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RESEARCH ARTICLE OPEN ACCESS

Protective effects of sodium copper chlorophyllin on chlorpyrifos-induced thyroid toxicity in adult female rats

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Abstract

Objective: Chlorpyrifos (CP), a commonly used organophosphorus pesticide, poses significant risks to human and animal health, particularly the thyroid gland. This study evaluated the protective effects of sodium copper chlorophyllin (SCC), a chlorophyll derivative with antioxidant and anti-inflammatory properties, against CP-induced thyroid toxicity in rats.

Method: Thirty adult female albino rats were randomly divided into five groups (n=6 each): Group 1 (Control), Group 2 (CP, 6.7 mg/kg), Group 3 (SCC, 50 mg/kg), Group 4 (CP + SCC 50 mg/kg), and Group 5 (CP + SCC 100 mg/kg). Treatments were administered orally for six weeks. Serum thyroid hormones (T3, T4, TSH), inflammatory cytokines (IL-10, TNF-α), and oxidative stress biomarkers [malondialdehyde (MDA), glutathione (GSH)] were measured. The thyroid tissues were examined histopathologically.

Results: CP exposure significantly decreased serum T3, T4, TSH, IL-10, and GSH, while increasing TNF-α and MDA ($p<0.05$). SCC alone did not significantly alter these parameters. Co-administration of SCC with CP markedly improved thyroid hormone levels, restored IL-10, TNF-α, and GSH, and reduced MDA in a dose-dependent manner. Histopathological findings confirmed the protective role of SCC.

Conclusion: SCC demonstrated antioxidant and anti-inflammatory effects that mitigated CP-induced thyroid damage in female rats, with the higher dose (100 mg/kg) providing greater protection. Further studies are needed to clarify the underlying molecular mechanisms and evaluate clinical relevance.

Keywords: Chlorpyrifos (CP), Sodium copper chlorophyllin (SCC), Thyroid toxicity, Oxidative stress, TNF-α, IL-10

Plain English Summary

Chlorpyrifos (CP) is a pesticide widely used to kill insects on crops, but it may also harm humans and animals. One of the organs most affected is the thyroid gland, which controls hormones and metabolism. This study tested sodium copper chlorophyllin (SCC), a natural plant pigment with antioxidant properties, to see if it could protect the thyroid from CP-induced damage. Thirty female rats were divided into five groups: some received only CP, some only SCC, and others received both for six weeks. After treatment, blood and thyroid tissues were analysed. The results showed that rats exposed to CP had lower thyroid hormone levels (T3, T4, TSH), lower glutathione (GSH, a natural antioxidant), and reduced IL-10 (an anti-inflammatory marker). At the same time, they had higher levels of malondialdehyde (MDA, a marker of oxidative damage) and TNF-α (a pro-inflammatory marker). When SCC was given with CP, these harmful effects were reduced: hormone levels and GSH improved, IL-10 increased, and both MDA and TNF-α decreased. Tissue studies confirmed that SCC protected the thyroid, with the higher dose showing stronger benefits. SCC may therefore help protect against pesticide-induced thyroid injury, but more research is needed before applying these findings to humans.

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Introductions

Chlorpyrifos (CP) is a widely used organophosphorus insecticide in agriculture and livestock production. Residues persist in soil, water, and food, raising major concerns about human and animal health (1, 2). CP exerts its toxicity primarily by inhibiting acetylcholinesterase, but also through oxidative stress and inflammatory responses that damage multiple organs, including the thyroid gland (3, 4, 5). Chronic exposure has been linked to reduced thyroid hormones and histopathological changes (6, 7).

Oxidative stress is a central mechanism of CP-induced thyroid dysfunction, characterised by increased reactive oxygen species (ROS), elevated lipid peroxidation, and altered cytokine balance (↑ TNF-α, ↓ IL-10) (8, 9, 10). Sodium copper chlorophyllin (SCC), a derivative of chlorophyll, exhibits antioxidant, anti-inflammatory, and chemoprotective properties (11, 12, 13). It scavenges ROS, inhibits DNA damage, and modulates immune response (14, 15, 16). However, its protective role against CP-induced thyroid dysfunction has not been systematically evaluated.

