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Evaluation of Resveratrol Protective Effects Against 4 Methylbenzylidene Camphor Induced Thyroid Toxicity in Albino Rats

Evaluación de los Efectos Protectores del Resveratrol contra la Toxicidad Tiroidea Inducida por 4-Metilbencilideno Alcanfor en Ratas Albinas

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SUMMARY: 4-Methylbenzylidene camphor (4-MBC) is a photo-absorbing UV filter prevalently used in cosmetics, which can be absorbed into circulation and cause systemic effects. It is known to act as an endocrine disruptor. Resveratrol is a well characterized antioxidants found in some fruits and plants. This study considers 4-MBC's potential effects on thyroid gland proliferation, inflammation and apoptosis and hormone levels and evaluated the expected protective potential of resveratrol. Thirty adult male albino rats were divided into control, treated (4-MBC), and protected (4-MBC + Resveratrol) groups. Treatments were administered for 35 days. We assessed serum T3, T4, and TNF-α levels, alongside histological and immunohistochemical (Caspase-3) analyses. 4-MBC treatment significantly reduced thyroid hormones (T3, T4) and increased inflammatory (TNF-α) and apoptotic markers (Caspase-3), causing follicular distortion. Co-administration of resveratrol significantly reversed these effects, restoring hormonal levels to near-normal values and preserving thyroid histology. 4-MBC induces thyroid dysfunction, inflammation, and apoptosis. Resveratrol effectively mitigates these alterations, suggesting its potential as a protective agent against environmental endocrine disruptors.

KEY WORDS: 4-MBC; Resveratrol; T3; T4; TNF-a; Caspase-3.

INTRODUCTION

4-Methylbenzylidene camphor (4-MBC), also known as Enzacamene, is commonly used in topically applied personal care products as a UV-B filter and may contribute to the prevention of skin cancer. Concentrations up to 4 % are approved for use in most European countries; however, its use is not permitted in Japan or the United States. Interestingly, 4-MBC has been detected in human urine samples even in the absence of sunscreen application, indicating potential exposure from alternative sources such as plastics, food packaging, and textiles (Murawski *et al*., 2021). As an organic camphor derivative, 4-MBC possesses strong photoprotective properties against UV-B radiation and is considered one of the most widely used UV filters in cosmetic formulations (Badia-Fabregat *et al*., 2012).

4-Methylbenzylidene camphor (4-MBC) is widely recognized as an endocrine disruptor that induces reproductive toxicity and anti-androgenicity. Its mechanisms include the inhibition of spermatogenesis, reduction of plasma 11-ketotestosterone levels, and downregulation of mRNA levels for hormone receptor genes such as hERβ and hERα (Liang *et al*., 2020). Furthermore, 4-MBC has been documented to affect placental and perinatal development. Given these broad endocrine effects, 4-MBC has also been implicated in thyroid disturbance (Yang *et al*., 2019).

4-MBC significantly impacts cell proliferation while inducing oxidative stress and apoptosis (Liang *et al*., 2020). Research suggests that its role as an endocrine disruptor involves interactions with estrogen and progesterone receptors, as well as macrophages, which trigger a systemic inflammatory cascade (Ao *et al*., 2018).

To our knowledge, this work represents the first evaluation specifically dedicated to 4-MBC-induced alterations in thyroid architecture and function. As 4-MBC continues to be discharged into the environment,

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understanding its biological consequences is essential for addressing potential public health risks.

Consequently, there is an increasing demand for a rigorous evaluation of 4-MBC's toxicological profile. This study aims to comprehensively synthesize current knowledge regarding the systemic effects of 4-MBC, with a specific focus on its impact on thyroid gland proliferation, inflammatory responses, apoptotic pathways, and hormonal regulation.

Resveratrol (3,4',5-trihydroxystilbene) is a natural polyphenol found in berries, grapes, peanuts, and other plants, where it acts as a phytoalexin to protect plants against pathogenic attack (Meng *et al*., 2021). Extensive research has highlighted its diverse therapeutic potential, particularly its well-documented antioxidant, anti-inflammatory, and antiproliferative effects (Kursvietiene *et al*., 2016).

