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Assessment of the Protective Effect of Rosemary Leaves Extract Against Oxidative Stress and Inflammatory Cytokines Induced by Cypermethrin in Male Rabbits

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ABSTRACT

Background & Objective: Rosmarinus officinalis (rosemary) is widely used in the daily diet and has recently gained increasing attention for its medicinal and pharmacological properties, particularly its antioxidant and anti-inflammatory potential. Cypermethrin (CYP) is a synthetic pyrethroid insecticide that induces oxidative stress and inflammation in biological systems. This study aimed to evaluate the protective efficacy of rosemary leaf extract against cypermethrin-induced oxidative stress, inflammatory response, and neurotoxicity in male rabbits.

Materials & Methods: Forty male rabbits were randomly divided into four groups (n=10 each).Group 1 (control): received 1 mL of corn oil. Group 2 (CYP): orally administered CYP at 66.5 mg/kg b.w (1/10 LD50).Group 3: received rosemary extract (100 mg/kg) + CYP (66.5 mg/kg).Group 4: received rosemary extract (200 mg/kg) + CYP (66.5 mg/kg). All treatments were administered orally once daily for five weeks. Serum levels of malondialdehyde (MDA), total antioxidant capacity (TAC), inflammatory cytokines (TNF-α and IL-6), and acetylcholinesterase (AChE) activity were measured.

Results: CYP exposure significantly increased MDA, TNF-α, and IL-6 levels and decreased TAC and AChE activity compared to the control group, indicating oxidative stress, inflammatory response, and neurotoxicity. Co-administration of rosemary extract with CYP resulted in a significant reduction in MDA and inflammatory cytokines, along with a marked increase in TAC and AChE levels compared to the CYP-only group.

Conclusion: The findings demonstrate that Rosmarinus officinalis possesses potent antioxidant, anti-inflammatory, and neuroprotective properties. Rosemary extract effectively mitigated cypermethrin-induced oxidative damage, inflammation, and cholinesterase inhibition in male rabbits.

Keywords: Rosmarinus Officinalis, Cypermethrin, Oxidative Stress, TNF-α, IL-6, TAC, AChE

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1. Introduction

Synthetic pyrethroids are insecticides that have been developed and used, but continuous and uncontrolled use of these chemicals has caused several health and environmental problems (1). pyrethroids are primarily affect the nervous system of the exposed organisms by inhibiting acetylcholinesterase (AChE) and raising acetylcholine levels in the cholinergic synapse. In general, the nerve membrane ionic channels represent the primary target of pyrethroid toxicity (2, 3). Also, pyrethroids induce oxidative stress, affect metabolic pathways and cause multiple organ dysfunction, pyrethroids type II includes cypermethrin (C22H19Cl2 NO3; known as α – CYP) are considered to be the most active forms, Cypermethrin is one of the extensively used pyrethroids as an ectoparasiticide in livestock, crops and public health programs, as a fast-acting neurotoxin to insects (4, 5). Roughly 0.1 % of the applied pesticides reach the target pests, and the rest spreads through water, soil, and food causing a broad spectrum, of neurotoxicity in mammals and long-lasting prolongation of sodium

permeability during excitation (6). This may cause severe repetitive nerve impulses in the sense organs and damage to the voltage-dependent sodium channel, prolonging its opening beyond usual. Therefore, there is a possibility of generating excessive reactive oxygen species (ROS) following exposure to CYP (7-9). CYP is absorbed via the gastrointestinal and respiratory tracts and confers discriminatory distribution into lipid-rich internal tissues (10). Cypermethrin is toxic substance, when exposure of CYP through ingestion or directly through dermal absorption this can be produce irritation, and itching at the skin and eyes, physiological impacts, neurotoxicity, reproductive toxicity and molecular toxicity (11, 12). Because there are thousands of potential medicinal plants that contain many bioactive substances and natural antioxidants strengthen the endogenous antioxidant defenses from reactive oxygen species (ROS) and restore the optimal balance by neutralizing the reactive species. It their critical role in disease prevention (13, 14), among these plants Rosmarinus officinalis, L is belonged to the family of Lamiaceae, known as rosemary which is evergreen herb and an aromatic that widely used as spice and flavoring in food (15, 16). It is the most herbs prevalence in various parts of the world. Rosemary leaves possess antioxidant and anti-inflammatory properties that are attributed to the presence of a variety of phenolic compounds such as carnosol, carnosic acid, ursolic acids, rosmanol, and caffeic acid. Rosemary leaves also used in the cosmetics industries due to mainly for their antioxidant and anti-inflammatory compounds (17-20). Nevertheless, few studies were done to manifest the hazardous effects of insecticide pollution on human health and animals and prophylaxis in Iraq. The current work aimed to evaluate the protective efficacy of rosemary leaf extract against adverse effects in male rabbits exposed to cypermethrin.

