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# The protective effect of rosemary in mitigating oxidative stress induced by Chlorpyrifos in rat kidney

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## Abstract

**Introduction:** Chlorpyrifos is an organophosphorus insecticide that can produce reactive oxygen species, and oxidative stress in the kidney. On the other hand, rosemary extract is an antioxidant playing a protective role against free radicals. Therefore, the aim of this study was to evaluate the oxidative stress in rat kidney due to the exposure to chlorpyrifos and to assess the antioxidant effect of two concentrations of 100 and 200 mg / kg rosemary extract on the nephrotoxicity caused by this pesticide.

**Material and method:** In the current study, 30 male Wistar Rats, weighing 200-220 g were used. They were randomly divided into five groups and selected as the study groups, each group including six rats for further comparison. They were as following groupd: Group I: served as control (received dissilated water, i.p) , Group II: the chlorpyrifos exposure group (13.5 mg / kg, i.p.), Group III: the group exposed to rosemary extract (100 mg / kg) by gavage, Group IV: the exposure group with rosemary 100 mg / kg concentration (by gavage) and after 30 minutes exposure to chlorpyrifos (13.5 mg / kg, i.p.) and Group V: simultaneous exposure of rosemary 200 mg / kg concentration (by gavage) and after 30 minutes exposure to chlorpyrifos (13.5 mg / kg, i.p.). After 2 and 4 weeks, the rats were weighed and killed. A section of kidney tissue was examined for changes of Malondialdehyde (MDA) and Glutathione peroxidase (GPx) and another section was tested for histopathological alterations.

**Results:** The results showed that chlorpyrifos significantly damaged the kidney tissue and altered the activity of GPx and MDA compared to the control group, as well as it damaged the kidney tissue. Moreover, the simultaneous exposure to chlorpyrifos and rosemary extract with concentration of 100 mg/kg significantly made modifications to the MDA and GPx levels. In addition, with the increasing of rosemary doses from 100 to 200 mg / kg, changes in the levels of MDA and GPx were closer to those of the control group.

**Conclusion:** The rosemary extract can reduce the oxidative stress caused by chlorpyrifos and, therefore, can be used to treat the poisoning caused by this pesticide.

**Keywords:** Chlorpyrifos, Oxidative Stress, Rosemary, Kidney Toxicity, Antioxidant.

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## 1. Introduction

Chlorpyrifos (O,O-diethyl-O-3,5,6-trichlor-2-pyridyl phosphorothioate; CPF) is one such broad spectrum insecticide that is indiscriminately being used throughout the world for a variety of agricultural and public health applications. Chlorpyrifos, in a manner analogous to most phosphorothioate insecticides, is metabolically activated to chlorpyrifos Oxon by the microsomal cytochrome P-450 drug-oxidizing system in liver. Researchers observed a high degree of lipid peroxidation and DNA single-strand breaks as a result of CPF intoxication in tissues of male rats, indicating increased oxidative stress and resultant tissue damage. Among the herbal extracts reported to have antioxidant activity, rosemary (Rosmarinus officinalis L.) is one of the most widely commercialized plant extracts; it is used as a culinary herb for flavoring and as an antioxidant in processed foods and cosmetics.

Although antioxidant compounds have been demonstrated to have beneficial effects on some parameters related to the cardiovascularRisk, the role of rosemary phenolic compounds on chlorpyrifos-induced oxidative stress has not been elucidated in the literature [1-8]. Therefore, the aim of this study was to evaluate the effect of two concentrations extracts (100 and 200 mg/kg) from rosemary (ROS) on the antioxidant status kidney of rats.

