spermatogenesis, though there is limited research specifically addressing its effects on sperm motility in descended testis conditions. Descended testes, or testes that have completed normal testicular descent during fetal development, are critical for optimal sperm production due to their maintenance at a slightly lower temperature than core body temperature. Any disturbance in this environment, whether due to temperature shifts, toxicants, or other stressors, can directly impact spermatogenesis and sperm function. In recent years, animal models have provided valuable insights into how pesticides like chlorpyrifos may affect sperm function and overall reproductive health. Despite this, most studies have focused on systemic effects or generalized reproductive toxicity, with few examining chlorpyrifos' specific impact on sperm motility in descended testis conditions.

This study aims to address this gap by evaluating the impact of CPF on sperm motility in male rats with descended testes. Using a controlled experimental model, we exposed male rats to varying doses of chlorpyrifos over a predetermined period and assessed its effects on sperm motility and other sperm quality parameters. We hypothesized that CPF exposure would impair sperm motility in a dose-dependent manner, potentially leading to compromised fertility. Additionally, we sought to understand whether chlorpyrifos exposure affects other aspects of sperm function, such as concentration and morphology, thereby providing a comprehensive view of its reproductive toxicity. This research holds considerable implications for public health, as pesticide exposure, particularly chlorpyrifos, is common among agricultural workers and populations in rural areas of India and other country of South east Asia. By understanding its effects on male reproductive health, this study aims to inform regulatory guidelines and promote safer agricultural practices to mitigate chlorpyrifos exposure. Furthermore, this research may contribute to broader discussions on pesticide use and the potential need for alternative pest control measures that pose less risk to reproductive health and overall well-being.

MATERIALS AND METHODS

Chemicals and reagents

In this study, all the reagents used were of analytical grade. Chlorpyrifos (CPF) was obtained from Devidayal (Sales) Limited, located in Mumbai, India.

Animals and experimental design

Adult healthy male Charles Foster rats aged 18-20 weeks and weighing 140-170 gm were used in the study. Animals were housed in polypropylene coated plastic cages containing paddy husk bedding and were maintain at an average room temperature of 25 ± 2° C with 12:12 h light and dark cycle. After 10 days of acclimatization, the rats were distributed to three groups. The rats of the first group (Group I) were received standard laboratory diet and were designated as control group. The rats of the second and third groups were designated as Treated I and Treated II. Treated I and Treated II received 5.4mg/BW/d (i.e., 20% LD_{50}) and 8.1 mg/BW/d (i.e., 30% LD_{50}) chlorpyrifos for 30 days duration. After completion of treatment, rats were immobilized with an approved anesthetic protocol then dissect and isolate the cauda epididymis for collection of semen. The blood was also collected for the determination of male reproductive hormone assay.

Procedure of the study of the sperm motility and morphology

A computer-assisted semen analysis (CASA) system was employed to study the parameters of sperm motility. To study rat semen quality, first immobilize the rats with an approved anesthetic protocol, then dissect and isolate the cauda epididymis. Place the epididymal tissue in a pre-warmed Petri dish containing a sperm buffer medium of Tyrode's albumin lactate pyruvate (TALP), and gently mince it to release sperm, incubating at 37°C for 10-15 minutes. Then dilute the sperm sample to 1-2 million sperm/mL for optimal concentration and load it into a CASA-compatible counting chamber. Calibrate the CASA system to rat sperm parameters (e.g., frame rate, pixel size) and maintain the sample chamber at 37°C. Adjust settings for tracking motility metrics like total motility, progressive motility, VCL, VSL, and VAP etc. Assess sperm morphology using eosin-nigrosin staining to observe defects. Analyze data across multiple samples for accurate results. CASA software summarizes motility, velocity, and morphology metrics, enabling interpretation of reproductive toxicity or fertility potential.

Hormonal study

Blood was collected from different groups of descended and cryptorchid rats and levels of serum testosterone and luteinizing hormone (LH) were measured by enzyme linked immunosorbent assay (ELISA) method using kits of ERBA Diagnostic GmbH, Mannheim, Germany.

Statistical analysis

The data were expressed in terms of mean ± SEM. The data were analysed by Student's 't' test, one way and two-way analysis of variance (ANOVA) and software 'R' wherever applicable. $^{a}P \leq 0.05$, $^{b}p \leq 0.01$, $^{c}p \leq 0.001$ were considered significant. The number of each experiment is indicated by the alphabet 'n' in the results.

