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Original Research Article

Possible Protective Effects of Propolis Compared to vitamin E on Sex Hormones in Male Adult Rats Exposed to Chlorpyrifos

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Abstract: ***Background*:** Chlorpyrifos (CF) is the most extensively used organophosphorus (OP) insecticide. Due to its widespread use in agriculture, residues of CF have been found in food crops and environmental samples such as air, leading to concern for potential risks to living beings. ***Objectives*:** The aim of the present study was to compare the protective roles of propolis versus vitamin E against chlorpyrifos-induced reproductive toxicity and oxidative stress in male rats. ***Methods***: A total of thirty-two adult male Wistar albino rats were randomly assigned into four groups (n = 8): control, chlorpyrifos-treated (6.7 mg/kg), chlorpyrifos + propolis (50 mg/kg) and chlorpyrifos + vitamin E (100 mg/kg). Treatments were given orally over six weeks. Serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and reduced glutathione (GSH) were assessed. Testicular tissue was also examined histopathologically. ***Results***: Chlorpyrifos exposure significantly reduced the serum LH and FSH levels, and markedly depleted GSH in comparison with the control group. Histopathological examination showed degenerative changes in the testicular tissue, loss of germ cells, and disrupted spermatogenesis. Propolis or vitamin E treatment restored normal testicular histological architecture, significantly increased antioxidant status, and maintained near-normal gonadotropin levels. However, the serum testosterone levels were not significantly affected within the experimental groups. ***Conclusion***: Chlorpyrifos-induced reproductive toxicity appears to be associated with oxidative stress and endocrine dysregulation. Propolis and vitamin E mitigated the adverse effects of exposure by enhancing antioxidant defenses and maintaining testicular architecture and hormone homeostasis.

Keywords: Chlorpyrifos, Propolis, Testis histopathology, Oxidative Stress, Glutathione.

INTRODUCTION

Chlorpyrifos (CF) is the most extensively used organophosphorus (OP) insecticide. Due to its widespread use in agriculture, residues of CF have been found in food crops and environmental samples such as air, leading to concern for potential risks to living beings [1–4]. In particular, chlorpyrifos ethyl is linked to numerous toxic effects including genotoxicity, teratogenicity, immunotoxicity, hepatotoxicity and changes in neurochemical and behavioral function [5, 6]. The principal toxic mechanism of OP compounds is via inhibition of acetylcholinesterase (AChE), the enzyme responsible for hydrolyzing acetylcholine (ACh). AChE inhibition generates considerable ACh accumulation at cholinergic synapses in both the peripheral and central nervous systems [7]. Nevertheless, toxic effects may also manifest even at exposure levels that do not significantly inhibit AChE, implying that other processes, such as oxidative stress and free radical production, are also implicated in these cases. Many studies have shown that oxidative stress is a major toxic effect induced by OPs [8]. Exposure to organophosphate pesticides can also interfere with endocrine regulation. During periods of acute stress, the hypothalamic–pituitary–adrenal (HPA) axis is activated and promotes release of vasopressin and corticotropin-releasing hormone from hypothalamic neurons, which ultimately the pituitary gland responds to by releasing adrenocorticotropic hormone (ACTH). ACTH stimulates adrenal cortex secretion of catecholamines, glucocorticoids, and mineralocorticoids [9, 10]. Previous reports have shown that exposure to organophosphate pesticides may increase ACTH secretion and thus lead to enhanced cortisol secretion [11].

A recent epidemiological study showed a link between organophosphorus insecticide exposure and diminished reproductive potential [12, 13]. Due to the frequent detection of residue from these pesticides on food products, animal studies have been conducted to explore the effects of low-dose CF exposure on reproductive function [8-14]. Long-term exposure to CF has been reported to decrease sperm count and motility while increasing the proportion of morphologically abnormal and immotile spermatozoa in rats [14, 15]. Increasing evidence suggests that oxidative stress plays a key role in chlorpyrifos-induced reproductive toxicity by promoting lipid peroxidation and depletion of endogenous antioxidants such as glutathione in testicular tissues [8-16]. Propolis (PR) is a resinous substance collected by honey bees from various plant sources and used to protect and seal honeycombs within the hive [17]. In recent years, propolis has gained considerable attention as a natural health-promoting supplement and has been incorporated into various food and beverage products worldwide. Numerous studies have reported that PR possesses diverse biological activities, including antimicrobial, antiviral, hepatoprotective, and renoprotective properties [18, 19].