Due to its diverse pharmacological profile, resveratrol has gained considerable attention for its potential role in managing chronic diseases such as cancer, diabetes, and cardiovascular disorders. Beyond its use as a dietary supplement, the clinical utility of resveratrol is currently being investigated across several completed and active clinical trials (Salehi *et al*., 2018).

In the context of the thyroid gland, there is currently sparse information regarding resveratrol's effects on glandular dysfunction. While it has been shown to suppress the proliferation of thyroid tumor cell lines (Giuliani *et al*., 2017), its role in addressing other forms of thyroid impairment remains under-explored.

The present work analyzes the impact of 4-MBC on thyroid structure specifically regarding hormonal balance, cellular proliferation, inflammation, and apoptosis with highlighting the expected therapeutic benefits of resveratrol.

MATERIAL AND METHOD

Animals

A total of 30 adult male albino rats, weighing between 150–180 g, were obtained and housed at the animal house of the College of Medicine at Qassim University. The rats were maintained in specific rooms with unrestricted access to standard rodent chow and drinking water. Following a one-week acclimatization period in the cage.

Chemicals and Dosage Preparation

All the chemicals used in this study, including the UV-filter 4-Methylbenzylidene camphor (4-MBC) and Resveratrol were analytical grade and purchased from Sigma-Aldrich Chemical Co. A daily oral dosage of 4-MBC (200 mg/kg body weight) and Resveratrol (100 mg/kg body weight, in 10 % DMSO) by gavage syringe for 35 days were applied in the study (Schlumpf *et al*., 2001; Hadi *et al*., 2022).

Experimental Design and Group Nomenclature

30 Adult male albino rats were categorized into 3 groups (n=10 rats each) as follows:

I. Control Group: subdivided into two subgroups (n= 5 rats each):
- Subgroup A: Rats received standard diet and 0.5 ml of distilled water (DW) orally by gavage syringe for 35 days.
- Subgroup B: Rats received 10 % Dimethyl sulfoxide (DMSO) (5 ml/kg) orally by gavage syringe for 35 days.

II. Treated (4-MBC) Group: Rats received 4-MBC (200 mg/kg body weight) orally by gavage syringe for 35 days.

III. Protected (4-MBC + Resveratrol) Group: Rats received 4-MBC (200 mg/kg body weight) and Resveratrol (100 mg/kg body weight, in 10 % DMSO) orally by gavage syringe for 35 days.

Evaluation Methods

Biochemical Assessments: At the conclusion of the 35 days, blood samples were collected via cardiac puncture from the left ventricle under anesthesia. Serum was immediately extracted and utilized to estimate the levels of tri-iodothyronine (T3), thyroxine (T4), and inflammatory marker tumor necrosis factor alpha (TNF-α).

Histological and Immunohistochemical Assessments

At the conclusion of the experiment, the thyroid glands were gently dissected and processed for light microscopic histological and immunohistochemical analyses. Thyroid specimens were fixed in 10 % neutral buffered formalin, processed, and embedded in paraffin.

A. Histological: 5 µm sections were stained with Hematoxylin and Eosin (H&E) for general morphology assessment.

B. Immunohistochemical Caspase-3 (apoptotic marker): The avidin-biotin complex technique was performed using primary rabbit anti-rat caspase-3 antibody. Positive reaction for caspase 3 was visualized as brown coloration in the cytoplasm and nuclei of follicular epithelium. Negative controls were done using the same steps except that

phosphate buffered saline was applied instead of the primary antibodies (Jackson *et al*., 2008).

Morphometrical Assessments

Ten non-overlapping fields from various areas of each group were used for counting positive mean area percent of Caspase-3 immunohistochemical (x400).

Ethical approval. Ethical clearance for the animal protocols used in this study was granted by the Committee of Research Ethics (Approval No.: 26-13-7), Deanship of Graduate Studies and Scientific Research at Qassim University, Saudi Arabia.