RESULTS AND DISCUSSION

Effect of CPF on kinematic parameter of sperm:

Testis, the main male reproductive organ produces sperm by the process of spermatogenesis and testosterone, a steroid hormone that plays a key role in male sexual development. Healthy motile sperm is extremely essential to fertilize an ovum. After the sperm undergoes capacitation in the vaginal fluid, if it fails to reach the fertilization site in the fallopian tube, fertilization will not occur, which can lead to infertility. Moreover, suppression of any stage of the complex process of spermatogenesis in the seminiferous tubule of the testis may lead to male infertility. In this study we have examined semen quality of the chlorpyrifos (CPF) exposed male rats as a holistic indicator about the toxic effects of CPF on male reproductive system function involving the key role of hypothalamus, pituitary and testis. In this experiment we have observed a significant reduction of % of overall motility, % of sperm progressive motility and average path velocity in CPF exposed group of rats compared to control groups of rats **(Figure 3: A; B; C).** Velocity and distance of straight line (VSL/DSL) sperm movements, distance average path (DAP) was also decreased dose dependently in CPF groups of rats compared to control groups of rats (**Figure 4: A; B; C).** Moreover, % of straightness (% STR) and velocity and distance of curve line sperm movement (VCL, DCL) were also decreased dose dependently in CPF exposed groups of rats (**Figure 5: A; B; C).**

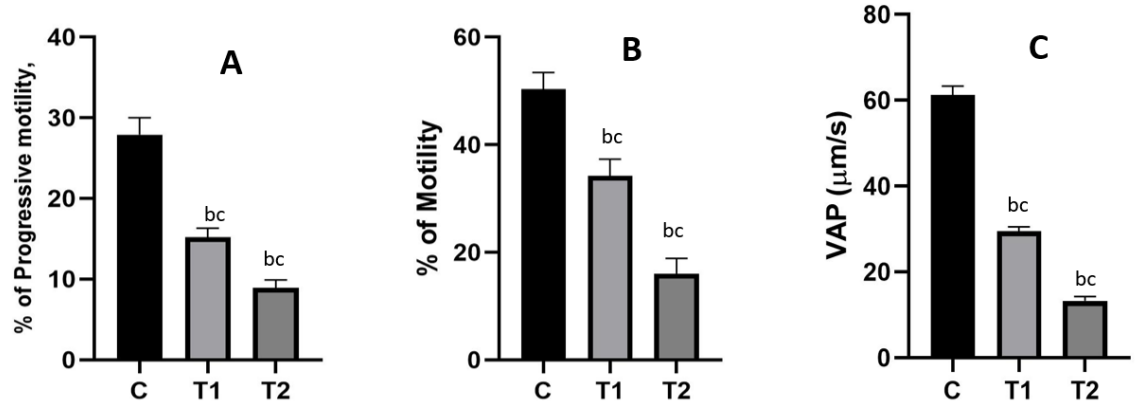

Figure 3: Bardiagrams showing the effects of CPF on the % of Progressive velocity (A); % of motility (B)and average path velocity (VAP) in control and treated groups of descended rats compare to control groups of rats. C: Control, T1: Treated 1(CPF applied 5.4mg/day/kg.BW for 30 days), T2: Treated2 (CPF applied 8.1mg/day/kg.BW for 30 days). Values are represented as Mean±SEM. [a] p<0.05, [b] p<0.01, [c] p<0.001 *vs.* control groups of rats. R-Right testis; L- Left testis. VAP-Velocity Average Path.