Both aqueous and alcoholic extracts of propolis have demonstrated anti-inflammatory, anticancer, and immunomodulatory activities [20]. In addition, several in vitro and in vivo investigations have confirmed that propolis extracts exhibit strong antioxidant properties by inhibiting free radical formation and lipid peroxidation, thereby protecting cells from oxidative damage [21, 22]. Experimental studies in rabbits have also shown that propolis supplementation can increase testosterone levels, reproductive organ weight, sperm motility, and sperm quality, while reducing oxidative stress markers [23]. Therefore, the present study aimed to evaluate the protective effects of propolis in comparison with vitamin E on reproductive hormones and oxidative stress in male rats exposed to chlorpyrifos. Furthermore, the study investigated whether propolis supplementation could mitigate pesticide-induced reproductive toxicity.

MATERIALS AND METHODS

Materials

Chlorpyrifos (CF) 48% technical concentrate (TC) was obtained from Nanjing, Jiangsu, China, and diluted in corn oil to prepare a 10% emulsion prior to administration. Propolis powder was obtained from a commercial herbal supplier in Erbil, Iraq, and stored in airtight containers at room temperature until use. Vitamin E (400 IU soft gelatin capsules) was manufactured by Mera Pharmaceuticals, Germany.

Animals

Thirty-two adult male Wistar albino rats weighing 170–200 g were used in the present study. The animals were obtained from the College of Veterinary Medicine, University of Kufa, and allowed to acclimatize for two weeks before the start of the experiment. Rats were housed in plastic cages (three animals per cage) with standard bedding. The animals were maintained under controlled environmental conditions at a temperature of 21 ± 3 °C and relative humidity of 50 ± 5%, with a 12 h light/dark cycle. Rats were provided with a standard pellet diet and had free access to drinking water throughout the study. The experiment was conducted in the animal facility of the College of Pharmacy, University of Basrah, in February 2025, for a total duration of six weeks.

Experimental Design

The thirty-two rats were randomly allocated into four experimental groups (n = 8 per group). Body weight measurements were recorded at the beginning of the experiment and monitored weekly during the treatment period. Group I (control): received corn oil (1 mL/kg/day) orally by gavage, group II (CF): received chlorpyrifos at a dose equivalent to 1/20 of the LD_{50} (6.7 mg/kg/day), administered orally in corn oil [24], group III (CF + PR): received chlorpyrifos (6.7 mg/kg/day) together with propolis (50 mg/kg/day) [25], and group IV (CF + VE): received chlorpyrifos (6.7 mg/kg/day) along with vitamin E (100 mg/kg/day) [26]. The selected doses were based on previously published studies. After six weeks of treatment, rats were fasted overnight and then sacrificed. Blood samples were collected through intracardiac puncture. Serum was separated by centrifugation at 5000 rpm for 10 minutes and stored at −20 °C until further biochemical analyses. Testicular tissues were excised, rinsed with physiological saline (0.9%), and longitudinally divided into two portions. The samples were fixed in 10% neutral buffered formalin for histopathological examination.

Biochemical Analysis

Luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone serum concentrations were measured using an automated immunoassay analyzer (Cobas e 411; Roche Diagnostics, Germany) following the manufacturer's instructions. Measurement of serum reduced glutathione (GSH) levels was performed using a competitive enzyme-linked immunosorbent assay (ELISA) kit (Reed Biotech, Wuhan, China) according to the manufacturer's protocol.

