

Investigating the of protective role *Cotula cinerea* extract against Chlorpyrifosinduced toxicological and biochemical alterations in rats

دراسة الدور الوقائي لمستخلص نبات *Cotula cinerea* ضد مبيد الكلوربيريفوس
المسبب لتغيرات سُمّية وبيوكيميائية لدى الجرذان

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الملخص:

تهدف هذه الدراسة إلى استقصاء التأثيرات الوقائية للمركبات الطبيعية المستخلصة من النبات الطبي *Cotula cinerea* ضد التغيرات البيوكيميائية الناتجة عن التعرض لمبيد الكلوربيريفوس لدى الجرذان. شملت الدراسة 30 جرذاً من ذكور سلالة ويستار البيضاء، حيث تم تعريضها لمبيد الكلوربيريفوس بجرعة 9 ملغ/كغ/اليوم، ثم عولجت لاحقاً بالمستخلص الميثانولي لنبات *Cotula cinerea* بجرعات 100 و 200 و 400 ملغ/كغ/اليوم عن طريق التجريع الفموي. بعد 25 يوماً من المعالجة، جُمعت عينات الدم لإجراء التحاليل البيوكيميائية المصلية، كما حُفظ الكبد لتقييم الوزن النسبي.

أسفرت عملية الاستخلاص الميثانولي عن مردود قدره 5.075%، مع تركيز من المركبات الفينولية بلغ 0.03 ± 0.142 ملغ مكافئ حمض الغاليك/مل. أدى التعرض للكلوربيريفوس إلى انخفاض وزن الجسم وارتفاع مستويات المؤشرات الرئيسية لوظائف الكبد والكلية، بما في ذلك AST, ALT, ALP, LDH، البيليروبين،

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والكوليسترول ($p < 0.05$ مقارنة بالمجموعة الشاهدة). في المقابل، لوحظ انخفاض في مستويات الكرياتينين وحمض البول، مما يدل على حدوث سمية كلوية وكبدية. غير أن المعالجة بمستخلص *Cotula cinerea* أظهرت تحسناً ملحوظاً في وظائف الكبد والكلية، مع استعادة وزن الجسم. تؤكد هذه النتائج النشاط البيولوجي الهام للمركبات الثانوية في نبات *Cotula cinerea* والتي لعبت دوراً أساسياً في التخفيف من التأثيرات السمية لمبيد الكلوربيريفوس، سواء في الدراسات المخبرية (*in vitro*) أو الحية (*in vivo*).

الكلمات المفتاحية: المركبات الفينولية، *Cotula cinerea*، المبيدات، الكلوربيريفوس، الإجهاد التأكسدي.

Abstract

This study explored the protective effects of natural compounds from the medicinal plant *Cotula cinerea* against biochemical changes caused by the pesticide Chlorpyrifos in rats. A total of 30 male Wistar albino rats were exposed to Chlorpyrifos (9 mg/kg/day) and subsequently treated with *Cotula cinerea* methanolic extract at doses of 100, 200, and 400 mg/kg/day via oral gavage. After 25 days of treatment, blood samples were collected for serum biochemical analysis, and the liver was preserved for relative weight assessment.

The methanol extraction process produced a yield of 5.075%, with a phenolic compounds concentration of 0.142 ± 0.03 mg AGE/ml (Gallic Acid Equivalents). Chlorpyrifos exposure led to a decrease in body weight and elevated levels of key liver and kidney markers, including AST (Aspartate Transaminase), ALT (Alanine Transaminase), ALP (Alkaline Phosphatase), LDH (Lactate Dehydrogenase), bilirubin, and cholesterol ($p < 0.05$ vs. control). In contrast, creatinine and uric acid levels were reduced, indicating nephrotoxicity and hepatotoxicity. However, treatment with *Cotula cinerea* extract showed improvements in liver and kidney functions and restored body weight. The findings emphasize the significant biological activity of secondary metabolites in *Cotula cinerea*, which played a key role in mitigating the toxic effects of Chlorpyrifos, both *in vitro* and *in vivo*.

Keywords: Phenolic compounds, *Cotula cinerea*, Pesticides, Chlorpyrifos, Oxidative Stress.