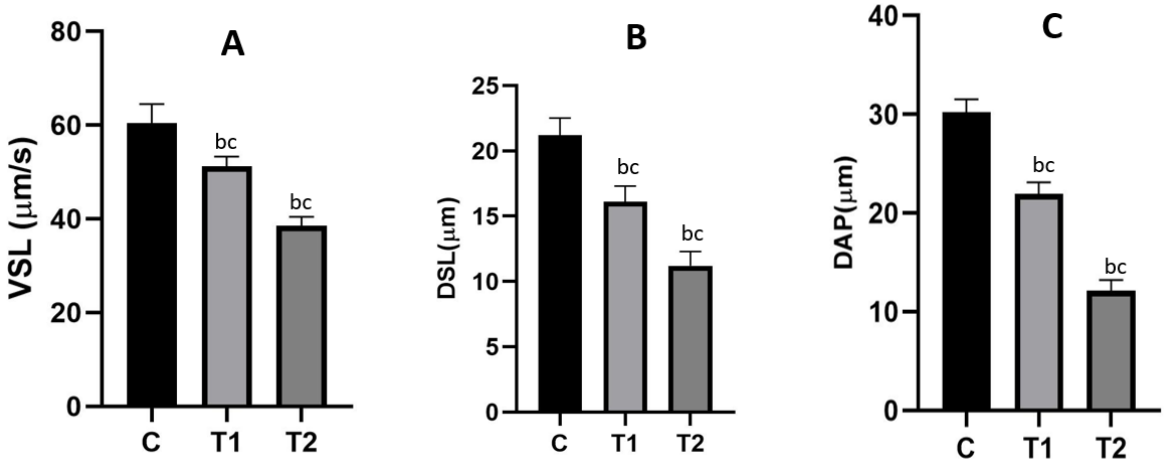

Figure 4: Bardiagrams showing the effects of CPF on the velocity straight line (µm/s)-VSL (A); Distance straight line(µm)-DSL (B) and Distance average path velocity (µm)DAP in control and treated groups of descended rats compare to control groups of rats. C: Control, T1: Treated 1(CPF applied 5.4mg/day/kg.BW for 30 days), T2: Treated2 (CPF applied 8.1mg/day/kg.BW for 30 days). Values are represented as Mean±SEM. [a] p<0.05, [b] p<0.01, [c] p<0.001 *vs.* control groups of rats. R-Right testis; L- Left testis. VSL- Velocity Straight Line; DSL-Distance Straight Line; DAP-Distance Average Path.

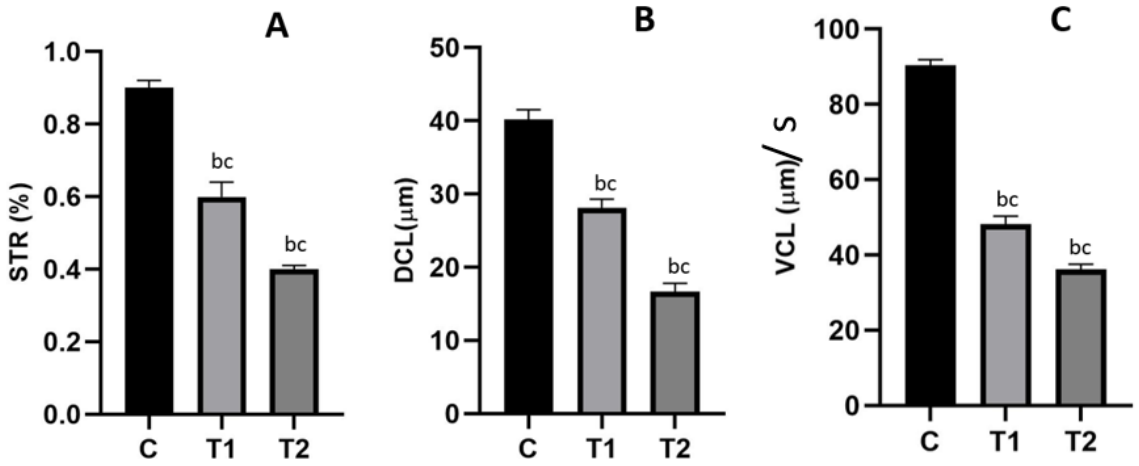

Figure 5: Bardiagrams showing the effects of CPF on the % of straightness-%STR (A); Distance curve line(µm)-DSL (B) and velocity curve line (µm)DAP in control and treated groups of descended rats compare to control groups of rats. C: Control, T1: Treated 1(CPF applied 5.4mg/day/kg.BW for 30 days), T2: Treated2 (CPF applied 8.1mg/day/kg.BW for 30 days). Values are represented as Mean±SEM. [a] p<0.05, [b] p<0.01, [c] p<0.001 *vs.* control groups of rats. R-Right testis; L- Left testis. STR-Straightness; DSL- Distance Curve Line; VCL- Velocity Curve Line (µm/s).