Histopathological Investigation

Testicular tissues were fixed in 10% formalin for approximately four days. The samples were subsequently dehydrated, embedded in paraffin, and sectioned into 4–5 µm thick slices. The sections were stained with hematoxylin and eosin (H&E). Microscopic evaluation was performed using a Genex light microscope with a 20× objective lens. Two tissue sections from each experimental group were examined for qualitative histopathological assessment.

Statistical Analysis

Statistical analyses were performed with GraphPad Prism, version 7.0, USA. Data are shown as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test was performed to evaluate differences within experimental groups. Group-based changes in body weight were measured with a paired t-test. Statistical significance was defined as a p-value < 0.05.

RESULTS

No mortality occurred in any experimental group during the study period. Moreover, the animals did not exhibit evident signs of toxicity such as lacrimation or tremors. Body weight increased significantly during the experimental period in all groups except the CF-treated group (Fig. 1).

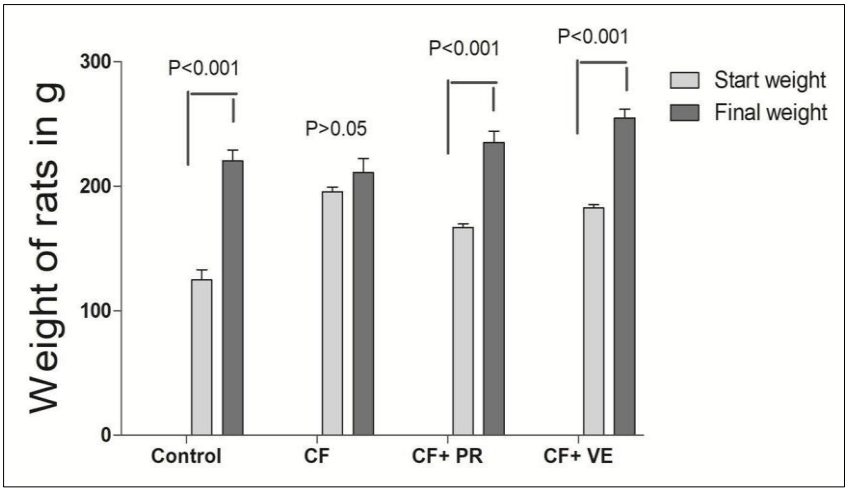

Fig. 1: Body weight changes in rats across the experimental groups during the study period. CF: chlorpyrifos; PR: propolis; VE: vitamin E. *p < 0.05

Treatment with chlorpyrifos produced a marked decline in serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels relative to the control group (p<0.01; Fig. 2 A&B). In contrast, rats receiving propolis or vitamin E alongside chlorpyrifos exhibited LH and FSH levels that remained close to those observed in the control animals. Serum testosterone concentrations did not differ significantly among the studied groups (p>0.05; Fig. 2 C).