Materials And Methods

Materials

Chlorpyrifos (CP) was obtained from Nanjing, Jiangsu, China, and sodium copper chlorophyllin (SCC) from Now Foods, USA. Other reagents included chloroform, formaldehyde, eosin, hematoxylin, and paraffin. Commercial ELISA kits (validated for rat serum) were used for hormone and biomarker analysis (8, 9).

Methods

Thirty adult female albino rats (12–14 weeks old, 150–230 g) were obtained from the University of Baghdad. Animals were housed under controlled conditions with standard food and water. After acclimatisation, they were randomly divided into five groups (n=6 each) (10, 11):

Group 1 (Control): distilled water.
Group 2 (CP): 6.7 mg/kg/day CP orally (1/20 LD50).
Group 3 (SCC): 50 mg/kg/day SCC orally.
Group 4 (CP + SCC 50 mg/kg): CP + SCC 50 mg/kg daily.
Group 5 (CP + SCC 100 mg/kg): CP + SCC 100 mg/kg daily.

Treatments continued for six weeks. At the end, blood and thyroid samples were collected for biochemical and histopathological analysis (12).

Biochemical Analysis

Serum samples were evaluated for thyroid function parameters and inflammatory markers. Levels of triiodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH), interleukin-10 (IL-10, a pro-anti-inflammatory cytokine), and tumour necrosis factor alpha (TNFα) were quantified using enzyme-linked immunosorbent assay (ELISA). All assays were performed following the manufacturer's protocols, utilising validated ELISA kits specifically designed for use in laboratory animals.

Histopathological Examination of Thyroid Tissue

Each rat's thyroid gland was meticulously dissected and fragmented. These were cleaned with distilled water to remove blood and tissue debris, then fixed in 10% formaldehyde to preserve cells. The samples were dehydrated in graded ethanol and embedded in paraffin wax for sectioning after fixing. Thin tissue slices (3-4 µm) were microtome-prepared and placed on clean glass slides. H&E was used to stain the sections for microscopic examination. A certified histopathologist used a light dissecting microscope to evaluate the samples, and an integrated camera system acquired representative photos.

Statistical Analysis

SPSS 26 was used for statistical analysis. The Shapiro–Wilk test determined data normality. Measurements are shown as mean ± standard error of the mean (SEM). One-way ANOVA was used to assess intergroup differences in normal datasets. The Kruskal–Wallis test was used for non-parametric comparisons when normality assumptions were broken. Results were graphed in GraphPad Prism. Statistical significance was indicated by p-value edges: ***$p < 0.001$, **$p < 0.005$, *$p < 0.05$.

Results

General Observations

No mortality or clinical signs of toxicity (e.g., lacrimation, tremors) were observed in any experimental group throughout the study period.

Body Weight Changes

All groups showed a gradual increase in body weight during the experimental period, except for the CP group, which exhibited a significantly lower gain compared to the control ($p < 0.05$). Co-treatment with SCC (50 or 100 mg/kg) attenuated this effect, with the higher dose showing greater improvement (Figure 1).

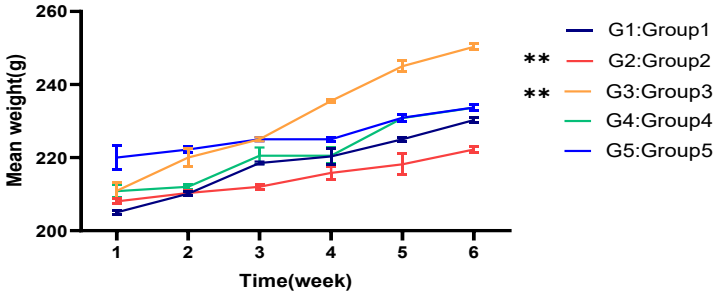

Figure 1: Comparative analysis of body weight changes among experimental rat groups. Data are presented as mean ± SEM (n = 6). *p < 0.05 vs. control.