## 2. Material and Methods

In the current study, dried and powdered leaves of the plant were obtained from the local herbal shop at Tehran, Iran. An amount of 50 g of grinded leaves of Rosemary was suspended in 200 ml of 70% ethanol to obtain the extract. And Commercial grade chlorpyrifos (diethyl 3,5,6-trichloro-2-pyridyl phosphorothioate, formulation EC40.8%) purchased from an agricultural used, Iran, after that 30 male Wistar Rats, weighing 200-220 g were used. Rats were acclimatized in the laboratory conditions for seven days before study initiation. The animals (Rats) were randomly divided into five groups, each group containing six rats for further comparison; after the period of acclimation, the rats were randomly assigned into five experiments (I- V) groups. In the control group, the distilled water was given by intraperitoneal (i.p). In groups of IV and V, the experimental rats were pre-treated once a day for seven consecutive days with Rosemary (100 mg/kg; by oral gavage). After seven days, rats were treated with Rosemary in different concentrations 100 and 200 mg/kg for IV and V groups, respectively, and before the administration of CPF only at a dose of 13.5 mg/kg (corresponding to 1/10 LD50 value: 135 mg/Kg). At the end of the experiment, 24 hours after the last exposure, all rats were weighed and anesthetized; after that, the rats were killed. The kidney tissue of rats was dissected out and rinsed in normal saline solution. One part of the kidney was homogenized in 4 volumes of phosphate-buffered saline (PBS), centrifuged. The supernatants stored at -70 0C until used for the biochemical studies. Also, a small slice of kidney tissue was fixed in 10% neutral formalin solution for histopathological examination purposes. Lipid peroxidation in the kidney was estimated on the basis of their Malondialdehyde (MDA) content according to the manufacturer protocol (ZellBio, Germany). This assay kit uses the MDA with thiobarbituric acid adduct formed by the reaction of Malondialdehyde and thiobarbituric acid (MDA-TBA) under high temperature. MDA was measured in acidic media and heat (90-1000C) colorimetrically at 535 nm. The values of MDA were expressed as μM. The determination of glutathione peroxidase (GPx) activity in the kidney homogenates was measured using ZellBio kits (ZellBio GmbH, Ulm, Germany). This assay kit was used to quantitative assay glutathione peroxidase activity on the basis of the colorimetric assay at 412 nm. For histological examination, the kidney tissue was separated and washed with saline and then fixed in 10% formalin. The tissues were then dehydrated and paraffin-embedded in ethanol. Paraffin-embedded samples were spiked with a five μM sample with a microtome (model 4055 Iran) and stained with hematoxylin-eosin stain. The stained sections were examined by light microscopy and photographed. At the level of tissue

sections, the pathological indices were scored on a single scale. Data were expressed using mean and standard deviation. All statistical analyses were performed using the SPSS 22 Version 22 software. Statistical significances were determined using one-way ANOVA followed by Tukey test for comparison between the data of the control and those of treatments. The value of $P<0.05$ was taken as a minimum level of significance.

## 3. Results and Discussion

In toxicological investigations, body weight is an important criterion for the assessment of toxicity. Generally, a decrease or increase of body weight than normal may be considered as a sign of toxicity, as shown in Fig. 1, during course of the current study. A significant increase in the body weights of control animals at the end of the study was observed in comparison to their initial weights. However, the weight gain of the rats intoxicated with CPF was much lower compared to the control group that these results are in line with many other studies. This weight loss can be explained by a metabolic imbalance or reduced dietary intake of the rats. Rosemary treatment in the concentration of 100 and 200 mg/kg to the Chlorpyrifos-treated rats also resulted in significant net body weight gain. However, this weight gain was somewhat lesser when compared to the control group. Similar protective effects of Rosemary in improving the body weight gain of the animals have been reported in other studies.

The findings showed that Rosemary has a positive effect on the antioxidant systems. In the current study, it was found that the administration of Rosemary dose-dependently reduced the harmful effects of CPF toxicity. These effects were observed in the improvement of kidney functions. The positive effect of Rosemary administration on lipid peroxidation is observed at 100 and 200 mg/kg, but at 200 mg/kg dosages of Rosemary seem to have a more prominent effect in reducing