INTRODUCTION

Chlorpyrifos is a widely used organophosphate pesticide employed in agriculture to protect various crops, including fruits, vegetables, and grains, from pests such as insects and nematodes. Its applications encompass nursery management, urban landscaping, and pest control, making it a vital tool in agricultural and non-agricultural settings [1, 2]. Chlorpyrifos

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is valued for its effectiveness and relatively low cost, contributing to its widespread use in integrated pest management strategies [3].

However, excessive use and improper application of Chlorpyrifos have raised significant environmental concerns. Residues have been documented to accumulate in soil and water sources, leading to contamination that harms non-target organisms, including beneficial insects, wildlife, and humans [4, 5]. The potential for Chlorpyrifos to leach into groundwater poses additional risks, making it a focal point in discussions about agricultural sustainability and environmental health [6].

The toxicity associated with Chlorpyrifos is primarily attributed to its neurotoxic effects, disrupting normal nerve transmission by inhibiting the activity of the enzyme acetylcholinesterase. This inhibition leads to the accumulation of acetylcholine at nerve synapses, resulting in prolonged stimulation of nerve cells and subsequent toxic effects [7]. Symptoms of chlorpyrifos poisoning can include dizziness, headache, nausea, respiratory distress, muscle twitching, and, in severe cases, seizures or coma [8]. Long-term exposure has also been linked to developmental and neurological issues in children, raising serious public health concerns [9]. Research indicates that exposure to Chlorpyrifos can induce oxidative stress, contributing to cellular damage and organ dysfunction. Studies have shown that it significantly increases hepatic lipid peroxidation and depletes antioxidant reserves, leading to elevated oxidative stress and liver injury [10]. Additionally, Chlorpyrifos has been reported to cause kidney toxicity, enhancing apoptotic activity and oxidative stress response in kidney tissues [11]. The growing body of evidence underscores the urgent need for stricter regulations on chlorpyrifos use to protect both environmental health and human safety.

Natural products, particularly those of plant origin, have long been regarded as a significant source of medicinal compounds. Statistics indicate that approximately 25% to 30% of medications used to treat various health conditions come from natural sources, including plants, animals, microbes, and fungi [12]. Furthermore, recent data from the pharmaceutical industry show that these natural products remain essential for developing new chemical entities, as their complex structures have evolved over millions of years to demonstrate high efficacy [13].

Among these promising natural products, antioxidants such as polyphenols have received considerable attention due to their diverse applications in pharmaceutical fields, including antibacterial [14], antifungal [15], antidiabetic [16], hepatoprotective [17, 18], and hematoprotective [19] properties, as well as their use in cosmetics and food for health benefits [20]. Additionally, there is a growing interest in natural alternatives for food preservation due to concerns that certain synthetic antioxidants may pose carcinogenic risks [21].

This study aims to evaluate the protective effects natural products from *Cotula cinerea* extract on the toxic effects by Chlorpyrifos in rat models.

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MATERIAL AND METHODS

Reagents and chemicals:

Sigma-Aldrich from the USA was used to purchase all chemicals and was of analytical quality. Chlorpyrifos pesticide was obtained from Bayer laboratory, and a commercial kit from Spinreact, Spain, was used for biochemical parameter determinations.

Plant material:

Leaves of *Cotula cinerea* were collected from Ben Guecha, El-Oued state, Algeria, and the plant was identified by Prof. Dr. Atef Chouikh (Faculty of Natural and Life Sciences, El-Oued University, Algeria). The medicinal herb *Cotula cinerea* leaves were cleaned, dried, and powdered, then kept at ambient temperature.

Plant extract preparation:

200 g of *Cotula cinerea* is macerated in methanol (2000 ml) for 24 h under agitation at room temperature and shade. The solvent (Distilled water) was removed from the extract with a rotary evaporator and then incubated at 60°C to dry entirely. For upcoming tests, after being weighed, the extract was kept in a refrigerator at 4°C [22]. The percentage yield of the *Cotula cinerea* extract was 5.075% w/w of the initial raw material.

Total phenolic compounds estimation:

Total phenolic compounds was calculated using the Folin–Ciocalteu method. Briefly, 0.1 ml of *Cotula cinerea* extract and 0.2 ml of Folin–Ciocalteu reagent were diluted with 3.16 ml of distilled water, and then 0.6 ml of sodium carbonate (20%) was added. After 120 min of incubation at ambient temperature, the absorbance was measured at 765 nm. Three different experiments were conducted to verify that the data were consistent. The amount of phenols in the extract, expressed as milligrams of gallic acid equivalent per ml of extract, was calculated [23].

