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RESEARCH ARTICLE

Antitoxic Effects of Fennel (*Foeniculum vulgare*) Extract Against Chlorpyrifos Induced Toxicity in Rabbits

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ABSTRACT

Chlorpyrifos, most widely used organophosphate insecticide, is extensively applied in agriculture to control pests but poses environmental and health issues when overused. The present study aimed to evaluate the toxic effects of chlorpyrifos on rabbits (*Oryctolagus cuniculus*) and protective potential of fennel seed extract. Twenty male rabbits were divided into four groups (A-D); group A served as control and treated with normal diet while group B was intoxicated with 1.5 mL/kg of chlorpyrifos. Group C and D were intoxicated with 1.5 mL/kg of chlorpyrifos and co treated with 5 mL and 10 mL of fennel seed extract respectively. After 96 hours, blood and organ samples were collected. However, fennel seed extract at a 10 mL concentration effectively normalized all measured parameters when co-administered with chlorpyrifos. Chlorpyrifos intoxication induced various histopathological alterations in cardiac tissues of rabbits including interstitial, pale acidophilic sarcoplasm, pyknotic nucleus, wide interstitial spaces and degenerated muscles, which were significantly ameliorated by fennel seed extract. Fennel seed extract contains bioactive compounds like Atropine and Phenolics which may reduce the toxic effects of chlorpyrifos. These findings may suggest that fennel seed extract has potential as an ameliorative agent against Organophosphate toxicity.

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INTRODUCTION

Pesticides are chemical compounds widely used to control and eliminate pests. Common types include fungicides, insecticides and herbicides are applied in agriculture for the pest management. However, their extensive and widespread usage has raised serious environmental and health issues. The American Chemical Society (2009) reported the existence of 50 million different compounds, with 2.5 million new compounds added annually (Hassaan and El Nemr, 2020). Pesticides can pollute habitat and ground water leading destruction, population decline and habitat loss of various animals and bird species. According to the German Environment Agency, increasing pesticide usage heavily disturb pollen transfer and insect pollinators leading to reduction in crop production and decline biodiversity (Koch *et al.*, 2023).

Long-term pesticides exposure through food and environment can affect the entire population via natural pest predators and using biodegradable chemicals (Muratkasimovna, 2023). The

uncontrolled use of pesticides has led to widespread exposure and contamination causing serious health risks such as poisoning and death of living organisms (Prathiksha *et al.,* 2023). Among these Organophosphate (OP) pesticides are hazardous and its residues cause food poisoning. Their lipophilic property allows partial mitigation via lipid-rich substances like milk. OPs primarily affect mammals including rabbit, mouse and rat, and domestic animals like dogs, cats and livestock to disrupt the adaptive immune mechanism in mammals (Bernal *et al.,* 2023).

Chlorpyrifos, one of mostly used organophosphate insecticides, cause endocrine disruption, neurological effects and cardiovascular diseases. Chlorpyrifos is used to control population of insects and pests in homes and fields (Hites, 2021). Chlorpyrifos exposure can also result from non-agriculture uses like turf maintenance on gulf courses, wood treatment and application o utility poles and railways (Nicholos *et al.,* 2024). In US, Chlorpyrifos was banned in 2001 for various residential usage, EPA revoked its agricultural use (2022) and this decision overhunted by Eighth Circuit Court of Appeals in 2023 (Nicholos *et al.,* 2024; Sellers *et al.,* 2025).

Due to chemical stability of chlorpyrifos, they persist in environment spread through soil, water and affect non-target organisms and its long-term exposure lead to dermal irritation, infertility and cancer (Hussain *et al,* 2023). Soil serves as the main reservoir and major route of environmental contamination for chlorpyrifos as rainfall and irrigation cause its leaching and runoff (Bhende *et al.,* 2022). *Foeniculum vulgare* Mill. (Fennel), a fragrant *Apiaceae* herb native to the Asia, is rich in nutrition and traditionally used to treat different endocrine, reproductive disorders and respiratory system (Noreen *et al.,* 2023). Fennel has showed various biological properties including antioxidant, anti-inflammatory, anti-microbial, anti-osteoarthritic, anticancer and analgesic (Ke *et al.,* 2021; Milenkovic *et al.,* 2022; Khammassi *et al.,* 2023). Phytochemical analysis of Fennel has demonstrated its minerals and vitamins presence such as riboflavin, Fe, Na, K, Ca, ascorbic acid and niacin (Endalamaw and Chandravanshi, 2015). A recent study showed that Fennel contains polyacetylene which may contribute to its ability to improve vision and to mitigate tumor cell proliferation (Kaveh *et al.,* 2023).