2. Materials and Methods

2.1 Procedure extraction of Rosemary Leaves

in our work Rosemary Leaves (Rosmarinus officinalis) were purchased from the market in Thi-qar city in Iraq, were cleaned, washed with distilled water and then dried at room temperature for two days under the shade, we prepared extract of leaves following ground into powder and then we take weighed 50g of crushed leaves were extracted with 200ml of ethanol 70% put in the round flask, for 12 hrs using reflex extractor. The extracted was filtering by using whatmann filter paper (No.31), the final product was left in the petri-dish under the shade, The dried extract was collected and kept in tight closed container and stored at 4ºC until using, the final of extraction was used in the current work with dose (100and200mg/ kg), this extract was prepared according to method described by Abbasi Oshaghi et al (21).

2.2 Animal's protocol and animals care

40 of male rabbits weighing (1250-1300 gms), were housed in the Animal House, two rabbits in each plastic cage for 14 days before the experiment within temperature (25±2 °C) and a 12-hour light / dark cycle, with food access and water available ad libitum. animals randomly distributed into four experimental groups, each group (10 rabbits) as following; Group I negative control animals were orally administered corn oil (1ml), Group II (group CYP) animals were orally exposure to CYP with dose 66.5 mg/kg b.w of 1/10 LD50 dissolved in corn Oil daily, Group III (Rosemary extract+ CYP) orally administered Rosemary extract 100mg/kg b.w plus 66.5 mg/kg b.w of CYP dissolved in corn oil daily, Group IV(Rosemary extract+ CYP): Orally administered Rosemary extract 200mg/kg b.w plus 66.5 mg/kg b.w of CYP dissolved in corn oil daily, the treatment continued for 6 weeks.

2.3 Samplings

Blood samples (about 5ml) were collected following 24 hours last dose of treatment by puncture of the heart from all groups. Samples were allowed to clot, then all samples were separated by centrifuge at 3000 rpm for 10 minutes obtain serum which was then stored at -20ºC until used for biochemical measurements.

2.4 Serum assays

Average of malondialdehyde (MDA) was estimated according to method of Ohkaw et al (22), Total antioxidant capacity (TAC) was measured according to method described by Bartosz (23), while, the inflammatory cytokines Tumor necrosis factor-alpha (TNF-a) and Interleukin-6 (IL-6) concentrations were evaluated by method of Naz et al (24) and Cauvi et al (25). Using ELISA procedure, serum acetylcholinesterase (AChE) enzyme activity was assessed according to method of Ellman et al (26).

2.5 Statistical Analysis

Mean values of serum indices were analyzed by one-way analysis of variance (ANOVA), and the obtained mean differences and standard deviations (mean ± SD) between treated and control groups. P-values ($P<0.05$) are considered statistically significant.

3. Result

As presented in Table 1, oral exposure to Cypermethrin (CYP) resulted in a significant increase in the oxidative stress biomarker malondialdehyde (MDA), accompanied by a substantial decrease in total antioxidant capacity (TAC) when compared to normal control animals.

Similarly, data in Table 2 showed a marked elevation in inflammatory mediators including TNF-α and IL-6 in CYP-exposed rabbits relative to the control group. Furthermore, Table 3 demonstrated a significant reduction in acetylcholinesterase (AChE) activity in CYP-treated rabbits in comparison to normal animals, indicating a neurotoxic effect. In contrast, rabbits treated with rosemary extract (100 and 200 mg/kg) along with CYP exhibited a significant decline in MDA and inflammatory cytokines, alongside a notable elevation in TAC and AChE levels compared to the CYP group alone.

These findings indicate the protective antioxidant, anti-inflammatory, and neuroprotective effects of rosemary extract.

Table 1. Serum MDA and TAC levels in control group and treated groups with Rosemary extract plus CYP in male rabbits.

Groups Parameter	Control 1ml corn oil	CYP (66.5mg/kg)	RE(100mg/kg) plus CYP 66.5mg/kg)	RE(200mg/kg) plus CYP 66.5mg/kg)
MDA µ mol / L	0.54±0.039 c	3.10±0.102 a	1.13±1.861 b	0.57±0.031 c
TAC µ mol / L	1.82±0.162 a	0.65±0.034 c	1.54±0.019 b	1.67±0.015 a

Data are expressed as mean ± SD (n=10). The different letters refer to significant difference ($p< 0.05$) CYP:cypermethrin, RE: Rosemary ethanolic extract

Table 2. Serum of TNFa and IL-6 in control group and treated groups with Rosemary extract plus CYP in male rabbits.