Thyroid Hormones

CP exposure (6.7 mg/kg) significantly reduced serum T3 (ng/dL), T4 (µg/dL), and TSH (µIU/mL) levels compared with the control group ($p < 0.05$). Administration of SCC alone (50 mg/kg) did not alter hormone levels. Co-treatment with SCC (50 or 100 mg/kg) restored T3, T4, and TSH in a dose-dependent manner, with the 100 mg/kg group approaching values comparable to controls (Figure 2).

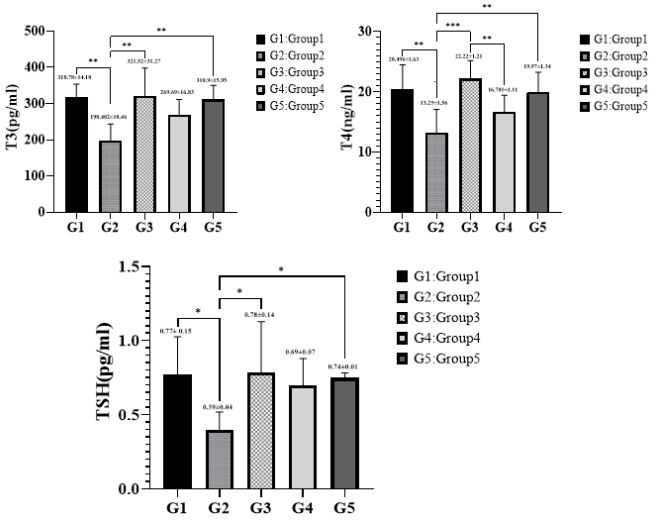

Figure 2: Effect on serum triiodothyronine (T3, ng/dL), thyroxine (T4, µg/dL), and thyroid-stimulating hormone (TSH, µIU/mL) levels in different groups. Values are mean ± SEM (n = 6) *p < 0.05, p < 0.01, *p < 0.001 vs. control

Inflammatory Cytokines

Rats exposed to CP exhibited a significant decrease in IL-10 (pg/mL) and a concomitant increase in TNF-α (pg/mL) compared to controls ($p < 0.05$). SCC alone had no significant effect. Co-administration of SCC with CP increased IL-10 and reduced TNF-α levels, with the high dose providing the most pronounced modulation (Figures 3 and 4).

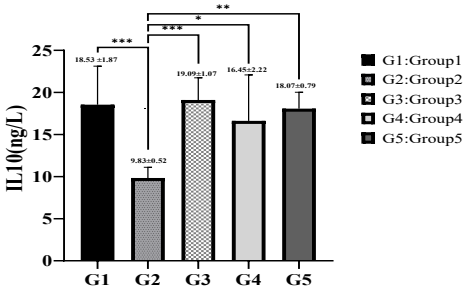

Figure 3: Effect on serum Interleukin-10 (IL-10, pg/mL) levels. Data are expressed as mean ± SEM (n = 6). *p < 0.05, p < 0.01, *p < 0.001 vs. control

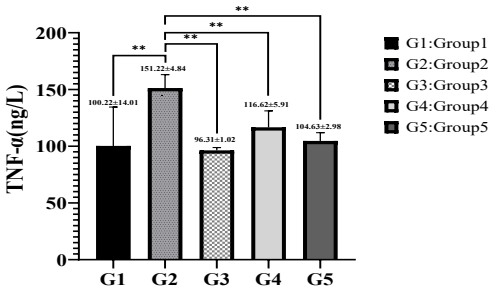

Figure 4: Effect on serum tumour necrosis factor-alpha (TNF-α, pg/mL) levels. Data are expressed as mean ± SEM (n = 6). *p < 0.05, p < 0.01, *p < 0.001 vs. control

Oxidative Stress Biomarkers

CP significantly elevated serum malondialdehyde (MDA, nmol/mL) levels and reduced glutathione (GSH, µmol/mL) concentrations relative to the control group ($p < 0.05$). SCC alone did not alter oxidative stress markers. However, SCC co-treatment markedly reduced MDA and increased GSH, with the higher dose yielding results close to control values (Figure 5).

