Antioxidant and Anti-Inflammatory Effects of Nano-Selenium against Cypermethrin-Induced Liver Toxicity

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Abstract

Cypermethrin (Cym) is a synthetic class II pyrethroid that is widely used and has a big risk to health. Cypermethrin produces oxidative stress and enhances inflammatory damage of liver. The present study was designed to investigate the ameliorating effects of NSe against Cym-induced hepatotoxicity in rats. For this purpose, twenty four male rats were divided into three groups. Group (I) was gavaged with Cym (control group), group (II) gavaged daily with Cym (1 mg/kg body weight), and group (III) gavaged with Cym + NSe (2.5 mg kg body weight/day, three times a week) for 21 days. Cypermethrin increased serum liver enzymes, oxidative stress and inflammatory markers. Administration of NSe significantly reduced the increased serum liver enzymes and inflammatory parameters and restored the antioxidant capacity in liver. Our results suggest that Nse exhibits promising hepatoprotective effects against Cym-induced oxidative damage and inflammation.

Keywords

Cypermethrin, Oxidative Stress, Anti-Inflammatory, Hepatoprotective, Nano-Selenium

1. Introduction

Nanotechnology is the dealing with materials on the atomic scale. Nanotechnology has been used in many areas such as diseases diagnosis and treatment. Minerals nanoparticles have been produced using nanotechnology. Few researches evaluated the efficiency of the nanominerals [1]. Nanoparticles (NPs) exhibit unique properties as great surface area, high absorption, lower toxicity.
Selenium (SE) is an essential trace element that is important for mammalian life as it regulates the functions of selenoproteins [3] [4]. Many selenoproteins have oxidoreductase activity and coordinate the redox balance [5] [6]. Se plays a significant role in antioxidant defense systems and it is important for fertility, growth, and immunity in human and animals [4]. Nano-selenium (NSe) showed high biological efficiency due to its good absorptive ability, high bioavailability, high surface activity and low toxicity comparing to other forms of Se [7]. NSe displays potent effects on upregulation of GPx and induction of glutathione S-transferase which resulted in less oxidative stress [8]. Several studies confirmed that NSe can act as an antioxidant with lower risk compared with ordinary selenium toxicity [9]. A previous study declared that 5 - 200 nm NSe has the ability to scavenge free radicals in a size-dependent manner [10].

Cypermethrin (Cym) is a synthetic class II pyrethroid widely used in agriculture, household in addition to animal husbandry to control insects and ectoparasites; however it shows risk to human and animal health as well as to environment [11] [12] [13]. Cypermethrin is quite toxic through ingestion or through dermal exposure [14]. Cypermethrin produces neurotoxicity by generating free radicals and reducing antioxidants defense mechanism [15] [16]. Cypermethrin metabolized in liver by CYP-450 enzymes through oxidative pathways producing reactive oxygen species, which leads to oxidative stress in mammals [17] [18].

Oral intoxication with Cym produces considerable oxidative stress in cerebral and hepatic tissues [19] [20]. Brain and muscles are greatly affected by Cym [21]. Cypermethrin targets the sodium channel and prevents its normal closing resulting in hyper excitation [22] [23]. Cypermethrin is a hepatotoxic pesticide as liver is the major site of pesticide metabolism [24]. Cypermethrin enhanced inflammatory damage by increased levels of pro-inflammatory cytokine IL-1 and tumor necrosis factor alpha TNF-α [25]. Cypermethrin may be up-regulates pro-inflammatory cytokines IL-1, IL-8, TNF-α and down-regulates anti-inflammatory cytokines IL-4, IL-10 and IL-13 [26].

To our knowledge there are many literatures clarifying the effects of Cym toxicity in relation to oxidative stress [24] [27] [28]. However, there are no articles discussing the role of NSe against Cym-induced oxidative liver damage. Therefore, the present study was designed to evaluate the antioxidant and hepatoprotective effects of NSe against Cym-induced oxidative stress and liver injury in rat.

2. Materials and Methods

2.1. Animals

Twenty four adult male albino rats (weight 155 ± 10 g) were obtained from animal house of the faculty of veterinary medicine, Suez Canal University and housed in stainless steel cages under controlled environmental conditions on a
12 hours light-dark cycle. Animals have received a commercial rodent diet (72% corn, 27% soya bean and 1% fish meal) and water *ad libitum* all over the whole experimental period (21 days plus 1 week acclimatization). This study was carried out according to the protocol of (Animal Care and Use Committee at Faculty of Veterinary Medicine, Suez Canal University).

