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Ameliorative Effects of Selenium on Monocrotophos Induced Hepatotoxicity in Male Rabbits

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Abstract:

Monochrotophos (MCP), a widely utilized organophosphate pesticide in agriculture, is highly risky to the hepatic health of most animal species. The present study was designed to examine the ameliorative effects of Selenium (Se) on MCP induced hepatotoxicity in male rabbits. A total of 25 normal male rabbits, weighing between 1200 and 1500 g, were randomly assigned to five groups. First group received normal saline and served as the control, second group orally received MCP (0.1 mg/kg bw), third group received MCP + Se (2µg/kg bw), fourth group received MCP + Se (4µg/kg bw) and fifth group received MCP + Se (8µg/kg bw) daily for 25 days. Blood samples were collected at the end of the treatment and analyzed for serum estimation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), total protein (T.P), albumin (Alb), total bilirubin (T.B), and direct bilirubin (D.B). Exposure to MCT caused a significant increase in serum levels of ALT and AST in MCT treated group (G2**:**133.2±1.303, 118±1.140 respectively) when compared to the control group (G1:23.2±0.836, 24.8±0.836 respectively)**,** while those of Se + MCP treated groups (G3:98.6±1.140**,** 91.2±1.303, G4: 81.8±1.303, 84.6±1.140 respectively) showed significant decrease comparing to that of the MCT treated group. Serum levels of Alb and T.P. of all treatment groups did not show any significant difference compared to the control group. Selenium as an antioxidant showed ameliorated effects against the toxicity of MCP in rabbits, which was dose dependent. Se mitigates MCP-induced liver damage and restored its serum biomarker levels. Further research is warranted to elucidate Selenium's protective mechanisms and their relevance to human health.

Keywords: Monochrotophos, Selenium, Antioxidant, Hepatotoxicity.

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INTRODUCTION

The use of pesticides and the resulting pollution is one of the most important and prominent problems of our time. Although using pesticides was an urgent necessity to protect agricultural crops from pests, as well as for good quality, speed up, and increase crop production, on the other hand, it might have affected the environment, which is represented in environmental pollution and health effects due to exposure to the toxic residues of these pesticides on human, animal, and plant tissues (Akhtar *et al.,* 2018; Tang *et al.*, 2021; Tudi *et al.*, 2021; Dhuldhaj *et al*., 2023). The study of pesticides and their effects has occupied a large space in the research aspect and has become of interest to many research centers and laboratories in an attempt to reveal their dangers and to find alternatives that have less impact on the environment, humans, and animals (AL-Hammadi *et al.,* 2021; Intisar *et al*., 2022).

Organophosphate (OP) pesticides are considered to be one of the most important factors leading to environmental pollution and damage to living organisms, especially when they are used indiscriminately (Kaushal *et al*., 2021; Pathak *et al.,* 2022). The problem of pesticide usage was further exacerbated in developing countries due to improper use of pesticides and absence of awareness (Dhuldhaj *et al.*, 2023). OPs make up about 90% of chemical pesticides, and they have been developed throughout the century (Boczkowski *et al.,* 2025).

Yemen is one of the developing countries that widely used pesticides in Qat cultivation (Rashed and Assakaf, 2024; Weir, 2021; Alsanoy and Alhakimi, 2021). The toxic effects of pesticides on humans' and animals' health were not discovered until 1932, when the World Health Organization classified OPs pesticides as highly toxic pesticides (Mahajan *et al*., 2022; Okolonkwo *et al.,* 2022). Rahman et al. (2021) proved the toxicity of OPs pesticides, as acute and chronic exposure to CPF pesticide caused an increase in free radicals such as H_2O_2, NO_2^-, and NO_3^- in the brain and liver of mice. These pesticides were considered to generate free radicals that adversely affect the cell membranes (Massoud *et al.,* 2022; Farkhondeh *et al.,* 2022). The toxic effect of OPs is mainly represented in their ability to inhibit the activity of AchE, the enzyme that activates the neurotransmitter Ach, which is the messenger between different parts of the parasympathetic nervous system (Ismail *et al.,* 2021; Ranjan *et al*., 2022).

Monochrotophos (MCP) is considered to be one of the most widely used OPs pesticides. Indiscriminate use of OPs pesticides affects the environment, plants, animals, and human health (Kumar, 2024). MCP has been shown to induce oxidative stress across various biological systems, leading to significant health impacts. Research indicates that exposure to monocrotophos results in increased lipid peroxidation, protein oxidation, and decreased antioxidant enzyme activities, which collectively contribute to cellular damage in tissues such as the kidneys and liver (Malik *et al*., 2021).