Statistical Assessments. Analysis was done using SPSS 19.0 and formulated as the mean ± SD. ANOVA was employed to ascertain the statistical significance, followed by Tukey's multiple comparison tests. $p \leq 0.05$ was deemed significant.

RESULTS

Biochemical Results

Serum hormonal analysis of tri-iodothyronine (T3), and thyroxine (T4), revealed a highly significant reduction ($P < 0.001$) in the treated group when compared to the control group Meanwhile, protected group illustrated a highly significant rise ($P < 0.001$) compared to the treated group.

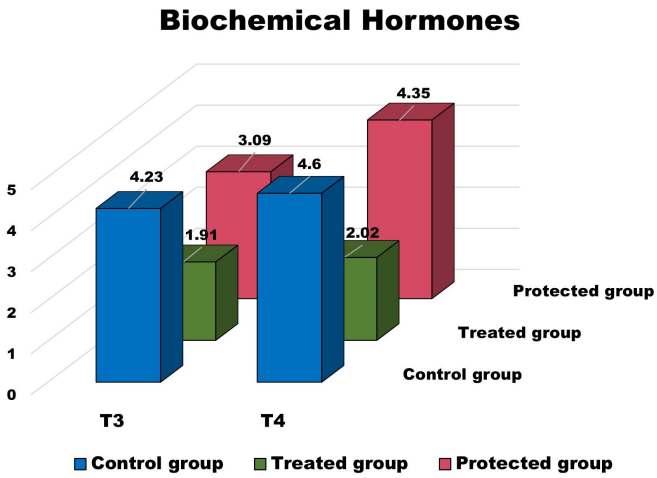

Fig. 1. Biochemical T3, and T4 hormonal analysis among the study groups.

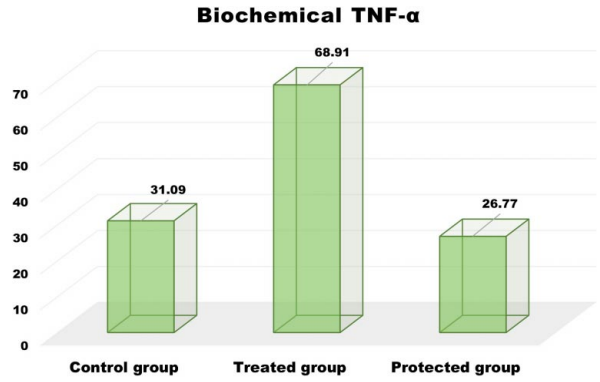

Fig. 2. Biochemical Tumor necrosis factor alpha (TNF-α) in the studied groups.

Table I. Biochemical hormonal analysis among the study groups.

	Control group (n = 10)	Treated group (n = 10)	Protected group (n = 10)	P value
T3				
Mean ±SD	4.23 ±1.25	1.91±0.55	3.09±1.45	<0.001[1]
Range	2.5–6.1	1.1–2.8	1.2–5.4	<0.001[2]
				0.072[3]
T4				
Mean ±SD	4.6 ± 1.3	2.02±0.6	4.35±1.25	<0.001[1]
Range	2.8–6.5	1.15–2.95	2.9–6.2	<0.001[2]
				0.612[3]

1. Comparing treated group vs. control group. 2. Comparing protected group vs. treated group. 3. Comparing protected group vs. control group.

Table II. Biochemical inflammatory marker Tumor necrosis factor alpha (TNF-a) (pg/ml) in the studied groups.

	Control group (n = 10)	Treated group (n = 10)	Protected group (n = 10)	P value
TNF-α				
Mean ±SD	31.09±8.5	68.91±12.1	26.77 ±9.2	<0.001[1]
Range	18.5–44.2	52.1–88.5	15.3–39.8	<0.001[2]
				0.285[3]

1. Comparing treated group vs. control group. 2. Comparing protected group vs. treated group. 3. Comparing protected group vs. control group.

There is no significant variation between protected and control groups (P=0.072, 0.612) in T3, and T4 respectively (Table I, Fig. 1).