Histopathological Findings

Histological analysis of thyroid tissue supported the biochemical findings

Control (G1) and SCC (G3) groups displayed normal thyroid architecture with intact colloid-filled follicles lined by cuboidal epithelial cells.

CP group (G2) showed severe pathological changes, including follicular cell necrosis, vacuolization, irregular follicle size, and inflammatory infiltration.

CP + SCC 50 mg/kg (G4) demonstrated partial restoration, with follicles mostly intact and only mild vacuolization.

CP + SCC 100 mg/kg (G5) showed near-complete preservation of thyroid morphology similar to controls.

For semi-quantitative assessment, histopathological alterations were scored on a 0–3 scale (0 = normal, 1 = mild, 2 = moderate, 3 = severe). The CP group showed the highest score (2.8 ± 0.2), while SCC co-treatment significantly reduced lesion severity in a dose-dependent manner (G4: 1.4 ± 0.3; G5: 0.6 ± 0.2; $p < 0.05$ vs CP) (Figure 6).

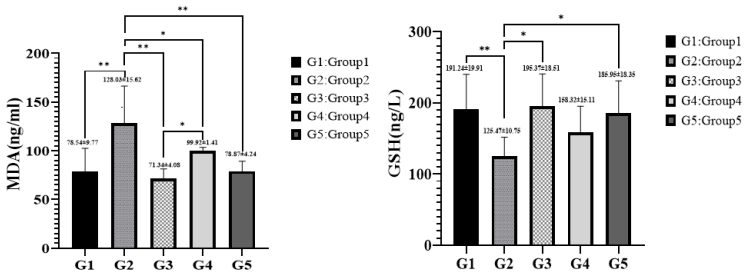

Figure 5: Serum oxidative stress markers: malondialdehyde (MDA, nmol/mL) and glutathione (GSH, µmol/mL) in experimental groups. Values are mean ± SEM (n = 6). *p < 0.05, p < 0.01, *p < 0.001 vs. control

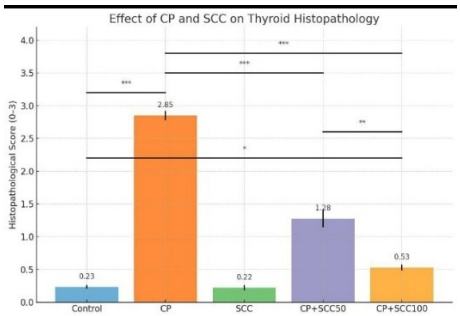

Figure 6: Histopathological scoring of thyroid tissue in experimental groups (0–3 scale). Data are presented as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test. *p < 0.05, p < 0.01, *p < 0.001

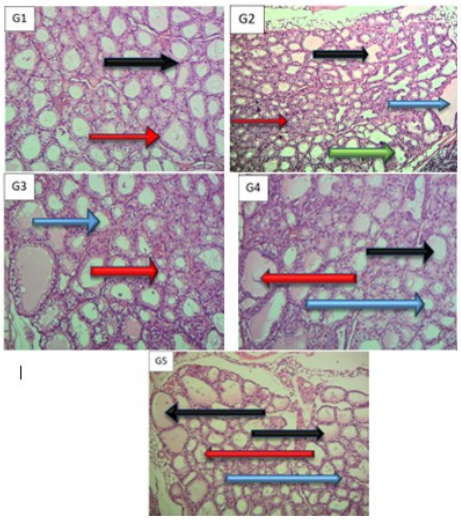

Figure 7: Representative histological sections of thyroid tissue (H&E, 200×). G1 (Control), G2 (CP 6.7 mg/kg), G3 (SCC 50 mg/kg), G4 (CP + SCC 50 mg/kg), G5 (CP + SCC 100 mg/kg)