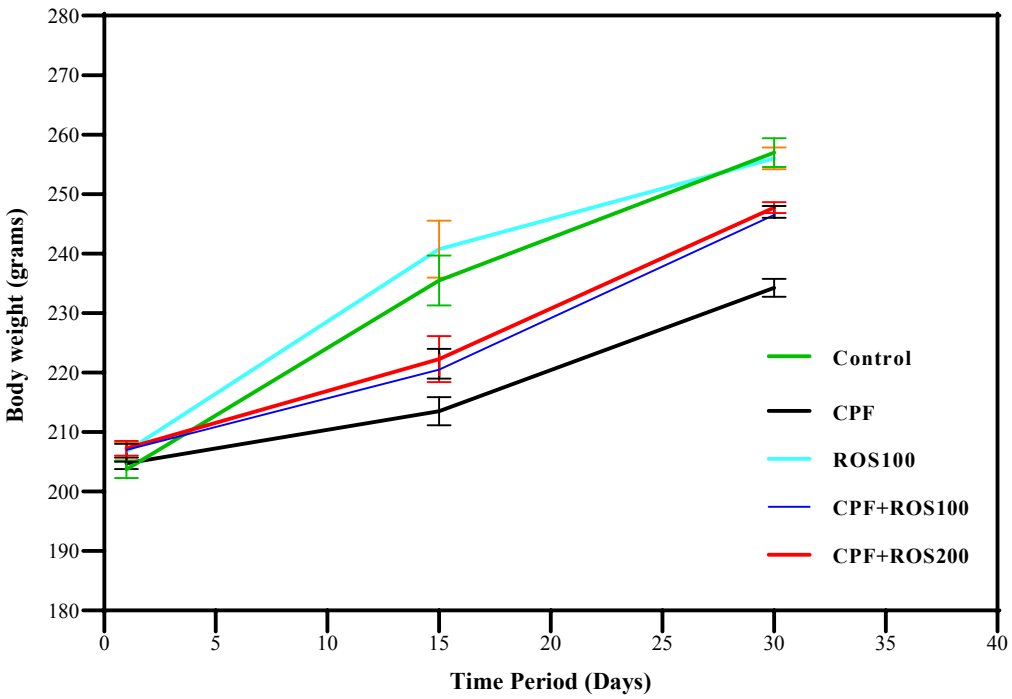


**Fig. 1.** The line chart shows the growth patterns of the control, chlorpyrifos-treated, Rosemary-treated, co-exposure to CPF and ROS (100 mg/kg), and co-exposure to CPF and ROS (200 mg/kg) animals. Rosemary-treated group and control group have a much higher net body weight gain throughout compared to the slower weight gained in chlorpyrifos-treated animals.

**Table 1.** Effects of Rosemary extract (100 and 200 mg/kg) on CPF-induced MDA contents in the liver, kidney, and serum tissues, *significantly different compared to control group at $p < 0.05$, **significantly different compared to CPF group at $p < 0.05$. @Significantly different compared to group IV

| | MDA (μM) | | |
|---|---|---|---|
| **Groups** | **Liver** | **Kidney** | **Serum** |
| Control | 4.51 ± 0.08 | 3.25 ± 0.09 | 1.21 ± 0.12 |
| CPF | 9.025 ± 0.06* | 8.05 ± 0.1* | 4.95 ± 0.5* |
| ROS (100 mg/kg) | 4.36 ± 0.04 | 3.05 ± 0.1 | 1.17 ± 0.45 |
| CPF + ROS (100 mg/kg) | 7.31 ± 0.17** | 6.7 ± 0.09** | 3.05 ± 0.51** |
| CPF + ROS (200 mg/kg) | 6.06 ± 0.11@ | 5.01 ± 0.08@ | 2.1 ± 0.19@ |

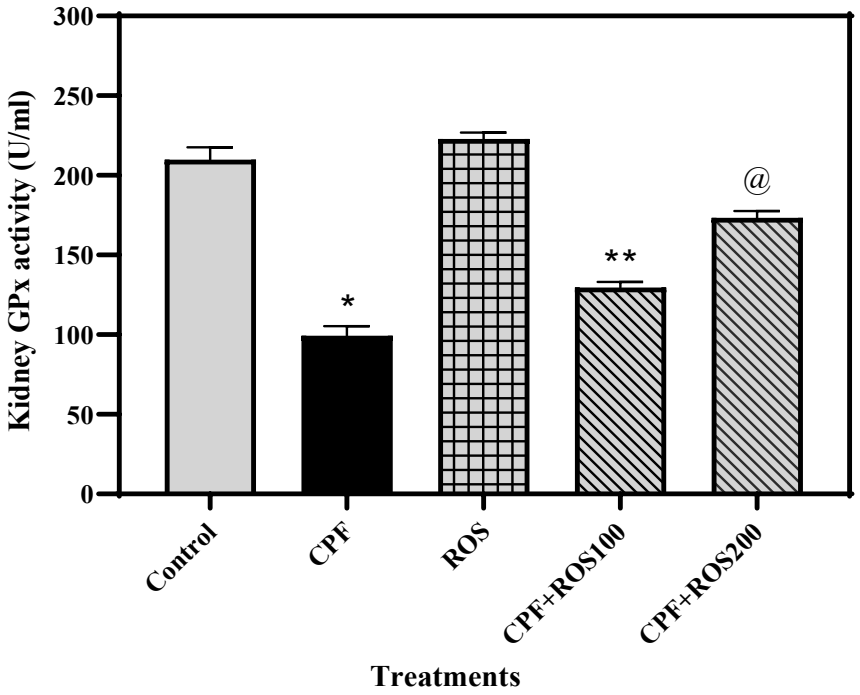


**Fig. 2.** ROS significantly increased GPx activity in kidney
*Significantly different compared to the control group at $p < 0.05$.
**Significantly different compared to the CPF group at $p < 0.05$.
@Significantly different compared to the group IV

the oxidant effect and supporting the antioxidant system (Table 1 and Fig. 2).

Examination of the sections of kidney tissues of the control and that of ROS treated revealed normal renal architecture (Fig. 3 A and C). Administration of CPF alone caused severe necrosis in kidney tubules, accumulation of inflammatory cells in the interstitial spaces, and congestion of renal vessels was also observed (Fig. 3B), these results could be due to its toxic effects by the generation of reactive oxygen species causing damage to different membrane components of the cells. These results are in line with a few previous published. Whereas, in group IV, co-exposure to CPF and ROS (100 mg/kg) caused moderate necrosis in tubules (Fig. 3D). On the other hand, sections of the kidney of rats from the group treated with CPF and ROS (200 mg/kg) caused a marked improvement in the structure of the kidney and showed the healthy structure of the kidney tubules like the normal group (Fig. 3E).