### 2.2. Chemicals

Cypermethrin and selenium were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA). NSe in the size range of 3 - 5 nm was prepared according to procedures described previously by [2]. Commercial assay kits for estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), albumin, total protein (TP), γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), catalase (CAT), reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) were purchased from (Biodiagnostic company, Cairo, Egypt). Tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP) and interleukin one beta (IL-1β) levels were evaluated using diagnostic kits (Roche Diagnostics, Cairo, Egypt).

### 2.3. Experimental Design and Sample Collection

Rats were randomly divided to three groups (n = 8) as follow:
- **Group I (negative control group):** orally received standard diet and tap water.
- **Group II (Cym group):** orally given Cym in a dose of 1/10 LD50 (1 mg/kg b.w) for 21 days by stomach tube.
- **Group III (Cym + NSe group):** rats orally administrated Cym at a dose of (1 mg/kg b.w) together with NSe at a dose of 2.5 mg/kg b.w (dose determination based on pilot work) by gastric tube once a day 3 times a week for 21 days.

At the end of treatment, all the rats were anesthetized with diethyl ether and blood samples were collected from the medial canthus of the eyes and sera were prepared by blood centrifugation at 3000 r/min for 15 min at 4 C and kept for estimation of serum liver parameters and inflammatory biomarkers. Liver tissues were removed immediately then homogenized in ice-cold sodium, potassium phosphate buffer (0.01 M, pH 7.4). The homogenates were centrifuged for at 4000 r/min for 10 min at 4 C and the supernatant was used for evaluation of antioxidant and oxidative stress markers.

### 2.4. Biochemical Analysis

The tested parameters were measured according to the directions of their referred methods: ALT and AST according to [29] by mixing the working reagent with the serum sample then read the absorbance by spectrophotometer, LDH [30]. The optical density was monitored at 340 nm using an UV-visible spectrophotometer (Shimadzu, Japan) and the decreased absorbance is due to oxidation of NADH to NAD1 which is proportional to the LDH activity, GGT [31]. Total protein was measured according to [32] the reagent containing copper salt in an
alkaline medium in presence of iodide and the resulted color intensity is proportional to the protein concentration, TB [33] bilirubin react with the Diaz reagent in presence of a surfactant then the absorbance was determined at 546 nm, Albumin [34] mix the reagent (Bromocresol green pH 4.2) with the samples and the standard then read the absorbance by spectrophotometer. CRP was measured using Roche/Hitachi cobas c311 automatic analyzer following the instructions of the manufacturer.

2.5. Inflammatory Cytokines

The Concentrations of IL-1β and TNF-α in the liver tissue homogenate were estimated by sandwich enzyme immunoassay technique using kits (R&D Systems, Europe, United Kingdom) specific for rats. A monoclonal antibody was coated onto a microplate then the standard and samples were mixed. Wash with buffer then add an enzyme-linked polyclonal antibody specific for rat cytokine. Re-wash then add a substrate solution and incubate for 30 minutes at room temperature before adding the stop solution. Optical density was determined at 450 nm using a microplate reader. The amount of amount of cytokines is proportional to the intensity of the developed color.

2.6. Liver Lipid Peroxidation and Antioxidant Parameters

MDA was evaluated spectrophotometrically according to [35] [36] by mixing the supernatant with 1 mL of 5% trichloroacetic acid and centrifuged at 2500 g for 10 min. Add 0.2 mL of the supernatant to 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 30% acetic acid (pH 3.5) and 1.5 mL of 0.8% thiobarbituric acid and heat for 30 min at 95°C and then cooled then centrifuged at 4000 g for 10 min. The absorbance was measured at 532 nm. GSH level was determined according to [37]. The method is depend on the development of a yellow color when 5,5' dithiobis-2-nitro benzoic acid is mixed with compounds containing sulfhydryl groups. The absorbance was measured at 412 nm. CAT activity was assessed by the method of [38]. CAT reacts with H₂O₂ then the reaction is stopped with CAT inhibitor. H₂O₂ reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone in presence of peroxidase forming a chromophore, the color intensity was measured spectrophotometrically at 510 nm. SOD activity was determined according to [39], 50 mL of the supernatant were added to 100 mL sodium pyrophosphate buffer (pH 8.3), 0.1 mL nitroblue tetrazolium and 0.1 mL of NADH followed by 0.01 mL of phenazine methosulfate. The absorbance was evaluated at 560 nm.