Protective role of *Cotula cinerea* extract estimation:

Animals and experiment design:

Thirty adult Wistar albino rats weighing 253.71 g $\pm$ 22.23 g were obtained from the Pasteur Institute of Algiers. The rats were housed in the Department of Molecular and Cellular Biology at El-Oued University, Algeria, under controlled conditions: temperature (24 $\pm$ 1 °C), photoperiod cycle (12 h light/12 h dark), with standard food and water available at all times during the investigation. Before use, the rats were acclimated for 15 days under identical lab conditions. The Institutional Animal Ethical Committee (IAEC) approved animal experiments and experimental protocols at the University of El Oued, Algeria. The rats were divided into five experimental groups of six animals each and received the following treatments for ten days:

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Group 1: Control group.

Group 2: Received daily Chlorpyriphos (9 mg/kg/J in oral gavage). [24]

Group 3: Received daily Chlorpyriphos (9 mg/kg/J in oral gavage) than *Cotula cinerea* extract (100 µl/kg/J in oral gavage).

Group 4: Received daily Chlorpyriphos (9 mg/kg/J in oral gavage) than *Cotula cinerea* extract (200 µl/kg/J in oral gavage).

Group 5: Received daily Chlorpyriphos (9 mg/kg/J in oral gavage) than *Cotula cinerea* extract (400 µl/kg/J in oral gavage).

Throughout the weeks of the study, body weight was frequently monitored.

Relative liver weight estimation:

After the rats were sacrificed, the liver was removed, cleaned with physiological saline, and weighed to calculate the relative liver weight using the formula: Relative liver weight = (Liver weight / Body weight) * 100% [24].

Blood collection for biochemical parameters analyses:

After 16 hours of feeding, the rats were sacrificed by decapitation following anesthesia with chloroform. Blood was then drawn, and the samples were placed into serum-separating tubes and centrifuged for 10 minutes at 3000 rpm to prepare them for biochemical analysis.

Biochemical parameters analyses:

(AST): Serum Aspartate Transaminase (ALT): Alanine Transaminase (ALP): Alkaline Phosphatase, (LDH): Lactate Dehydrogenase, (γ-GT): Gamma-Glutamyl Transferase, Glucose, Cholesterol, Triglycerides, Total Protein, Urea, Uric Acid, Creatinine, Total Lipids, Direct Bilirubin, Indirect Bilirubin, and Ionogram: Ca (Calcium), K (Potassium), Mg (Magnesium), Na (Sodium).

Statistical analysis:

A student t-test (using Minitab® 13 software) was conducted to analyze the obtained data. P-values of 0.05 or below were considered significant. The results of each experiment were presented as the mean and standard deviation (S.D.).

RESULTS

Total phenol content:

The total phenol content of *Cotula cinerea* extract was estimated using the equation ($Y = 15,827x - 0,0635$, $r^2 = 0.09922$) and expressed as Gallic Acid Equivalents (GAE). It was 0.03 ± 0.142 mg GAE/ml of the *Cotula cinerea* aerial part extract.

Protective properties of *Cotula cinerea* extract estimation:

Relative liver weight:

The results of relative liver weight show that body weight was affected by the Chlorpyriphos pesticide and the plant extract. Additionally, Group 2 (treated with Chlorpyriphos) showed liver hypertrophy. In contrast, the extract of *Cotula cinerea*, when administered at different doses in combination with Chlorpyriphos pesticide, reduced the elevated liver weight. The results of relative liver weight are summarized in Table 1.

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Table 01: Rats' relative liver weight treated by *Cotula cinerea* extract.

Groups	Body weight (g)	Liver weight (g)	Relative liver weight (%)
Group 1	268.68 ±24.87	9.04 ±0.58	3.37 ±0.00
Group 2	268.99 ^{NS} ±23.29	9.15 ^{NS} ±1.68	3.39 ^{NS} ±0.00
Group 3	259.39 ^{NS} ±23.80	8.036 ^{NS} ±1.15	3.11 ^{NS} ±0.00
Group 4	283.75 ^{NS} ±25.65	9.492 ^{NS} ±1.00	3.34 ^{NS} ±0.00
Group 5	264.08 ^{NS} ±14.82	8.23 [*] ±0.74	3.22 ^{NS} ±0.00

Results are presented as the mean ± S.E. (Standard Error) (n = 6). (*) p≤0.05, (**)p≤0.01 and (***)p≤0.001. (N.S.)p > 0.05 as compared with Chlorpyriphos pesticide. Model group. bp < 0.05 as compared with a normal control group.