Discussion

This study demonstrates that chlorpyrifos (CP) induces thyroid toxicity in female rats through oxidative stress and inflammatory disruption, as evidenced by suppressed thyroid hormones (T3, T4, TSH), elevated malondialdehyde (MDA), depleted glutathione (GSH), and imbalanced cytokines (↓IL-10, ↑TNF-α). Sodium copper chlorophyllin (SCC) co-treatment mitigated these effects dose-dependently, with the higher dose (100 mg/kg) providing near-complete restoration of thyroid function and histology.

The protective effects of SCC align with its established dual role as an antioxidant and anti-inflammatory agent. By scavenging CP-induced reactive oxygen species (ROS) (8), SCC reduces lipid peroxidation (↓MDA) and preserves endogenous antioxidants (↑GSH). Concurrently, it modulates immune responses, elevating anti-inflammatory IL-10 while suppressing pro-inflammatory TNF-α, a mechanism corroborated by Jin et al. (12) in bone marrow studies. The superior efficacy of the 100 mg/kg dose may reflect saturation of antioxidant pathways (e.g., Nrf2 activation) or enhanced binding affinity to ROS, as observed in dose-response studies of chlorophyllin derivatives (15, 17). Notably, Lanfer-Marquez et al. (13) reported a linear dose-effect relationship for SCC's radical-scavenging capacity up to 150 mg/kg in rodents, supporting our dose selection.

Compared to other CP protectants like selenium (18) or melatonin (19), SCC offers broader activity, neutralising ROS, chelating metal ions, and stabilising membranes via its porphyrin structure (14, 16). This multi-target action likely underlies its robust histological preservation, even at lower doses (50 mg/kg).

Limitations and Future Directions

This study has some limitations. The sample size was relatively small (n = 6 per group), which may limit generalizability. Only female rats were included, so sex-related differences were not assessed. The study duration was limited to six weeks. Finally, molecular and genetic mechanisms were not investigated. Future research should address these limitations. Future work should explore SCC's pharmacokinetics and dose optimisation in mixed-sex cohorts.

Conclusion

SCC at 100 mg/kg effectively counters CP-induced thyroid damage by restoring redox balance and cytokine homeostasis. Its dose-dependent efficacy, supported by prior pharmacokinetic data (15), positions it as a promising adjuvant against pesticide toxicity.

List of Abbreviations

CP: Chlorpyrifos
ELISA: Enzyme-Linked Immunosorbent Assay
H&E: Haematoxylin and Eosin
IL-10: Interleukin-10
ROS: Reactive Oxygen Species
SCC: Sodium Copper Chlorophyllin
SEM: Standard Error of the Mean
SPSS: Statistical Package for the Social Sciences
T3: Triiodothyronine
T4: Thyroxine
TNF-α: Tumour Necrosis Factor Alpha
TSH: Thyroid-Stimulating Hormone

Declarations

Ethics approval and consent to participate

All animal procedures were conducted in compliance with the international ethical guidelines for health-related research involving

humans and animals, as outlined by the Council for International Organisations of Medical Sciences (CIOMS) in collaboration with the World Health Organisation (WHO). Additionally, protocols adhered to the principles set forth by the World Organisation for Animal Health (OIE). The experimental design received formal approval from the Ethics Committee of the University of Basra, College of Pharmacy (Approval No. EC71, dated January 1, 2024).

Consent for publication
All the authors gave consent for the publication of the work under the Creative Commons Attribution Non-Commercial 4.0 license.

Availability of Data
Data for this work is available from the authors and may be provided upon reasonable request.

Conflict of interest
None

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Authors' contributions
All authors equally contribute to the manuscript.

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