2.7. Statistical Analysis

Data were collected and presented as mean ± standard error S.E. for 8 rats per group. The Statistical significances were determined by one-way ANOVA followed by Bonferroni’s test for multiple comparisons using SPSS version 21 software package (SPSS, Inc, Chicago, Illinois, USA). The level of statistical signi-
ficance was acceptable at \( P \leq 0.05 \).

3. Results

3.1. Effects on Liver Function

The effects of Cym intoxication and the protective effects of NSe on serum biochemical parameters are shown in Figure 1. There was a significant increase (\( P \leq 0.05 \)) in serum liver integrity biomarkers (AST, ALT, LDH and \( \gamma \)-GT) in Cym group compared to the negative control group. Also, there was a significant increase (\( P \leq 0.05 \)) in total bilirubin. Serum total protein and albumin were significantly decreased (\( P \leq 0.05 \)) in Cym intoxicated rats compared with the untreated control group. Treatment with NSe restored serum albumin and total protein levels and inhibited the rise of liver enzymes and bilirubin.

3.2. Oxidative Stress and Antioxidant Parameters

The effects of Cym intoxication as well as ameliorating effects of NSe on lipid peroxidation and antioxidant parameters are shown in (Table 1). Results revealed a significant increase (\( P \leq 0.05 \)) in hepatic levels of MDA and a significant decrease (\( P \leq 0.05 \)) in liver GSH, SOD and CAT levels in Cym intoxicated rats compared to the negative control group. Concomitant treatment of intoxicated

![Figure 1. Serum enzyme activities and biochemical parameters in the control and treated groups (percentage of changes from the control group). Cym; Cypermethrin, NSe; Nano-selenium, ALT; Alanine aminotransferase, AST; Aspartate aminotransferase, GGT; \( \gamma \) glutamyl transferase, TP; Total protein, TB; Total bilirubin and LDH; Lactate dehydrogenase.](image)

| Groups       | Parameters | MDA (nmol/g) | GSH (mg/g) | Catalase (U/g) | SOD (U/g) |
|--------------|------------|--------------|------------|----------------|-----------|
| Control      | 31.6 ± 3.10| 58.8 ± 2.90  | 1.71 ± 0.07| 436 ± 37.5     |           |
| Cym          | 103 ± 7.98*| 39.9 ± 2.76* | 0.64 ± 0.01*| 239 ± 22.3*    |           |
| Cym + NSe    | 53.3 ± 4.08*| 47.2 ± 2.67* | 0.94 ± 0.03*| 354 ± 29.6*    |           |

Values are expressed as means ± SE; \( n = 8 \). Cym; Cypermethrin, NSe; Nano-selenium, MDA; Malondialdehyde, GSH; Reduced glutathione, CAT; Catalase and SOD; Superoxide dismutase. Superscript letters indicate a significant difference at \( P \leq 0.05 \). * indicates a significant difference from control group; † indicates a significant difference from Cym group.
rats with NSe induced a significant decrease (P ≤ 0.05) in liver MDA levels along with a significant increase (P ≤ 0.05) in GSH, SOD and CAT levels compared with the Cym group.

3.3. Inflammatory Markers

Cym enhanced liver inflammation as indicated by increased IL-1β, TNF-α and CRP levels in Cym intoxicated group compared with control (P ≤ 0.05). However, treatment with NSe significantly reduced (P ≤ 0.05) levels of these inflammatory parameters (Table 2).

4. Discussion

Cypermethrin is a worldwide synthetic pyrethroid that has been recognized to be toxic in acute and chronic states for human and animals [40]. Cypermethrin is metabolized in liver and this may lead to hepatic damage and inflammation by producing reactive oxygen species causing oxidative stress [17] [18] [19] [24]. Oxidative stress is a major cause of various toxicities [41].

Results of this study revealed that oral administration of Cym induced pronounced negative effects on liver tissue. This was manifested by increased levels of serum liver integrity enzymes, lipid peroxidation, pro-inflammatory markers, as well as decrease in antioxidant defense system.

In the present study, oral administration of Cym increased the leakage of liver function markers (ALT, AST, GGT and LDH) into serum; it also raised blood TB. There was a significant decrease in serum total protein and albumin. These results are in agree with [23]. TB, ALT, AST, GGT and LDH indicators of the hepatic function status and their excessive leakage into the blood is usually associated with impaired hepatocellular function and disintegration of hepatic cell membrane [42] [43]. The damage of liver cells by Cym may disturb protein metabolism and synthesis in liver resulting in a decrease of plasma proteins [21].