Groups Parameter	Control 1ml corn oil	CYP (66.5mg/kg)	RE(100mg/kg) plus CYP 66.5mg/kg)	RE(200mg/kg) plus CYP 66.5mg/kg)
TNFα ng/ml	23.19 ±1.86 c	36.55±2.97 a	28.39 ±2.9 b	25.48 ±1.47 c
IL-6 pg/ml	7.03 ±0.74 b	16.97±1.61 a	8.26 ±1.23 b	7.80 ±1.63 b

Data are expressed as mean ± SD (n=10).The different letters refer to significant difference ($p<0.05$) CYP:cypermethrin RE: Rosemary ethanolic extract

Table 3. Serum cholinesterase enzyme in control group and treated groups with Rosemary extract plus CYP in male rabbits.

Groups Parameter	Control 1ml corn oil	CYP (66.5mg/kg)	RE(100mg/kg) plus CYP 66.5mg/kg)	RE(200mg/kg) plus CYP 66.5mg/kg)
Cholinesterase U/L	3058.08±14.4 a	2514.87±52.59 c	2882.1±19.89 b	3026.40±46.73 a

Data are expressed as mean ± SD (n=10). The different letters refer to significant difference ($p< 0.05$) CYP: Cypermethrin RE: Rosemary ethanolic extract

4. Discussions

The current findings revealed that CYP exposure induced oxidative stress, as indicated by elevated MDA and reduced TAC levels. This may result from enhanced lipid peroxidation and depletion of endogenous antioxidant defenses driven by excessive reactive oxygen species (ROS) production and mitochondrial dysfunction. These results are in accordance with previous studies (2, 27-30). CYP also triggered a significant rise in pro-inflammatory cytokines (IL-6 and TNF-α), reflecting activation of the acute inflammatory response and ROS generation, which leads to oxidative damage to cellular structures including lipids, proteins, and DNA. This aligns

with earlier reports (31-33). Additionally, a positive correlation between MDA and inflammatory cytokines, and a negative correlation between TAC and these mediators, indicated a link between oxidative stress and inflammation in CYP-treated animals. Treatment with rosemary extract markedly alleviated oxidative stress and inflammation, likely due to its high levels of phenolic compounds and flavonoids such as carnosol and carnosic acid, which possess potent antioxidant and anti-inflammatory activities. Rosemary extract scavenges ROS, stabilizes cell membranes, and enhances antioxidant defenses. These observations agree with previous studies (16, 17, 34-41). Moreover, the reduction in AChE activity in CYP-treated animals confirms its neurotoxic potential, consistent with earlier findings (42-44). Rosemary extract restored AChE activity, supporting its neuroprotective role likely due to its polyphenolic and diterpene constituents (19, 45-47). Collectively, rosemary extract demonstrated potent antioxidant, anti-inflammatory, and neuroprotective effects against CYP-induced toxicity.

5. Conclusion

The present study demonstrated that *Rosmarinus officinalis* extract exerts significant protective effects against cypermethrin-induced toxicity. These protective effects were evident through reduced lipid peroxidation (MDA), elevated total antioxidant capacity (TAC), suppression of inflammatory cytokines (TNF-α and IL-6), and improved acetylcholinesterase (AChE) activity. The antioxidant, anti-inflammatory, and neuroprotective properties of rosemary may be attributed to its rich content of phenolic compounds and flavonoids. Therefore, rosemary extract may serve as a promising natural therapeutic agent against oxidative stress, inflammation, and neurotoxicity caused by pesticide exposure.

6. Declarations

6.1 Acknowledgments

The authors would like to express their sincere appreciation to the laboratory staff and technical team for their valuable assistance and support throughout this study.

6.2 Ethical Considerations

All experimental procedures involving animals were conducted according to institutional ethical guidelines and approved by the relevant Ethics Committee. This study used the procedures agreed upon by the Standing Committee for Scientific Research Ethics at University of Thi-Qar/ College of Pharmacy.

6.3 Authors' Contributions

Shireen Ali Hasan conceptualized, supervised the study, and collected data, performed experiments, did data analysis and reviewing manuscript. Ahlam A. Al Rikaby helped in computational analysis and was involved in proofreading the manuscript.

6.4 Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

6.5 Fund or Financial Support

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

6.6 Using Artificial Intelligence Tools (AI Tools)

The authors were not utilized AI Tools.

7. Publisher's Note

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