It has been reported that within the body cells there was a protective system of cellular protective antioxidant enzymes (CPAE), which has the ability within certain limits to protect cells from the harmful effects of free radicals. However, the increase of these radicals in the body of the organism might limit or reduce and inhibit the effectiveness of the activity of these protective enzymes (Martemucci *et al.,* 2022; Kiokias and Oreopoulou, 2021; Zhou *et al*., 2022; Sadiq, 2023). Some studies have revealed that there were compounds and chemical elements that limited the effect of these free radicals and thus protected cells from their harmful effects by scavenging these radicals before they reached the cell membrane and nullifying their effect. These compounds and chemical elements are known as antioxidants (Engwa *et al.*, 2022; Chaudhary *et al*., 2023; Pisoschi *et al*., 2021; Sadiq, 2023). It has been reported that vitamins A, C, and E, as well as the elements Zn, have the ability to strengthen the protective systems of cells through their activation of CPAE, in addition to acting as an inhibitor for the formation of free radicals, as they were considered to be antioxidants

(Hossain *et al.*, 2021; Ponnampalam *et al*., 2022).

Several studies indicated that Selenium (Se) is one of the chemical antioxidant elements and prevents the formation of free radicals in the body, which might indicate that it has a great protective effect within the body to prevent many diseases and activates CPAE inside the cells. Thus, in turn might reduce the effect of free radicals resulting from the decomposition of pesticides (Vaghari-Tabari *et al.*, 2021; Barchielli *et al.*, 2022). Se plays a crucial antioxidant role in the human body, contributing significantly to cellular defense mechanisms against oxidative stress (Gorini *et al*., 2021; Huang *et al*., 2022; Zhang and Wei, 2023). As a trace element, it is essential for the synthesis of selenoproteins, such as GSH, SOD, CAT and GPx, which are vital for neutralizing reactive oxygen species (ROS) and maintaining redox homeostasis (El-Megharbel *et al*., 2021; Huang *et al*., 2022; Basegmez, 2023). Selenium supplementation has been linked to improved metabolic health, particularly in chronic diseases like diabetes and cardiovascular disorders, by enhancing antioxidant defenses and reducing inflammation (Hamza and Diab, 2020; Adeniran *et al.*, 2021; Bjørklund *et al.*, 2022; Basegmez, 2023).

Hence, the present study was aimed at studying the ameliorative role of Se against the toxic effects of MCP on the liver of male rabbits.

MATERIALS AND METHODS

Compounds used

Monocrotophos (MCP)

The pesticide was obtained from the Agriculture Office in Al-Dhalea Governorate, where we obtained a can of 100 ml with a concentration of an active substance of 40%. The pesticide was prepared as follows: 0.25 ml of Novacron was added to 1 liter of distilled water so that this solution contains 100 mg of the active substance MCP, and therefore, every 1 ml of the solution contains 0.1 mg of the active substance MCP.

Selenium

Soft gel Selenium yeast supplements used in this study, each soft gel contains 200 µg of Selenium yeast, the product name is Selevyte Selenium, manufactured by *Vitane Pharma*, USA. It was purchased from the pharmacy of Al-Thawra Hospital, Sana'a, Yemen. The dosage was prepared by dissolving each softgel in 200 ml of distilled water so that each 1 ml of water contains 1µg of Se.

Experimental animals

Twenty-five male rabbits were used in this study. Animals weighing between 1200 and 1500 g, were purchased from the center of Al-Dhalea Governorate. They were housed in cages (60 cm high, 100 cm wide, and 100 cm long) containing husk as the bedding material and had *ad libitum* access to water and food. The whole experiment was carried out at the same environmental conditions under a 12 h light and 12 h dark schedule. The study protocol was approved by the Animal Ethics Committee of Biological Science Department, Faculty of Science, Sana’a University (ethical code: BAHSS101).

Animals were kept for a period of 25 days for adaptations before the commencement of the experiment and divided into five groups (5 animals each) and administered orally for 25 days as follow:

The 1st group (G1): Rabbits were given a daily oral dose of 5 ml of normal saline and served as a negative control group.

The 2nd group (G2): Rabbits were given a daily oral dose of 0.1 mg/kg bw of MCP dissolved in 1 ml of distilled water and served as a positive control group.