Chlorpyrifos causes various metabolic and hormonal alterations in animals while Fennel contains bioactive compounds with potential antioxidant properties that may counteract the harmful effects of pesticides. Therefore, this study was aimed to investigate the hormonal imbalances induced by chlorpyrifos exposure and to assess antitoxic effect of fennel extract in rabbits highlighting its potential use as a natural protective agent against pesticide toxicity.

MATERIALS AND METHODS

Animal handling: Adult male rabbits were purchased from local market and housed them in animal facility of University of Education Vehari. Each rabbit was weighing approximately 1 kg and placed in cages under controlled environment at 27 ℃ ± 1.

Fennel extraction: Fennel seeds were collected, air-dried, ground into fine powder, subjected to the ethanol extraction and dried again followed by Soxhlet extraction method of material extraction (Rodríguez-Blázquez *et al.,* 2025). Phytochemical analysis was referenced from previous studies to strengthen the studies (Table 1).

Grouping of animals: A total of twenty rabbits were divided into four groups with five rabbits per group. The body weight of each rabbits was recorded before treatment. Chlorpyrifos was administrated at 1.5 mL/kg of to each rabbit, a standard dose to induce toxic effects. To examine the anti-toxic effect of Fennel, fennel extracts were administrated to rabbits. The treatment groups were:

1) Group A (control): received normal diet and distilled water.
2) Group B: treated with 1.5 mL/kg chlorpyrifos orally throughout the day.
3) Group C: treated with 1.5 mL/kg chlorpyrifos plus 5 mL fennel extract orally once daily.
4) Group D: treated with 1.5 mL/kg chlorpyrifos plus 5 mL fennel extract orally twice daily.

Histopathological analysis: The hearts were excised from rabbits, weighed and preserved in formalin. Tissue samples were cut and embedded around 1 cm^3 and placed in cassettes for histological processing. The sample-loaded cassettes were run through the following pattern of alcohol concentrations (30%, 50%, 70% and 90%) to test for tissue dehydration. After two xylene treatments, the samples were cleaned before being impregnated with molten paraffin wax at 58ºC. The tissues were embedded in a mold filled with fresh molten wax and allowed to solidify.

Paraffin blocks were sectioned, trimmed, frozen and fixed to the cassettes. The tissues were sliced using a rotating microtome (Shandon, Thermo, CD-2235) fixed with a microtome blade at 3–4 µm thickness. In warm water, the segmented strips were expanded and shaped. After placing tissues on the slides, egg albumen was used to mount them. After that, they were submerged in warm water, causing the wax to melt and adhere the tissue to the slide. Slides were stained using hematoxylin and eosin (H&E) method,

in which hematoxylin stains the nucleus indigo and eosin stains the protoplasm red. Tissue sections were examined under compound microscope for histopathological analysis (Khan *et al.,* 2021).

RESULTS

Phytochemical Analysis of Fennel: Phytochemical analysis of Fennel extract revealed the presence of various bioactive compounds with significant antioxidant potential. It showed significant level of phenol (38.2 ± 2.3), flavonoids (122.1 ± 1.4) and flavonols (205.2 ± 2.3) known for their cardio-protective properties (Table 1). Similarly, *F. vulgare* contained a prominent proportion of nitrogen-free extract (43.44 ± 1.82) and folates (270). These findings are aligned with the previous studies, suggesting that fennel extract contribute to the anti-oxidant and protective effects against the pesticides-induced toxicity.

Table 1: Phytochemical analysis of fennel (*F. vulgare*) extract reported in previous studies

Compounds	Percentage	Reference (s)
Phenols (mg EGA/g)	38.2±2.3	Cherbal *et al.*, 2023 Ogbona *et al.*, 2024
Flavonoids (mg EQ/g)	122.1±1.4	Ni'ma and Lindawati, 2022 Salami *et al.*, 2017 Cherbal *et al.*, 2023
Flavonols (mg EQ/g)	205.2±2.3	Yashin, 2017 Cherbal *et al.*, 2023
Nitrogen free extract (%)	43.44±1.82	Mehra *et al.*, 2021
Folates (mg/kg)	270	Mehra *et al* ., 2021

Histopathological analysis of heart: Rabbits intoxicated with 1.5 mL/kg chlorpyrifos exhibited histopathological alterations in heart including interstitial fibrosis, wide interstitial spaces, pyknotic nucleus, degenerated cells and congestion. These alterations were partially reduced with 5 mL/kg fennel extract and mitigate completely with 10 mL/kg fennel extract as shown in Fig. 1.