Serum analysis of the inflammatory marker, Tumor necrosis factor alpha (TNF-a), demonstrated a highly significant increase ($P < 0.001$) in the treated group when compared to the control group. While, the protected group showed a highly significant decrease ($P < 0.001$) compared to the treated group. There is no significant difference between protected and control groups (P=0.285) (Table II, Fig. 2).

Histological, Immunohistochemical, Morphometrical and Statistical Results

H&E- Sections stained with H&E in the control group, show normal histological architecture of thyroid glands. They consist of thyroid follicles lined with cuboidal follicular epithelium with rounded nuclei and filled with acidophilic colloid material with peripheral small vacuoles. There are notable interfollicular cells between the follicles (Fig. 3A).

H&E sections of the treated group show most follicles with a notable decline in colloidal material, others appear with empty lumen. The thyroid follicles illustrate exfoliation, distortion, and vacuolation. A congested blood vessel is also noted (Fig. 3B).

Sections stained with H&E in the protected group reveal restoration of the normal thyroid follicles structure. However, there are minimal follicles that still distorted; others appear with empty lumen (Fig. 3C).

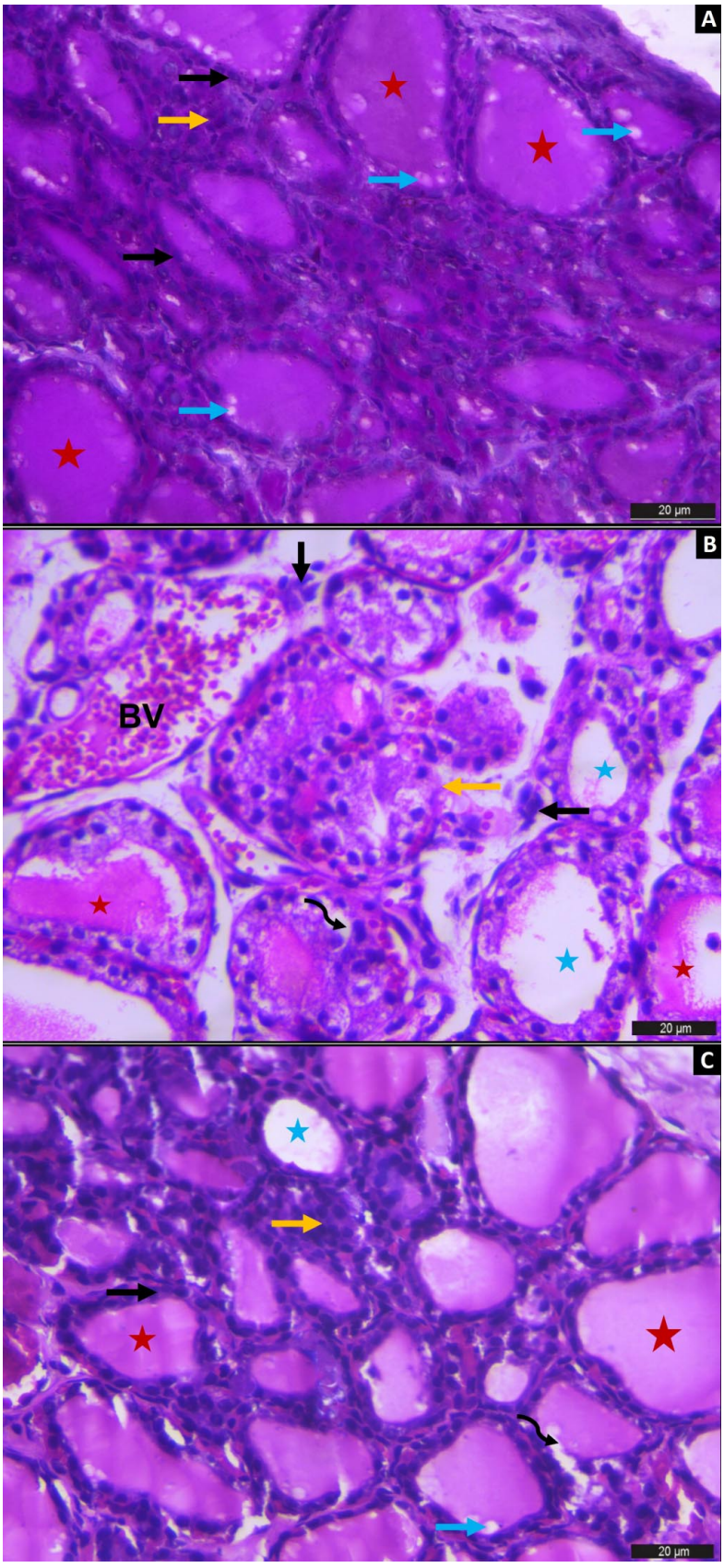

Fig. 3. Histopathological H&E staining sections of the thyroid gland (x400). A. Control group reveals normal thyroid follicles filled with acidophilic colloid material (red star) with peripheral small vacuoles (blue arrow). The follicles lined with cuboidal follicular epithelium with rounded nuclei (black arrow). Note; Interfollicular cells (yellow arrow) in-between the follicles. B. Treated group shows most follicles appear with an obvious decline in colloidal material (red star), others appear with empty