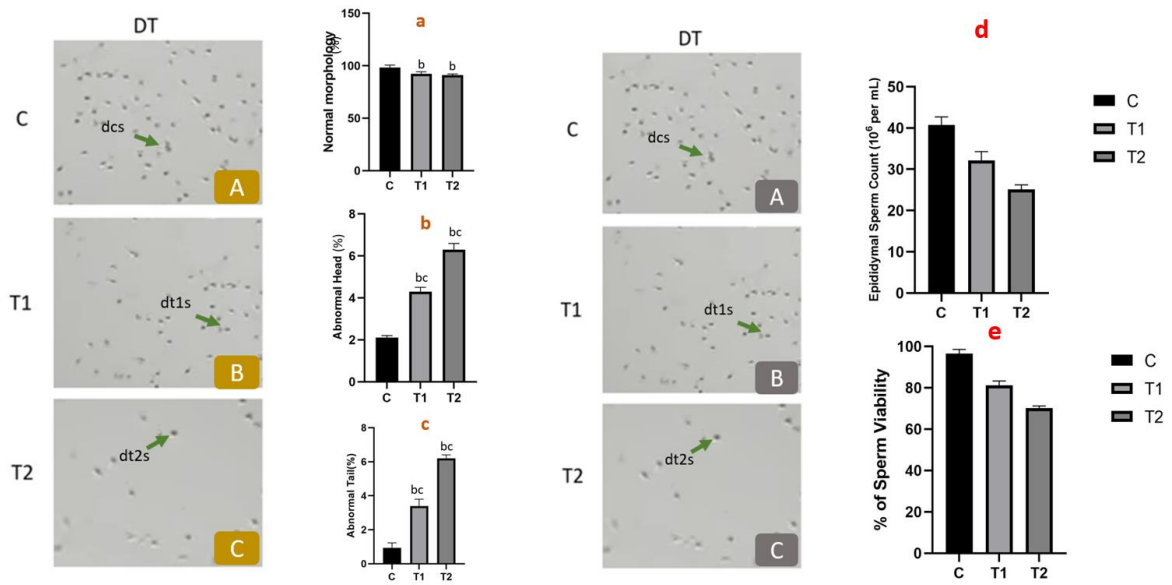

Figure 6: Bardiagrams showing the effects of CPF on the normal morphology (a); abnormal head (b); abnormal tail (c); epididymal sperm count (d); % of sperm viability of control and treated groups of descended rats compare to control groups of rats. C: Control, T1: Treated 1(CPF applied 5.4mg/day/kg.BW for 30 days), T2: Treated2 (CPF applied 8.1mg/day/kg.BW for 30 days). Values are represented as Mean±SEM. [a] p<0.05, [b] p<0.01, [c] p<0.001 *vs.* control groups of rats. R-Right testis; L-Left testis.

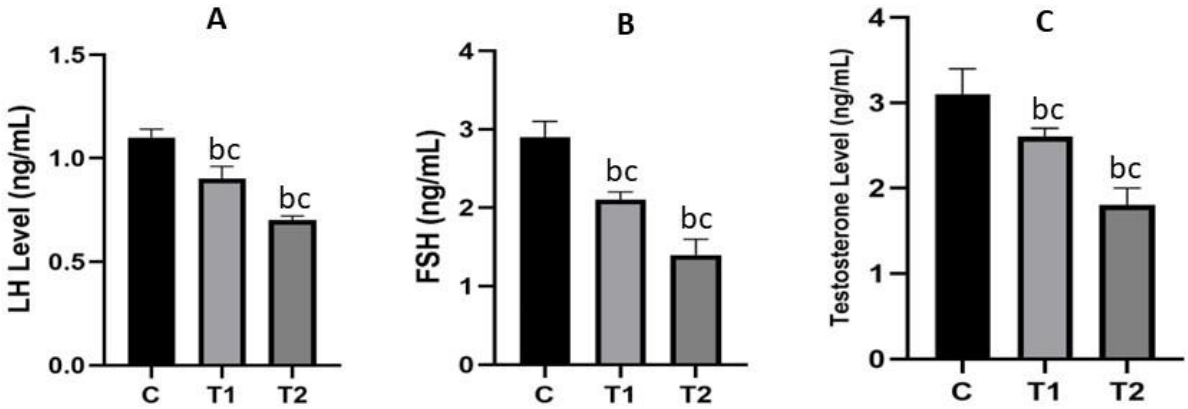

Figure 7: Bardiagrams showing the changes in male reproductive hormone levels. (A-LH; B-FSH; C-Testosterone) of descended testes of male charles foster rats. Values are Mean± SEM. [a] P<0.01; [b] P<0.001; [c] P<0.05 Vs corresponding control groups of rats. C: Control, T1: Treated 1(CPF applied 5.4mg/day/kg.BW for 30 days), T2: Treated2 (CPF applied 8.1mg/day/kg.BW for 30 days).