Biochemical parameters analyses:

- Liver and renal function profile

Figure 1 illustrates the impact of the *Cotula cinerea* extract on the activity of serum enzymes, including Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), Gamma-Glutamyl Transferase (GGT). The findings revealed a notable reduction in serum enzyme activity after administering the Chlorpyrifos pesticide to the rats. However, rats pretreated with different doses of *Cotula cinerea* extract showed a substantial decline in serum enzyme activity.

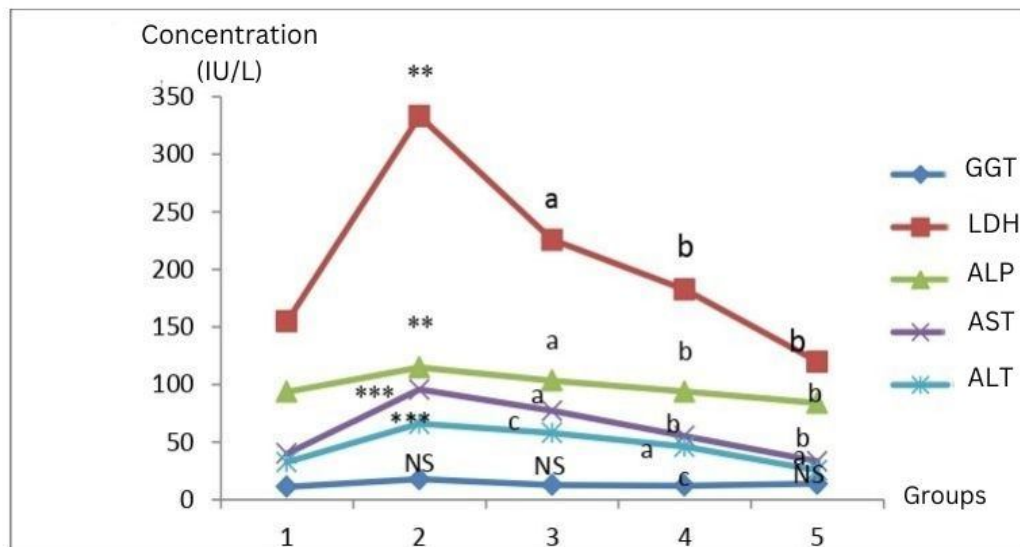


Figure 1: Impact of *Cotula cinerea* extract on serum enzyme activity in rats exposed to Chlorpyrifos toxicity.

Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), Gamma-Glutamyl Transferase (GGT). Results are presented as the mean ± S.E. (Standard Error) (n = 6). P > 0.05: (N.S.) No significant difference compared to Group 1 (Normal control group). P > 0.05: (N.S.) No significant difference compared to Group 2 (Chlorpyrifos pesticide group). P = 0.05: (*) Significant difference compared to Group 1. P = 0.05: (c) Significant difference compared to Group 2. P ≤ 0.01: (**) Highly significant difference compared to Group 1. P ≤ 0.01: (a) Highly significant difference compared to Group 2. P ≤ 0.001: (***) Very highly significant difference compared to Group 1. P ≤ 0.001: (b) Very highly significant difference compared to Group 2.

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After evaluating enzyme activity related to liver function, the effect of administering *Cotula cinerea* extract on liver health parameters, such as direct bilirubin and indirect bilirubin, was assessed in rats exposed to Chlorpyrifos pesticide. As shown in Table 02, The application of Chlorpyrifos leads to an increase in both conjugated (direct) and unconjugated (indirect) bilirubin levels. However, the results vary depending on the concentration of the pesticide used. In contrast, treatment with plant extract at different concentrations consistently produces a statistically significant reduction in the levels of the metabolites studied.

Table 02: Impact of *Cotula cinerea* extract on liver parameters in rats exposed to Chlorpyrifos toxicity.