lumen (blue star). There is notable exfoliated follicular epithelium (black arrow), distorted follicles (yellow arrow), and follicular vacuolation (arched arrow). A congested blood vessel (BV) is also observed. C. Protected group illustrates restoration of the normal thyroid follicles that lined with cuboidal follicular epithelium (black arrow) with rounded nuclei and filled with acidophilic colloid material (red star) with peripheral small vacuoles (blue arrow). Interfollicular cells (yellow arrow) can be seen in-between the follicles. There are minimal follicles that still appear distorted (arched arrow) others appear with empty lumen (blue star).

Immunohistochemical Caspase-3

Control group reveals a negative Caspase-3 expression in the cytoplasm and nuclei of follicular epithelium (Fig. 4A). While, the treated group demonstrates a strong positive cytoplasmic and nuclear reaction (Fig. 4B). The protected group demonstrates a very mild positive cytoplasmic and nuclear reaction (Fig. 4C).

Quantitative analysis of the mean area percent of positive immunoreactive Caspase-3 cells revealed a notable high significant elevation ($P < 0.001$) in the treated group when compared to the control group. However, the protected group displayed a high significant reduction ($P < 0.001$) compared to the treated group. There is no significant difference between protected and control groups (P=0.284) (Table III, Fig. 4D).

Table III. Mean area percent of immunohistochemical Caspase-3 in the studied groups.

	Control group (n = 10)	**Treated group** (n = 10)	**Protected group** (n = 10)	**P value**
Caspase-3				
Mean ±SD	6.1±2.1	24.7±5.5	9.7±2.5	<0.001[1]
Range	3.2–9.5	15.2–34.2	3.8–11	<0.001[2]
				0.284[3]

1. Comparing treated group vs. control group. 2. Comparing protected group vs. treated group. 3. Comparing protected group vs. control group.