It was documented that free radical-mediated oxidative stress is responsible for the toxicity of pesticides [20] [44]. In the current experiment, the oxidative stress that attributed to free radical production may be concerned in hepatic damage by Cym [19]. Cypermethrin intoxication induces lipid peroxidation,

| Groups          | Parameters          | Value       |
|-----------------|---------------------|-------------|
|                 | IL1-1β (pg/mL)      | 3.21 ± 0.37 |
|                 | TNF-α (pg/mL)       | 8.93 ± 0.26 |
|                 | CRP (mg/L)          | 4.44 ± 0.39 |
| Control         |                     |             |
| Cym             |                     | 5.34 ± 0.46*|
|                 |                     | 13.4 ± 0.61*|
|                 |                     | 7.84 ± 0.55*|
| Cym + NSe       |                     | 3.91 ± 0.48*|
|                 |                     | 9.32 ± 0.23*|
|                 |                     | 5.24 ± 0.43*|

Values are expressed as means ± SE; n = 8. Cym; Cypermethrin, NSe; Nano-selenium, IL1-β; interleukin one beta, TNF-α; tumor necrosis factor alpha and CRP; C-reactive protein. Superscript letters indicate a significant difference at P ≤ 0.05. * indicates a significant difference from control group; † indicates a significant difference from Cym group.
Oxidative Stress and a marked decrease in glutathione peroxidase activity [45]. Cypermethrin induced a significant increase in the level of the oxidative stress biomarker (MDA) and decreased GSH antioxidant capacity in liver tissue as well as decreasing the activities of hepatic SOD, CAT. These effects are incriminated in hepatic oxidative stress and toxicity. This brings to mind that Cym-mediated hepatic damage occurred as a result of excessive production of reactive radicals and consumption of antioxidants. These results are in agreement with those obtained by many other researchers [24] [28] [46] [47].

Reduced glutathione is a non-enzymatic antioxidant which plays an important role in protecting cells against free radicals and detoxifying xenobiotics by scavenging of ROS [48]. SOD and CAT are considered the first line of defense against the reactive intermediates. Superoxide dismutase is responsible for scavenging the superoxide radicals while; CAT is functioning to neutralize the hydrogen peroxide radical. In our study, the significant reduction of hepatic antioxidants (GSH, SOD and CAT) may reveal the utilization of them in combating the pro-oxidants generated in Cym metabolism and act as a marker of tissue degeneration and damage [47] [49] [50].

Results revealed that Cym enhanced inflammatory damage indicated by increase of serum concentrations of the inflammatory marker CRP and pro-inflammatory cytokines IL-1β and TNF-α. Toxic substances mediate inflammatory response through activation of macrophages and induction of pro-inflammatory cytokines [51]. Abdou et al., (2019) stated that TNF-α is responsible for the induction and progression of the inflammatory response and the production of inflammatory mediators, such as IL-1 and IL-6 [52]. Inflammatory reactions are mediated by IL-1β and TNF-α through neutrophil activation [53]. CRP is a protein produced by liver cells in acute cases of inflammation, infection and tissue damage [54]. These findings are in consistence with [28] [55] [56]. Oxidative stress is attributed in the pathogenesis of inflammation by activation of redox-sensitive transcription factors that control the gene expression of pro-inflammatory mediators and antioxidants [57].

Our results proved that administration of NSe was significantly modulated the raised liver enzymes and inflammatory markers to be nearly similar to control group; these findings may be due to maintaining of the hepatocytes integrity or regeneration of injured hepatocytes [58]. Also, treatment with NSe reduced MDA and elevated GSH contents as well as antioxidant enzymes (CAT and SOD) in liver tissue in (Cym + NSe) compared with Cym group. These findings verify the restorative effects of NSe on liver tissue that could be due to the ability of NSe to minimize the Cym-induced oxidative stress by reduction of free radicals production and thereby inhibiting the free radical chain reactions. The antioxidant activity of NSe was established by other researchers [7] [24] [59]. Many studies showed the potential protecting effects of Se and its nano form against oxidative stress, DNA damage and apoptosis that attributed to the significant role of Selenium in the improvement of the antioxidant defense mechanisms and
free radicals scavenging capacity [59] [60] [61].

5. Conclusion

The current study confirmed the hepato-protective effects of NSe against Cym toxicity. These effects may be due to its antioxidant capacity. Finally, NSe could be helpful for people and animals suffering from or at risk of Cym exposure.

Ethics Statement

This study was carried out according to the guiding principles of the Scientific Ethical Committee, Faculty of Veterinary Medicine, Suez Canal University.

Conflicts of Interest

The authors declared that they have no conflict of interest.

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