Parameters	Groups				
	Group 1	Group 2	Group 3	Group 4	Group 5
Indirect bilirubin (mg/L)	7.9 ±0.86	11.22 ^{**} ±1.01	10.24 ^{NS} ±0.55	8.26 ^b ±0.53	6.05 ^c ±1.13
Direct bilirubin (mg/L)	0.75 ±0.52	3.06 ^{***} ±0.57	2.29 ^{NS} ±0.35	1.25 ^a ±0.58	0.51 ^a ±0.05

Results are presented as the mean ± S.E. (Standard Error) (n = 6). P > 0.05: (N.S.) No significant difference compared to Group 1 .(Normal control group). P > 0.05: (N.S.) No significant difference compared to Group 2(Chlorpyrifos pesticide group). P = 0.05: (*) Significant difference compared to Group 1. P = 0.05: (c) Significant difference compared to Group 2. P ≤ 0.01: (**) Highly significant difference compared to Group 1. P ≤ 0.01: (a) Highly significant difference compared to Group 2. P ≤ 0.001: (***) Very highly significant difference compared to Group 1. P ≤ 0.001: (b) Very highly significant difference compared to Group 2.

Administration of the chlorpyrifos pesticide caused a significant increase in the renal parameters analyzed. Table 03 presents the effects of *Cotula cinerea* extract in mitigating the damaging effects of Chlorpyrifos. The serum uric acid measurements reveal a significant difference among the groups: an increase in the control group (32.32 mg/L) and a marked decrease across the subsequent other groups, with levels of 24.8 mg/L, 23.38 mg/L, 19.76 mg/L, and 20.47 mg/L, respectively. A reduction in creatinine levels is particularly noted in the second group (5.75 mg/L), while the remaining four groups exhibit relatively stable values ranging between 6 mg/L and 7 mg/L. As for urea, no significant effect of the pesticide or the plant extract at a concentration of 100 mg/L is observed. However, urea levels are reduced at 200 mg/L and 400 mg/L concentrations, decreasing from 0.35 g/L to 0.30 g/L.

Table 03: Impact of *Cotula cinerea* extract on renal parameters in rats exposed to Chlorpyrifos toxicity.

Parameters	Groups				
	Group 1	Group 2	Group 3	Group 4	Group 5
Uric acid (mg/L)	32.32 ±2.4	24.8 [*] ± 2.64	23.38 ^{NS} ±4.5	19.76 ^c ±3.2	20.47 ^c ±1.68
Creatinine (mg/L)	7.10 ±1.85	5.75 ^{NS} ±0.51	6.57 ^{NS} ±1.45	7.22 ^c ±3.05	6.57 ^{NS} ±0.38
Urea (mg/L)	0.41 ±0.07	0.40 ^{NS} ±0.02	0.40 ^{NS} ±0.02	0.35 ^{NS} ±0.03	0.30 ^c ±0.02

Results are presented as the mean ± S.E. (Standard Error) (n = 6). P > 0.05: (N.S.) No significant difference compared to Group 1. (Normal control group). P > 0.05: (N.S.) No significant difference compared to Group 2 (Chlorpyrifos pesticide group). P = 0.05: (*) Significant difference compared to Group 1. P = 0.05: (c) Significant difference compared to Group 2. P ≤ 0.01: (**) Highly significant difference compared to Group 1. P ≤ 0.01: (a) Highly significant difference compared to Group 2. P ≤ 0.001: (***) Very highly significant difference compared to Group 1. P ≤ 0.001: (b) Very highly significant difference compared to Group 2.

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The results for the other serum parameters, cholesterol, triglycerides, total lipids, total proteins, and blood glucose, are presented in Table 04. The results indicate an increase in plasma levels of cholesterol, triglycerides, total lipids, and total proteins in the group treated with Chlorpyrifos compared to the control rats. The toxic effect on serum glucose levels is reflected in a decrease. On the other hand, the *Cotula cinerea* extract, across varying concentrations, significantly reduces these increases ($P \geq 0.05$).

Table 04: Impact of *Cotula cinerea* extract on otherparameters in rats exposed to Chlorpyrifos toxicity.

Parameters	Groups				
	Group 1	Group 2	Group 3	Group 4	Group 5
Cholesterol(mg/L)	0.55 ±3.4	0.62 ^{NS} ±0.06	0.5 ^{NS} ±0.0	0.59 ^{NS} ±0.04	0.56 ^{NS} ±0.5
Triglycerides,(mg/L)	0.92 ±0.00	1.24 ^{NS} ±0.2	0.86 ^{NS} ±0.2	0.98 ^c ±0.16	0.65 ^c ±0.1
Total lipids (mg/L)	31.95 ±3.46	41.63 ^{NS} ±9.66	30.2 ^{NS} ±6.64	32.85 ^{NS} ±6.4	29.47 ^c ±3.35
Total proteins(mg/L)	66.8±2.38	71.66 ^{NS} ±2.96	66.88 ^a ±1.80	65.88 ^c ±1.25	61.75 ^c ±1.76
Blood glucose(mg/L)	0.99±0.10	0.91 ^{NS} ±0.19	0.87 ^{NS} ±0.09	0.78 ^{NS} ±0.10	0.90 ^{NS} ±0.06