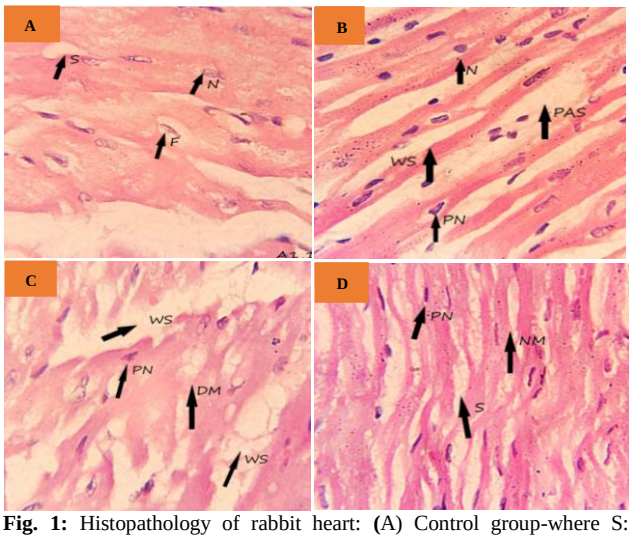

Fig. 1: Histopathology of rabbit heart: **(**A) Control group-where S: sarcoplasm, N: vesicular nucleus, F: Interstitial fibrosis; (B) Chlorpyrifos group-where WS: wide interstitial space, PAS: pale acidophilic sarcoplasm; (C) Chlorpyrifos +5 mL/kg Fennel- PN: pyknotic nucleus, WS: wide interstitial spaces, DM: degenerated muscles, (D) Chlorpyrifos + 10 mL/kg Fennel with NM: Normal myocytes, S: sarcoplasm and PN: pyknotic nucleus**.**

DISCUSSION

Pesticides are widely used chemicals, based on mechanism of action and target specificity; they are classified into organochlorines, carbamates, pyrethroids, neonicotinoids and organophosphates (Aloizou *et al.,* 2020). Pesticides, particularly organophosphates including Chlorpyrifos widely used in agriculture and public areas, are highly toxic and cause cancer, malformation, infertility, neurotoxicity, hormonal imbalances and other health disorders (Wolejko *et al.,* 2022).

Fennel is an edible, aromatic plant that is widely grown for its essential oil and traditional uses, is a great source of anti-bacterial and ant-oxidant agents even through its stalk (Ogbonna *et al.,* 2024). Phytochemical analysis of *F. vulgare* in this study revealed the notable presence of bioactive compounds, flavonoids, flavonols, phenols, nitrogen-free extract and folates (Table 1), aligning with the previous studies. Fennel constituents highlighted the antioxidant and protective effects against various pesticides-induced toxicity.

In the present study, chlorpyrifos exposure induced the prominent histopathological alterations in cardiac tissues of the treated groups (B, C and D) compared to control group (A). Group B, which intoxicated with chlorpyrifos alone showed significant number of alterations compared to groups C and D (Fig. 1). The most prominent histopathological alterations observed in this study were pyknotic nucleus, wide interstitial space, pale acidophilic sarcoplasm, vascular congestion and degenerated muscles. These alterations were dose-dependent, with damage severity increasing with Chlorpyrifos concentration.

High chlorpyrifos exposure induced oxidative stress in earthworms and triggered upregulation of anti-oxidant defenses following the same dose-dependent exposure pattern (Kumar *et al.,* 2024).These findings are consistent with the previous reports (Peritore *et al.,* 2023) observed that male rat intoxicated with fungicide highlighted severe cardiac disruption including myofibril loss, inflammatory cells infiltration, myocardial tissue separation, cardiomyocytes vacuolization and pale acidophilic sarcoplasm.

Similarly, a study reported degenerated muscle fiber, vessel congestion, tissue necrosis and wide interstitial spaces in albino rats exposed to ethion toxicity (Devi *et al.,* 2025). This study evaluated the hormonal imbalances and histopathological alterations induced by chlorpyrifos exposure and assessed the protective effects of fennel extract in rabbits suggesting it a natural agent to reduce chlorpyrifos-induced toxicity.

Conclusion

Exposure to chlorpyrifos induced various significant health issues including cardiac tissue damage. The findings of this study depicted both the toxic effects of chlorpyrifos and the protective potential of *F. vulgare*. Fennel possesses antioxidant properties attributed to its bioactive components such as atropine, enhances cardiac health, functioning of targeted organism and improves the blood biochemical profile in rabbits exposed to chlorpyrifos.

Conflict of Interest: The authors have no competing interests.

Authors' contribution: MS, SA, AJ, and HT executed the experiments. SI, AA, AB, MM KA Analyzed the data, write up and revised the manuscript.

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