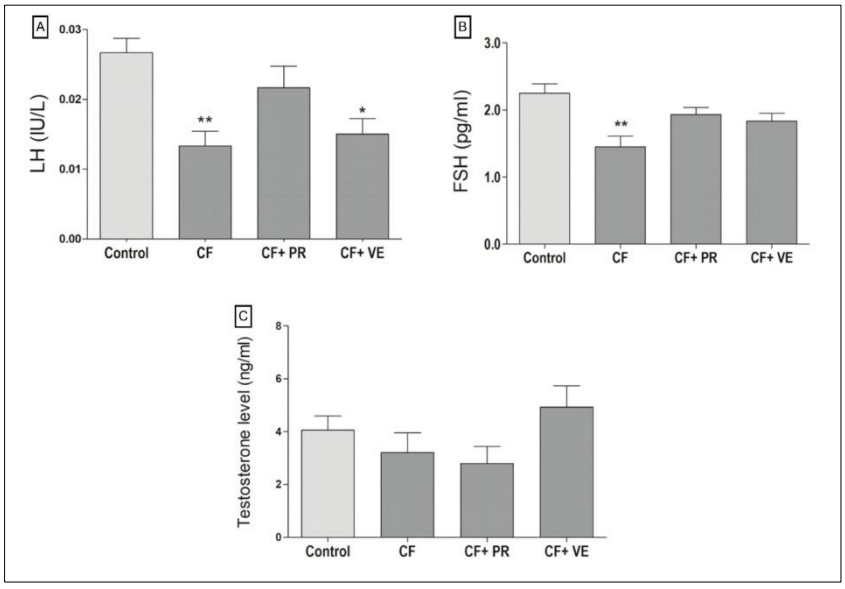

Fig. 2: Influence of chlorpyrifos treatment with or without propolis or vitamin E on reproductive hormone levels in male rats: (A) luteinizing hormone (LH), (B) follicle-stimulating hormone (FSH), and (C) testosterone. CF: chlorpyrifos; PR: propolis; VE: vitamin E. *p<0.05, **p<0.01 vs. control

As illustrated in (Fig. 3), chlorpyrifos exposure caused a pronounced depletion of serum reduced glutathione (GSH) when in comparison with the control group (p<0.001). Conversely, animals receiving propolis or vitamin E together

with chlorpyrifos showed a significant improvement in GSH levels relative to the CF-only group ($p<0.001$ and $p<0.01$, respectively).

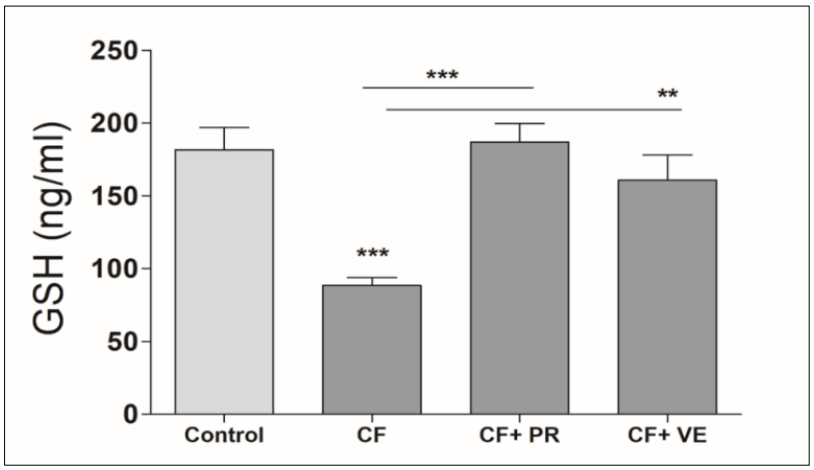

Fig. 3: Serum reduced glutathione (GSH) concentrations in rats exposed to chlorpyrifos with or without antioxidant treatment. CF: chlorpyrifos; PR: propolis; VE: vitamin E. **p < 0.01, ***p<0.001.

Histopathological Examination of the Testis

Histopathological examination of testicular tissue revealed normal histological architecture in the control group, characterized by well-organized seminiferous tubules and active spermatogenesis (Fig. 4A). In contrast, the CF-treated group (6.7 mg/kg) showed marked degenerative changes, including germ cell loss and cytoplasmic vacuolization (Fig. 4B). Treatment with propolis (50 mg/kg) improved the testicular structure, with seminiferous tubules appearing nearly normal and restoration of spermatogenic activity (Fig. 4C). Similarly, vitamin E treatment (100 mg/kg) showed noticeable restoration of seminiferous tubule structure and germinal epithelium in comparison with the CF group (Fig. 4D).