Results are presented as the mean ± S.E. (Standard Error) (n = 6). $P > 0.05$: (N.S.) No significant difference compared to Group 1. (Normal control group). $P > 0.05$: (N.S.) No significant difference compared to Group 2 (Chlorpyrifos pesticide group). $P = 0.05$: (*) Significant difference compared to Group 1. $P = 0.05$: (c) Significant difference compared to Group 2. $P \leq 0.01$: (**) Highly significant difference compared to Group 1. $P \leq 0.01$: (a) Highly significant difference compared to Group 2. $P \leq 0.001$: (***) Very highly significant difference compared to Group 1. $P \leq 0.001$: (b) Very highly significant difference compared to Group 2.

- Ionogramparameters

Administration of Chlorpyrifos pesticide caused a significant increase in the serum biochemical parameters analyzed. The effects of *Cotula cinerea* extract on mitigating the damaging effects of Chlorpyrifos are presented in Figure 2. Calcium, potassium, and sodium levels increased in the second group compared to the control group. A significant decrease is observed between the second and third groups, and this variation remains statistically significant ($p \geq 0.05$). Serum magnesium levels do not exhibit notable changes, fluctuating between 22 and 21 mg/L, except for a significant decrease between the fourth and fifth groups.

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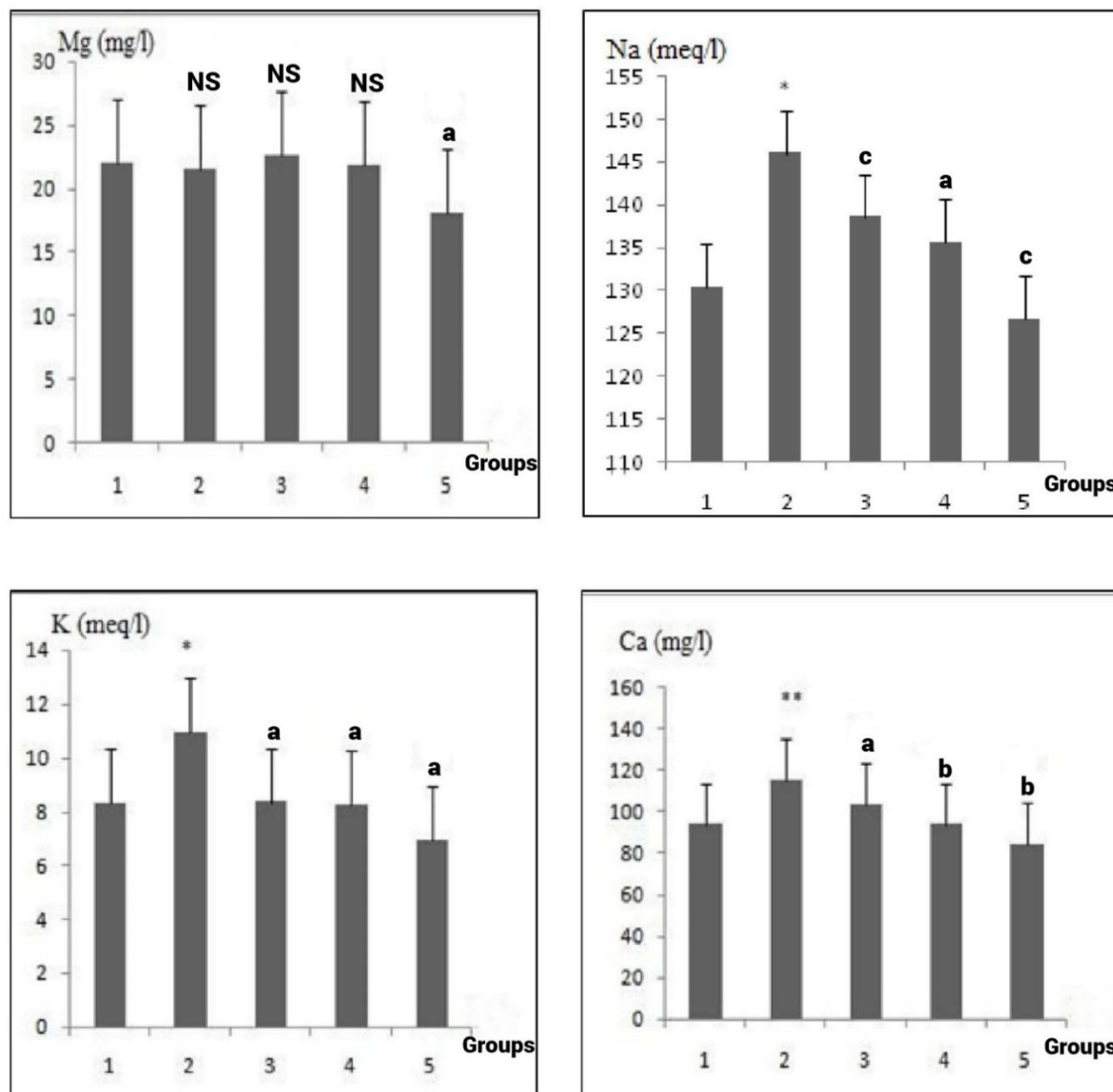