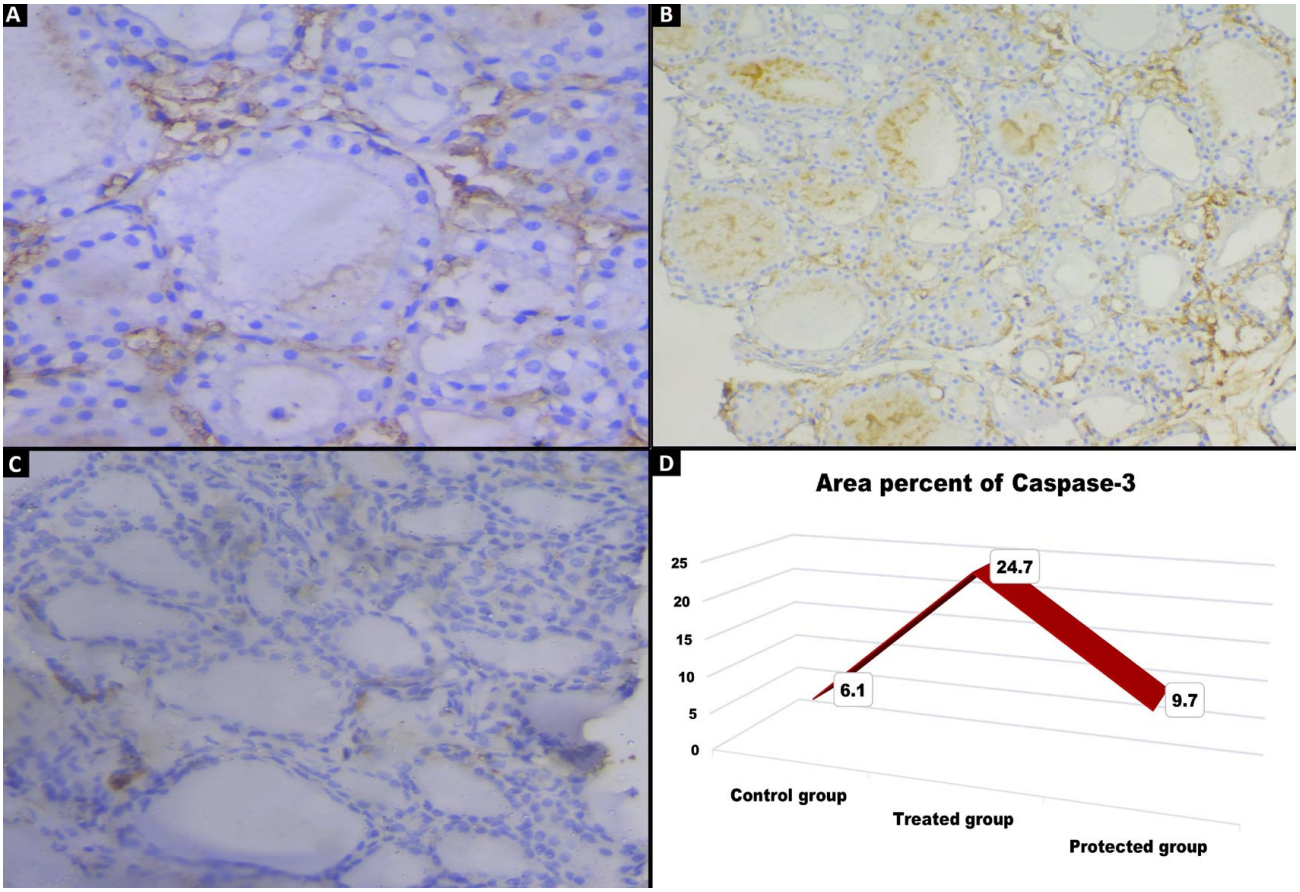

Fig. 4. Immunohistochemical staining for apoptotic marker Caspase-3 (x400). A. Control group reveals a negative Caspase-3 expression in the cytoplasm and nuclei of follicular epithelium. B. Treated group demonstrates a strong positive cytoplasmic and nuclear reaction. C. Protected group demonstrates a very mild positive cytoplasmic and nuclear reaction. D. Mean area percent of Caspase-3 in the studied groups.

DISCUSSION

The present study investigated the potential thyroid-disturbing effects of 4-MBC and evaluated the protective role of resveratrol in an albino rat model. Our findings demonstrate that 4-MBC significantly impairs thyroid function and structure, while resveratrol administration offers substantial protection against these alterations.

4-Methylbenzylidene camphor is a widely used UV filter in personal care products. Current results showed a significant reduction in serum T3 and T4 levels in rats treated with 4-MBC. This aligns with previous reports identifying 4-MBC as an endocrine disruptor (Murawski *et al*., 2021). The reduction in thyroid hormones suggests that 4-MBC may interfere with the hypothalamic-pituitary-thyroid (HPT) axis or directly inhibit the synthetic machinery within thyroid follicles. Morphological observations supported this, showing distorted follicles, exfoliation of the follicular epithelium, and a marked decline in colloidal material (Murawski *et al*., 2021). In contrast to the current finding, Ao *et al*. (2018) demonstrated that 4-MBC has thyroid hormone regulation effect.