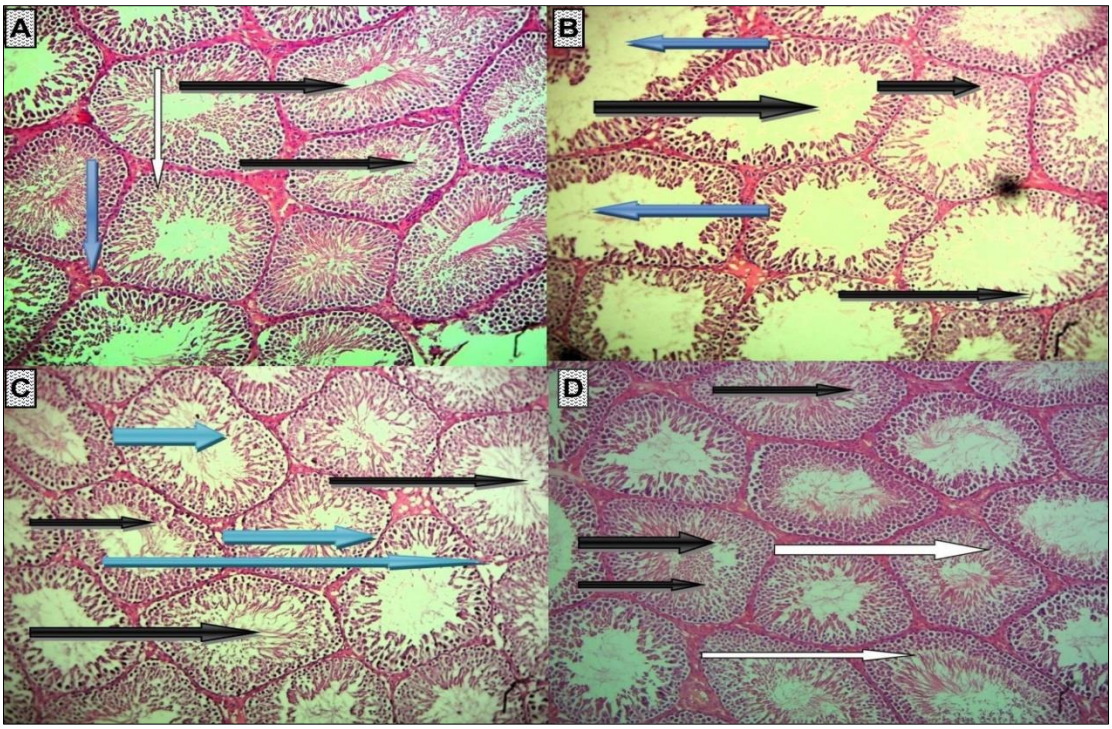

Fig. 4: Microscopic section of rat testis (H&E, 20×). (A) The control group shows normal seminiferous tubules full of spermatozoa (black arrows), with normal architecture of germinal cell proportion (white arrows) and Leydig cells (blue arrows). (B) CF group (6.7 mg/kg) showing severe germ cell loss, vacuolization, and impaired spermatogenesis (black arrow); seminiferous tubules with negative tubular differentiation and repopulation indices, as well as severe edema and infiltration of immune mononuclear cells (blue arrow). (C) CF + PR group (50 mg/kg) showing seminiferous tubules morphologically within normal limits and similar in size (blue arrow), with a lot of spermatids (black arrow). (D) CF + VE group (100 mg/kg) showing restoration of seminiferous tubule structure (white arrow) and germinal epithelium layers along with the presence of spermatids (black arrow). Abbreviations: CF = chlorpyrifos; PR = propolis; VE = vitamin E.

DISCUSSION

Organophosphorus pesticides are among the most widely used pesticides in agriculture. Their extensive use has significant impacts on the environment and poses potential risks to humans and other organisms. These compounds can easily enter the human body through the food chain, as well as through direct exposure to contaminated air and dust containing pesticide residues and plant fragments [27, 28]. Chronic exposure, even at low levels, to these pesticides can cause a wide range of harmful effects on human health like metabolic, reproductive, cardiovascular and endocrine disorders [29]. The results of the present study demonstrated a significant increase in body weight among all experimental groups from the start to the end of the experiment, with no significant increase in body weight for the CF-treated group. This attenuated body weight gain was likely attributable to oxidative stress triggered by chlorpyrifos exposure [30]. Such observations are consistent with experimental studies, in which treatment with chlorpyrifos was linked to reduced body weight gain by the treated animals [31].