Figure 02: Impact of *Cotula cinerea* extract on ionogram parameters in rats exposed to Chlorpyrifos toxicity.

Ca (Calcium), K (Potassium), Mg (Magnesium), and Na (Sodium) Results are presented as the mean $\pm$ S.E. (Standard Error) (n = 6). P > 0.05: (N.S.) No significant difference compared to Group 1. (Normal control group). P > 0.05: (N.S.) No significant difference compared to Group 2 (Chlorpyrifos pesticide group). P = 0.05: (*) Significant difference compared to Group 1. P = 0.05: (c) Significant difference compared to Group 2. P $\leq$ 0.01: (**) Highly significant difference compared to Group 1. P $\leq$ 0.01: (a) Highly significant difference compared to Group 2. P $\leq$ 0.001: (***) Very highly significant difference compared to Group 1. P $\leq$ 0.001: (b) Very highly significant difference compared to Group 2.

DISCUSSION

Previous studies have demonstrated that solvents like methanol, ethanol, and water, as well as their mixtures in various ratios, are widely recognized as the most effective solvents for maximizing the extraction and recovery of phenolic compounds from plant materials. These solvents, individually or in combination, play a crucial role in breaking down plant cell

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walls, thereby facilitating the release of bioactive compounds, such as phenolics, known for their antioxidant and therapeutic properties [25, 26, 27]. Among these, methanol has been particularly noted for its efficiency in extracting a broad range of phenolic compounds due to its polarity and ability to dissolve hydrophilic and hydrophobic components.

In this study, methanol was specifically selected as the solvent to obtain the crude extract from the leaves of *Cotula cinerea* plant species. Methanol's high extraction efficiency ensures that a significant portion of the plant's bioactive components, including phenolic compounds, are preserved in the crude extract, making it suitable for subsequent biological and chemical analyses. The choice of methanol aligns with its widespread use in similar phytochemical studies, where maximizing the yield of active compounds is essential for evaluating their potential pharmacological applications.

Generally, the levels of dry extracts can vary between plants within the same family based on factors like growth conditions, maturity, processing, storage conditions, and extraction methods [28]. Other research supports this, showing that environmental and developmental factors significantly impact plants' concentration of bioactive compound[29, 30, 50].

Beyond these quantitative aspects, any extraction method must also ensure the quality of the extract, particularly its bioactivity. Studies have shown that extraction techniques play a crucial role in preserving the integrity of bioactive compounds [31, 32]. In this study, the maceration method with agitation was used to speed up the extraction process and minimize solvent contact time with the extract while maintaining the bioactivity of the compounds. Additionally, carrying out the extraction at room temperature and removing the solvent under reduced pressure helps maximize the recovery of compounds and prevent their degradation or modification, which can happen with the high temperatures used in other methods.[33]

Among the studied parameters, the significant increase in the relative liver weight indicates hepatomegaly induced by the pesticide Chlorpyrifos at a 9 mg/kg/day concentration. This liver abnormality is commonly reported in the literature as a response to chemical exposure, particularly pesticides. Several studies have specifically addressed the effects of Chlorpyrifos on liver health, demonstrating that prolonged exposure can lead to liver hypertrophy and damage due to the liver's central role in detoxification [34, 35]. Research indicates that Chlorpyrifos can induce oxidative stress and inflammation in liver tissues, critical factors contributing to hepatotoxicity [36, 37]. Hepatomegaly is often associated with cellular inflammation and oxidative stress, both of which contribute to tissue damage and represent adaptive responses to the toxicity of chemical compounds.