We observed a significant elevation in TNF-a levels in the 4-MBC treated group. TNF-a is a primary pro-inflammatory cytokine that can exacerbate tissue damage. The increase in TNF-a suggests that 4-MBC induces a systemic or localized inflammatory response within the thyroid gland. This is consistent with studies by Ao *et al*. (2018), which suggested that organic UV filters could trigger inflammatory cascades in human macrophages.

The immunohistochemical results revealed a strong positive reaction for Caspase-3 in the 4-MBC treated group, indicating a high rate of programmed cell death (apoptosis) in the follicular epithelium. The induction of oxidative stress by 4-MBC is a likely mechanism for this apoptosis, as reactive oxygen species (ROS) are known to activate the caspase cascade (Yang *et al*., 2019).

Moreover, Lax *et al*. (2023), showing interesting effects of 4-MBC on cell proliferation, invasion, induction of apoptosis and production of reactive oxygen species.

The most significant finding of this study is the restorative effect of resveratrol. Rats co-administered with resveratrol showed serum T3, T4, and TNF-a levels that were nearly identical to the control group. Furthermore, the histological architecture was largely preserved, and Caspase-3 expression was significantly reduced.

Resveratrol's protective effects are likely due to its well-documented antioxidant and anti-inflammatory properties (Kursvietiene *et al*., 2016). By scavenging ROS and inhibiting pro-inflammatory cytokines like TNF-α, resveratrol stabilizes the thyroid follicular environment. Moreover, Ray *et al*. (2020) confirmed that resveratrol is a well characterized antioxidant that can allevi-ate nonylphenol induced oxidative stress and restore antioxidant enzymes level.

Interestingly, while some studies suggest resveratrol can have its own anti-thyroid effects at high doses (Giuliani *et al*., 2017), our study demonstrates that at a dosage of 100 mg/kg, it acts as a potent protective agent against exogenous chemical disruptors like 4-MBC.

CONCLUSION

4-MBC acts as a potent thyroid disruptor by reducing hormone levels, inducing inflammation, and triggering apoptosis. Resveratrol effectively mitigates these toxic effects, suggesting its potential as a dietary supplement to protect against environmental endocrine disruptors.

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SALAMA, R.M. & ABOUKHALIL, R.E. Evaluación de los efectos protectores del resveratrol contra la toxicidad tiroidea inducida por 4-metilbencilideno alcanfor en ratas albinas. *Int. J. Morphol., 44(3)*:766-772, 2026.

RESUMEN: El 4-metilbencilideno alcanfor (4-MBC) es un filtro UV foto absorbente de uso común en cosméticos, que puede ser absorbido por el torrente sanguíneo y causar efectos sistémicos. Se sabe que actúa como disruptor endocrino. El resveratrol es un antioxidante bien caracterizado que se encuentra en algunas frutas y plantas. Este estudio considera los posibles efectos del 4-MBC sobre la proliferación, la inflamación y la apoptosis de la glándula tiroides, así como sobre los niveles hormonales, y evalúa el potencial protector esperado del resveratrol. Treinta ratas albinas macho adultas se dividieron en grupos control, tratado (4-MBC) y protegido (4-MBC + Resveratrol). Los tratamientos se administraron durante 35 días. Evaluamos los niveles séricos de T3, T4 y TNF-α, junto con análisis histológicos e inmunohistoquímicos (Caspasa-3). El tratamiento con 4-MBC redujo significativamente las hormonas tiroideas (T3, T4) e incrementó los marcadores inflamatorios (TNF-α) y apoptóticos (Caspasa-3), causando distorsión folicular. La coadministración de resveratrol revirtió significativamente estos efectos, restaurando los niveles hormonales a valores casi normales y preservando la histología tiroidea. El 4-MBC induce disfunción tiroidea, inflamación y apoptosis. El resveratrol atenúa eficazmente estas

alteraciones, lo que sugiere su potencial como agente protector contra los disruptores endocrinos ambientales.

PALABRAS CLAVE: 4-MBC; Resveratrol; T3; T4; TNF-α; Caspasa-3.

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