The 3rd group (G3): Rabbits were given a daily oral dose of 0.1 mg/kg bw of MCP dissolved in 1 ml of distilled water + 2 µg/kg bw of Se dissolved in 2 ml of distilled water.

The 4th group (G4): Rabbits were given a daily oral dose of 0.1 mg/kg bw of MCP dissolved in 1 ml of distilled water + 4 µg/kg bw of Se dissolved in 4 ml of distilled water.

The 5th group (G5): Rabbits were given a daily oral dose of 0.1 mg/kg bw of MCP dissolved in 1 ml of distilled water + 8 µg/kg bw of Se dissolved in 8 ml of distilled water.

At the end of the experimental period, rabbits were fasted all night, then sacrificed by cervical decapitation next day morning, after that blood samples were collected into sterilized tubes for biochemical analysis.

Biochemical Parameters

The activities of serum ALT and AST were assayed by the method as described by Khanam et al. (2016). Serum Alb was estimated according to the methods of Doumas et al. (1971), and T.P. in the serum was measured according to the methods of Gornal et al. (1949). Serum T.B. and D.B. were determined by the colorimetric method, which was described by Tietz (1976). Using kits purchased from Biodiagnostic Company, Dokki, Giza, Egypt.

Statistical analysis

The mean values of each parameter were computed considering data on 5 rabbits per group and expressed as mean ± S.D. One-way analysis of variance (ANOVA) was conducted to determine differences between groups, followed by Duncan's new multiple range test (DMRT) as post-hoc test using SPSS version 22. Differences were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

ALT and AST serum levels

Table (1) showed that male rabbits of G2 group that treated with MCP (0.1 mg/kg bw) exhibit a significant increase in serum ALT and AST levels (133.2 U/L, 118 U/L respectively) with a percentage of 474% increase in ALT serum level and 375.8% increase in AST serum levels when compared to that of the control group (23.2 U/L), which indicates severe toxic effect of MCP pesticide in the liver of rabbits**.** This result might be attributed to the increase in the permeability of the cell membrane that leads to the release of transaminases into the bloodstream because of the degeneration and necrosis of hepatocytes, which might be attributed to ROS induced by MCP. Our findings are consistent with the findings of some recent studies carried out on the toxic effect of MCP (Chahal, 2023; Uwamahoro *et al.,* 2024). The increase in serum ALT and AST levels indicates liver tissue damage that might have resulted in the leakage of these enzymes into the bloodstream (Vishawanath *et al.*, 2023; Ren *et al*., 2025). Besides, Sharma and Bansal, (2022) and Massoud et al. (2022) confirmed that Malathion an organophosphates (OPx) pesticide inhibit the activity of CPAE, which plays an active role in protecting the cell from various toxic effects and accelerate the processes of LPO that create free radicals, thus raising the rate of TBARS, which is the oxidizing of free radicals of membrane lipids in rats. Due to that most important pillars of the durability of the cellular membrane have been destroyed, which means that the cytoplasm exits from its natural surroundings to the outside of the cell causing death of the cell (Sule *et al.,* 2022; Rahman *et al.,* 2021; Singh *et al.,* 2022).

In this study, MCP-treated rabbits were treated with three different doses of selenium; low (2 µg/kg bw) in G3, medium (4 µg/kg bw) in G4 and high (8µg/kg bw) in G5 that have been used as an antioxidant to evaluate their effects on the hepatotoxicity induced by MCP. Table (1) showed that treatment with low, medium and high doses of Se in MCP- treated rabbits of G3, G4 and G5 groups caused a significant increase in ALT and AST serum levels comparing with control groups and a significant decreased in serum ALT and AST enzyme levels when compared with MCP- treated rabbits of G2 group. The percentage decreased in ALT and AST serum levels following treatment with the low, medium and high doses of Se in MCP-treated rabbits was 26.39%, 39%, and 48% for ALT and 23%, 27%, and 46% for AST respectively which was dose-dependent. The findings of the present study were in agreement with the results of Sharma and Bansal, (2021), Ozturk Kurt et al. (2022), Sharma and Bansal, (2022) and Kumar et al. (2024) who observed that treatment with OPs pesticides supplemented with Se decreased serum enzyme levels of ALT and AST when compared to OPs pesticide treated animals.