A decrease in serum enzyme levels of AST, ALT, PAL, and γ -GT was observed in rats treated with the plant extract of *Cotula cinerea*. This plant is known for its hepatoprotective properties, attributed to its rich content of bioactive compounds that can mitigate liver damage. The serum enzymes AST, ALT, PAL, and γ -GT are synthesized in the cytoplasm of cells and released into circulation in cases of cellular damage. These enzymes are considered good indicators of hepatocellular cytolysis and liver function. Elevated levels of liver enzymes, particularly AST, ALT, and γ -GT, are often associated with metabolic and/or toxic effects of various substances, including pesticides like Chlorpyrifos [38, 39]. Notably, PAL is

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involved in phenolic metabolism, and its elevation can indicate stress responses in the liver [40]. Similarly, increased γ -GT levels are often linked to oxidative stress and liver injury [41]. In our case, a variation in serum levels of AST, ALT, PAL, and γ -GT was noted between the groups of rats intoxicated with the insecticide and those treated with three doses of the methanolic extract of *Cotula cinerea* compared to untreated intoxicated rats. Our results align with recent studies indicating that treatment with natural extracts, such as *Cotula cinerea*, can mitigate the elevation of liver enzymes in intoxicated subjects.[43 ,1]

Serum bilirubin concentrations, on the one hand, are among the most sensitive biomarkers directly involved in assessing the extent of liver damage and toxicity. On the other hand, elevated plasma bilirubin levels (both total and direct) indicate liver dysfunction. Our results show increased bilirubin levels in rats administered the pesticide compared to control rats. These observations are consistent with recent studies indicating that exposure to pesticides like Chlorpyrifos leads to liver dysfunction, including elevated bilirubin levels due to the hepatotoxic effects on liver cells [44, 45]. This increase is associated with inflammation and oxidative stress mechanisms activated by prolonged pesticide exposure [46]. In this context, bilirubin is a key marker for predicting liver damage related to chemical toxicity, including exposure to agricultural pesticides.[48 ,47]

The biochemical metabolites, including triglycerides, cholesterol, renal markers (creatinine, urea, uric acid), and glucose, appear significantly affected by toxic inducers, such as Chlorpyrifos pesticide. Exposure to pesticides leads to disturbances in lipid levels and renal markers, as seen in various experimental studies. The administration of pesticides induces hepatotoxicity in rats, manifesting as an increase in plasma cholesterol and triglyceride levels. Recent studies suggest that these alterations in the lipid profile are due to changes in the activity of enzymes involved in lipid metabolism.[49 ,34]

Furthermore, secondary metabolites, particularly polyphenols found in *Cotula cinerea*, are crucial in mitigating these toxic effects. Polyphenols, known for their potent antioxidant properties, help combat oxidative stress and lipid peroxidation caused by pesticide exposure. They modulate lipid metabolism by influencing the activity of enzymes critical to lipid biosynthesis and degradation, thereby improving lipid profiles and reducing overall toxicity [49, 51]. Additionally, polyphenols offer nephroprotective effects by scavenging free radicals and reducing inflammation, protecting against renal damage induced by pesticides [52, 53]. These findings suggest that *Cotula cinerea* could effectively alleviate both hepatic and renal toxicity triggered by pesticide exposure through its rich polyphenol content.

CONCLUSION

This study indicated the protective role of natural compounds, particularly phenolic compounds from *Cotula cinerea*, in mitigating biochemical changes caused by chlorpyrifos exposure. Chlorpyrifos led to increased liver enzyme activity, bilirubin levels and disrupted electrolyte balance, indicating oxidative stress. However, *Cotula cinerea* extract effectively reduced this stress and restored biochemical balance due to its antioxidant properties. These findings suggest *Cotula cinerea* as a promising natural solution to pesticide toxicity, highlighting the need for further research into its bioactive components and their detoxification mechanisms, paving the way for safer approaches to managing pesticide-related health risks.

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Conflicts of interest

The authors declare no conflicts of interest.

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