Effect of CPF on sperm morphology, viability, concentration and reproductive hormones:

A significant increase in the abnormal head and tail of the sperm was observed in CPF exposed groups of rats compared to control rat (**Figure 6: a; b; c).** Exposure to chlorpyrifos in rats with descended testes led to a significant decrease in sperm count and viability compared to the control group **(Figure 6: d; e).** The reduced sperm count observed in the CPF-exposed group may be due to lowered testosterone levels resulting from decreased GnRH-FSH-LH output[12].

In rats with descended testes exposed to CPF, there was a significant reduction in LH, FSH, and testosterone levels compared to the control group **(Figure 7: A; B; C).** These findings suggest that CPF may impair testicular function, likely by inhibiting the release of gonadotropins (LH and FSH) from the anterior pituitary and testosterone from Leydig cells in the testes. The decrease in blood gonadotropin levels (LH and FSH) may result from CPF-induced suppression of the genes responsible for LH and FSH production, which are crucial for gonadotropin release and steroidogenesis. CPF appears to cause prolonged suppression of neural transmission in the hypothalamic neural circuit by inhibiting brain AChE, impairing the synthesis and/or release of GnRH from the hypothalamus and subsequently reducing the synthesis and/or release of gonadotropins (LH and FSH) from the pituitary. A low LH concentration in the blood may contribute to reduced testosterone levels, as LH directly stimulates Leydig cells to produce testosterone. Consequently, the observed decrease in reproductive hormones (LH, FSH, and testosterone) could be due to disruption of the hypothalamo-pituitary-gonadal (HPG) axis. Additionally,

CPF-induced oxidative stress in the pituitary gland and testes may further reduce reproductive hormone levels. In CPF-treated rats, low testosterone levels in the blood may stem from oxidative stress–induced damage to Leydig cells, which are responsible for testosterone secretion. This suggests that CPF-induced neuroendocrine disruption of the HPG axis, along with oxidative stress–mediated degeneration of the pituitary gland and testes, likely contributes to the decline in male reproductive hormones (LH, FSH, and testosterone)[13-15].

Exposure to chlorpyrifos (CPF) in rats with descended testes led to a significant reduction in sperm count, motility, viability compared to the control group (Figure 4: A; B; C; D). The lower sperm count observed in the CPF-exposed group may result from reduced testosterone levels due to decreased GnRH-FSH-LH output. Similarly, the lower FSH levels in CPF-treated rats contributed to the reduction in sperm count, as FSH plays a critical role in sperm production. Additionally, oxidative stress induced by CPF in the testes may further decrease sperm count by generating excessive reactive oxygen species (ROS) in the seminiferous tubules, creating an unfavourable environment that hinders spermatogenesis [16-18].

The plasma membrane of spermatozoa, rich in polyunsaturated fatty acids (PUFA), is vulnerable to ROS generated by CPF exposure in the seminiferous tubules. ROS attack the PUFA-rich membrane of spermatozoa, but sperm cells employ antioxidant defenses like SOD, catalase, and ascorbic acid to counteract CPF-induced oxidative stress[19-21]. CPF disrupts testicular antioxidant defense mechanisms by reducing scavenging enzymes and glutathione, which increases free radicals in testicular tissue and impairs DNA damage repair, contributing to reduced sperm count. CPF exposure also appears to induce germ cell apoptosis via ROS, potentially suppressing gonadal function and further decreasing sperm count. Sperm motility, essential for fertilization, is another critical factor influenced by CPF exposure. In this study, CPF-exposed rats with descended testes showed significantly reduced sperm motility compared to controls. This inhibition of motility may result from lower ATP levels in spermatozoa, as CPF affects the enzymes involved in oxidative phosphorylation, leading to reduced ATP production. Since ATP is required for forward sperm movement, low ATP levels hinder motility, impairing fertilization and potentially causing infertility [22-25].