The current study showed that serum LH and FSH levels were reduced significantly by chlorpyrifos treatment. These hormones are important for the regulation of testicular function and spermatogenesis via the hypothalamic–pituitary–gonadal axis [32, 33]. The decrease observed might be due to the neurotoxic and oxidative action of organophosphate pesticides that can disrupt endocrine regulation and pituitary functioning [34, 35]. Previous studies have shown comparable reductions in gonadotropin levels with chlorpyrifos exposure in male rats, consistent with reproductive endocrine dysfunction [36, 37]. Conversely, both propolis and vitamin E supplementation in chlorpyrifos-administered rats maintained normal LH and FSH levels. This protective effect could relate to the antioxidant activity of these agents. Propolis is a natural resinous substance rich in flavonoids and phenolic compounds that exhibit strong antioxidant and anti-inflammatory activities, which may help protect tissues from oxidative damage [38]. Similar protective effects of propolis against oxidative stress and reproductive toxicity have been reported in experimental studies [14-25]. Likewise, vitamin E is a well-known lipid-soluble antioxidant that protects cellular membranes from oxidative injury by neutralizing reactive oxygen species [39], and its protective role against pesticide-induced toxicity has been demonstrated in previous studies [26]. Interestingly, serum testosterone levels were not significantly altered among the experimental groups. This finding suggests that the exposure conditions used in the present study may have primarily affected gonadotropin secretion rather than directly impairing Leydig cell steroidogenesis. It is also possible that the CF dose used in this study (6.7 mg/kg) was not sufficient to induce a significant reduction in testosterone levels. Similar observations have been reported in experimental studies where organophosphate exposure altered pituitary hormones, whereas higher doses were required to significantly affect testosterone production [37-40].

Oxidative stress appears to play an important role in chlorpyrifos-induced reproductive toxicity. The significant depletion of GSH observed in chlorpyrifos-treated rats indicates increased oxidative stress and impaired antioxidant defense mechanisms. Reduced glutathione is one of the most important intracellular antioxidants and plays a key role in protecting cells from oxidative damage. Organophosphate pesticides have been reported to increase the generation of reactive oxygen species, leading to lipid peroxidation and depletion of antioxidant molecules such as GSH [16-41]. Propolis and vitamin E treatment, significantly improved GSH levels versus the chlorpyrifos-treated group. These seem to suggest that both of these agents have protective effects through their antioxidant mechanisms. Propolis is known to be rich in flavonoids and phenolic compounds, which have the ability to improve endogenous antioxidant defenses and decrease oxidative damage [42]. Similarly, vitamin E is a chain-breaking antioxidant able to inhibit lipid peroxidation within biological membranes [43]. Histopathological findings in the present study further supported the biochemical results. Chlorpyrifos exposure resulted in prominent degenerative changes in testicular tissue: germ cell loss, vacuolization and altered spermatogenesis. These changes may be linked to oxidative stress and disruption of hormonal regulation induced by pesticide exposure [44]. These findings corroborate those reported in previous studies examining chlorpyrifos-mediated reproductive toxicity in experimental animals [45, 46].

Conversely, propolis and vitamin E ameliorated the histology of testicular tissues in rats by maintaining structural integrity of seminiferous tubules and restoring spermatogenic cells. These results suggest that antioxidant properties of propolis and vitamin E may contribute to maintaining the structure and function of testicles in conditions of pesticide-induced oxidative stress [47, 48].

CONCLUSION

Chlorpyrifos exposure causes male reproductive toxicity primarily through oxidative stress and disturbance of the hypothalamic–pituitary–gonadal axis in rats. The combination of propolis and vitamin E proved to have protective effects by restoring antioxidant status and maintaining testicular structure, as well as hormonal balance. These results indicate that antioxidant agents can reduce pesticide-induced reproductive damage.

Ethical Approval

All experimental procedures were conducted in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals. Ethical approval was obtained from the Ethical Committee of the College of Pharmacy, University of Basrah (approval number EC89, November 2024).

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Conflict of Interest: The authors declare that they have no conflicts of interest.

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