## 4. Conclusion

Protective effects afforded by Rosemary in animals treated with Chlorpyrifos. The data suggest that Rosemary treatment in chlorpyrifos-treated animals afforded protection owing to its antioxidant properties and thus maintaining the structural as well as functional disturbances in the kidney. Further studies are needed to determine the exact mechanism of rosemary extract in the body of the animal and human specimens. One of the strengths of this study is the use of commercial poison, which is currently being used in the market and agriculture. From this point of view, the results of the study can be generalized to the workplace, but it should be kept in mind that the extract was used purely in the study, and some measures should be considered for use in the workplace.

## 5. Acknowledgment

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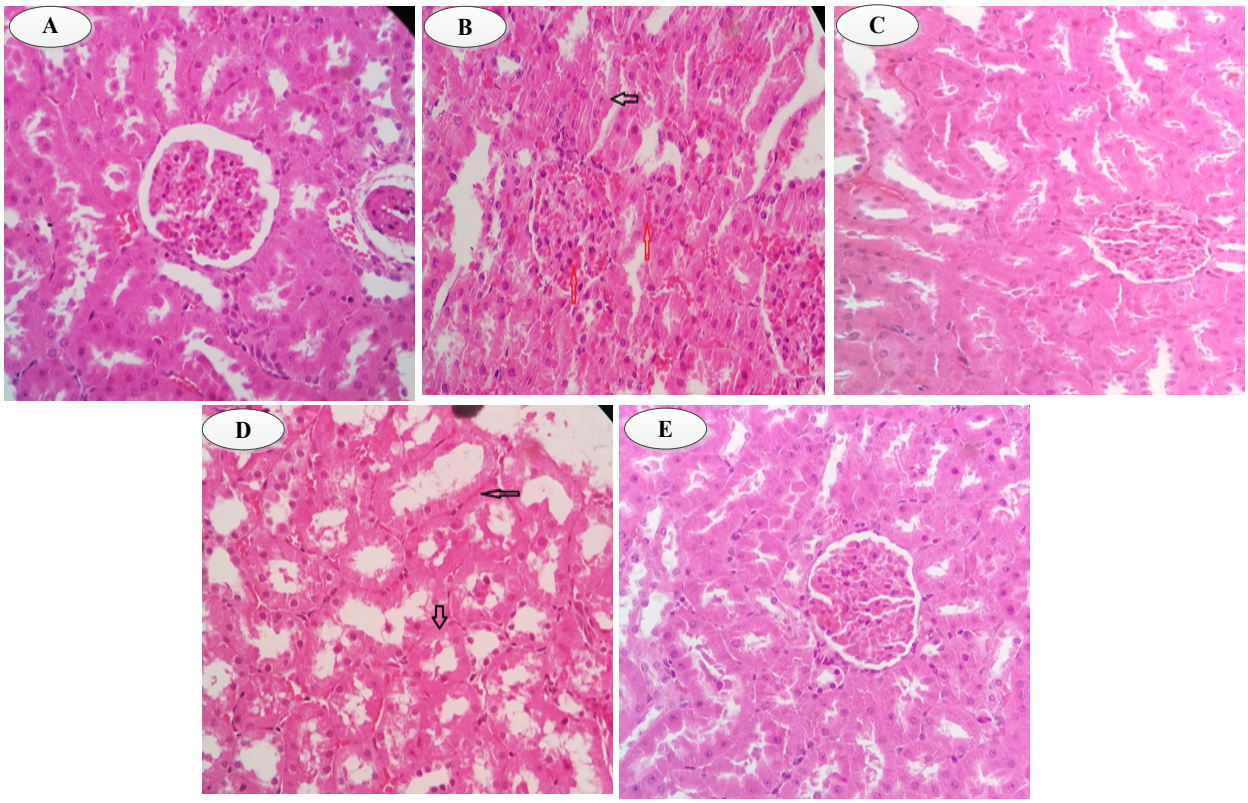


**Fig. 3.** Representative histological images of kidney slices **A:** Control rat showing normal kidney architecture. **B:** Rats treated with CPF showing severe necrosis in tubules (red arrow), accumulation of inflammatory cells in the interstitial spaces and congestion of renal vessels (black arrow) **C:** rats treated with ROS (100 mg/kg) revealed normal kidney architecture **D:** CPF (13.5 mg/kg) + ROS (100 mg/kg) showed partial necrosis in tubules (black arrow). **E:** CPF (13.5 mg/kg) + ROS (200 mg/kg) showed normal structure of the kidney tubules.

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