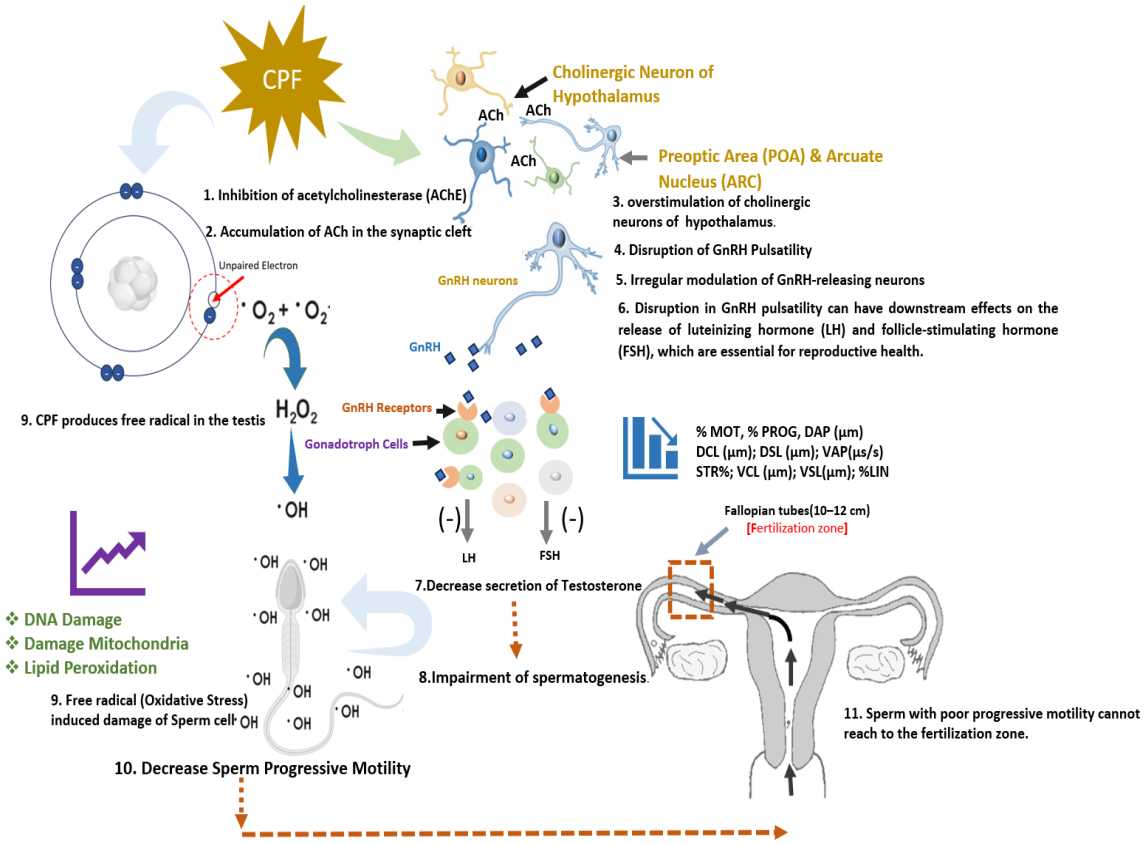

Figure 8: Chlorpyrifos (CPF) disrupts hypothalamic function and induces oxidative stress, impairing sperm motility and viability. This leads to reduced progressive motility, preventing sperm from reaching the fertilization zone and hindering fertilization.

Exposure to CPF directly interferes with the hypothalamic neural circuit by inhibiting acetylcholinesterase (AChE), resulting in the accumulation of acetylcholine (ACh) in the synaptic cleft and causing overstimulation of the hypothalamic cholinergic neurons. Overstimulation of the hypothalamic cholinergic neurons causes disruption of GnRH pulsatility and irregular secretion GnRH.

Disruption of GnRH pulsatility can have downstream effects on the release of LH and FSH which are essential for

maintenance spermatogenesis (**Figure 8**). Chlorpyrifos (CPF) generate excessive free radicals. These reactive oxygen species (ROS) cause damage to cellular structures, including lipids, proteins, and DNA, disrupting normal cellular function. In the male reproductive system, this oxidative imbalance particularly affects sperm cells, which are highly susceptible due to their limited antioxidant defense mechanisms. The free radicals generated by CPF lead to lipid peroxidation of the sperm membrane, impairing motility and structural integrity. Additionally, oxidative stress can result in DNA fragmentation within sperm cells, reducing their viability and compromising fertility[26-29]. This mechanism highlights the potential of CPF to adversely impact on spermatozoa and decrease the sperm progressive motility. As a result, capacitated sperm become incapable of progressing toward the fertilization zone, thereby hindering the fertilization process.

CONCLUSION

The findings suggest that CPF impairs the motility of the sperm in descended rats and induce asthenospermia by oxidative stress induced damage of the spermatozoa in the testis. These adverse effects on spermatozoa may also result from CPF-induced suppression of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion from the anterior pituitary, along with reduced testosterone production from the testes. This disruption seems to arise from changes in the regulatory balance of the hypothalamic-pituitary-testicular axis (HPT).

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