Our results demonstrated that Se supplementation significantly attenuated MCP-induced hepatotoxicity, as evidenced by reduced serum ALT and AST levels. This protective effect is attributed to Selenium's essential role in activating glutathione peroxidase (GPx). As a key component of GPx's catalytic site, selenium enables the enzyme to neutralize MCP-generated reactive oxygen species (ROS) and lipid peroxides. By converting these toxic metabolites into inert compounds, the Se-GPx axis mitigates oxidative damage to hepatocyte membranes and organelles, thereby preserving cellular integrity and normalizing liver enzyme profiles. This mechanism aligns with the observed restoration of hepatic function in Se-treated groups (Kunwar and Nayak, 2025).

Table 1. The ameliorated effect of Selenium against the toxic effect of MCP on the serum levels of ALT and AST in male rabbits.

Groups		ALT U/L	AST U/L
G1	Control group	23.2±0.836	24.8±0.836
G2	MCP treated group	133.2±1.303*	118±1.140*
G3	(MCP +Low dose of Se)	98.6±1.140*°	91.2±1.303*°
G4	(MCP + Medium dose of Se)	81.8±1.303*°	84.6±1.140*°
G5	(MCP + High dose of Se)	68.8±0.836*°	64.6±1.341*°

Values are expressed as mean ± standard deviation (n = 5). *p < 0.05: Difference was significant when compared with the control group (G1), °p < 0.05: Significant difference compared with the MCP-treated group (G2), NS: Not significant.

G1: Control group, G2: MCP treated group (0.1 mg/kg bw), G3: (0.1mg MCP+2 µg/kg bw Se), G4= (0.1mg MCP+4 µg/kg bw Se), (G5= (0.1mg MCP+8 µg/kg bw)

Alb and T.P serum levels

Table (2) showed that serum levels of albumin (Alb) and total protein (T.P) in MCP- treated male rabbits of G2 did not show any significant difference when compared with that of the control group (G1).

Table 2. The ameliorated effect of Se against the toxic effect of MCP on serum levels of T.P and Alb in male rabbits.

Groups		T.P g/dl	Alb g/dl
G1	Control group	6.48±0.083	4.18±0.130
G2	MCP treated group	6.38±0.130^{NS}	4.52±0.083^{NS}
G3	(MCP +Low dose of Se)	6.48±0.083 ^{NS}	4.4±0.083 ^{NS}
G4	(MCP + Medium dose of Se)	6.42±0.083 ^{NS}	4.32±.130 ^{NS}
G5	(MCP + High dose of Se)	6.52±0.083 ^{NS}	4.06±0.89 ^{NS}

Values are expressed as mean ± standard deviation (n = 5). *p < 0.05: Difference was significant when compared with the control group (G1), °p < 0.05: Significant difference compared with the MCP-treated group (G2), NS: Not significant.

G1: Control group, G2: MCP treated group (0.1 mg/kg bw), G3: (0.1mg MCP+2 µg/kg bw Se), G4= (0.1mg MCP+4 µg/kg bw Se), (G5= (0.1mg MCP+8 µg/kg bw)

These results were in agreement with the findings of Malik et al. (2021) and Singh et al. (2022) who studied the effect of MCP in rats. While studies on other types of OPs pesticides are in agreement with the finding of the present study (El Okle *et al.,* 2022; Wisudanti *et al.,* 2024; Saleem *et al.,* 2025). The results highlight the complexity of assessing liver's health by measuring protein levels in the blood because serum levels of Alb and T.P might not change significantly despite liver injury, suggesting that Alb and T.P levels can remain stable even when liver function is compromised (Sun *et al.,* 2019; Malik *et al.*, 2021; Singh *et al.,*2024). Similar to that result rabbits treated with low, medium and high of Se following MCP treatment did not show any significant difference in serum levels of Alb and T.P in rabbits comparing to that of the control and MCP- treated groups, which might be due to the fact that the MCP dose was not enough to affect the targeted cells that produced albumin and total protein.

T.B and D.B serum levels

Table (3) showed that either rabbit's serum levels of T.B or D.B were increased significantly with percentage increases of 61% and 173% respectively, following treatment with MCP in G2 rabbits when compared to that of the control group. The results of the present study were in agreement with the studies of Mohapatra et al. *(*2021), Chabane et al. (2022), and Fu et al. (2023) that reported that MCP had a toxic effect on the liver of male rabbits. Therefore, liver injury impairs the normal bilirubin metabolism by inhibiting the conjugation and excretory processes, causing bilirubin accumulation in the serum. Also, inflammation due to MCP treatment caused swelling or obstruction of the bile ducts, also inhibiting bile flow and resulting in cholestasis, which increases bilirubin levels. MCP can also accelerate the breakdown of red blood cells, increasing bilirubin production above hepatic clearance capacity. Such hepatotoxic actions of MCP are in line with many studies showings its ability to cause liver damage, impair protein metabolism, and inhibit enzymatic processes involved in bilirubin clearance.

With regard to the rabbits of G (3), (4), and (5), that treated with low, medium and high doses of Se following treatment with MCP, the mean serum levels of T.B and D.B of each group showed non-significant difference when compared to the control group and a significant decrease which was dose dependent with a percentage decrease of 14%, 32%,34% respectively for T.B serum levels and 52%, 54%, 57% respectively for D.B serum levels when compared to MCP treated group (G2). Such decreased might be attributed to the antioxidant effect of Se in its three different doses against the induced toxic effect of MCP in such groups which was dose dependent, as the dose of Se increased the antioxidant effect against MCP toxicity decreased. Our results are consistent with prior research as it is in agreement with the findings of Adikwu et al. (2020), Tichati et al. (2020), Du et al. (2022), and Khokhar et al. (2022) who reported that Se had a protective or ameliorative role against diverse manifestations of toxic chemically induced hepatotoxicity. Hence, we conclude that Se had an ameliorative effect against the hepatotoxicity of MCP pesticide and mitigates its toxicity in a dose-dependent manner.

Table 3. The ameliorated effect of Se against the toxic effect of MCP on serum levels of T.B and D.B in male rabbits.

Groups		T.B mg/dl	D.B mg/dl
G1	Control group	1.018±0.046	0.23±0.011
G2	MCP treated group	1.64±0.270*	0.63±0.007*
G3	(MCP +Low dose of Se)	1.4±0.1*°	0.30±0.008*°
G4	(MCP + Medium dose of Se)	1.12 ±0.083*°	0.29±0.008*°
G5	(MCP + High dose of Se)	1.08±0.083*°	0.27±0.008*°

Values are expressed as mean ± standard deviation (n = 5). *p < 0.05: Difference was significant when compared with the control group (G1), °p < 0.05: Significant difference compared with the MCP-treated group (G2), NS: Not significant.

G1: Control group, G2: MCP treated group (0.1 mg/kg bw), G3: (0.1mg MCP+2 µg/kg bw Se), G4= (0.1mg MCP+4 µg/kg bw Se), (G5= (0.1mg MCP+8 µg/kg bw)

In this study, the results of the indicators in the last three groups (G3, G4, and G5) indicated that treatment with Se showed a protective effect against the toxicity of MCP, although this effect was not complete, as the three different doses of Se were not enough to nullify the toxic effect of MCP, but the dose-dependent effect caused by Se confirmed its protective role as an antioxidant even when it is given in lower doses. The results are consistent with prior research that has documented the protective or ameliorative properties of Se against diverse manifestations of chemically induced hepatotoxicity. Se has been evidenced to confer protection against cadmium-induced hepatotoxicity in murine models (Du *et al*., 2022), as well as hepatic damage resulting from the concomitant administration of isoniazid and rifampicin (Adikwu *et al.*, 2020). Comparable protective effects have been observed against 5-fluorouracil-induced hepatotoxicity in albino rats (Adikwu and Ebinyo, 2020) and methothrexate-induced hepatic toxicity in mice (Khokhar *et al*., 2022). Moreover, Se has exhibited hepatoprotective efficacy against acetaminophen (Al-Doaiss, 2020), 2,4-dichlorophenoxyacetic acid (Tichati *et al.*, 2020), and cyclophosphamide (Abdel Magid *et al.*, 2021) induced hepatic injuries. Additionally, research has demonstrated that Se mitigates hepatotoxic consequences induced by doxorubicin (Cengiz *et al*., 2021) and carbon tetrachloride in rats (Ebaid *et al.*, 2021). These cumulative findings substantiate the characterization of Se as a formidable antioxidant and hepatoprotective agent with the capacity to alleviate oxidative stress and cellular injury within the liver instigated by various toxic agents..

CONCLUSION

This study confirms that Se as an antioxidant, significantly mitigates MCP-induced hepatotoxicity in male rabbits, which was a dose-dependent. MCP exposure elevated the activities of liver enzymes GPT, GOT and bilirubin, indicating severe hepatic injury. Se supplementation effectively reduced these toxic impacts, with the highest dose (8 µg/kg b.wt) providing the highest protection. The findings of the present study support that Se has an antioxidant and hepatoprotective activities against organophosphate pesticide toxicity and its health risks. Further studies need to be conducted to further characterize its protective mechanisms and therapeutic applications.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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