Protective effect of pumpkin extract on oxidative stress induced by Amiodarone in hypothyroid rats

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Abstract

This work aimed to study the protective effect of pumpkin plant extract against oxidative stress induced by amiodarone in male rats with hypothyroidism. The experiment was conducted at the College of Education for Women, University of Anbar, starting in December 2024 and lasted for 30 days. A total of 40 adult male white rats were randomly allocated into five categories, each one consisting of 8 pets, and were selected based on their similar weights. Following the conclusion of the acclimatization duration, which spanned one week, the rats had therapy dosed orally by tube feeding daily for 30 days. The therapies administered were tailored to the specific requirements of the experiment: (G1) (Negative group), healthy control group, (G2): (Positive group), treated with Amiodarone orally (5 mg/kg), (G3): treated with pumpkin plant extract (200 mg/kg) and amiodarone (400 mg/kg), (G4) treated with pumpkin plant extract (300 mg/kg) and amiodarone (400 mg/kg), (G5): treated with pumpkin plant extract (400 mg/kg) and amiodarone (400 mg/kg), Measurements include T3, T4, TSH, MDA measurement by ELISA kit, and with a histological examination of the thyroid gland. T3 levels recorded (0.949 ±0.02), (0.742 ±0.09), (0.850 ±0.04), (0.836 ±0.02), and (0.846 ±0.05) ng/dl, T4 levels were 1.951 ±0.22, 2.412 ±0.10, 1.320 ±0.31, 2.125 ±0.09, and 2.316 ±0.12 µg/dl, TSH levels were 0.275 ±0.04, 0.154 ±0.01, 0.209 ±0.03, 0.245 ±0.05, and 0.268 ±0.06 (µIU/ml). MDA levels were 41.73 ±4.50, 60.31 ±4.53, 47.87 ±6.28, 49.21 ±8.67, 48.46 ±3.82 µmol/, for G1 to G5 respectively. Regarding the weights of the animals before and after treatment, the results indicate the presence of significant differences at the probability level. The results showed that amiodarone caused a decrease in T3 and TSH levels, and an increase in T4 and oxidative stress (MDA) levels. In contrast, pumpkin extract helped gradually improve T3 and TSH levels and reduced MDA, especially at higher doses. Results indicate that pumpkin extract possesses antioxidant and thyroid-modulating properties, making it a promising candidate for mitigating amiodarone-induced oxidative toxicity in hypothyroidism.

Keywords: Thyroid gland, Amiodarone, Pumpkin extract, ELISA kits

Introduction

Hypothyroidism is a condition in which the body lacks sufficient thyroid hormone. The thyroid hormone plays a fundamental role in the development, differentiation, proliferation and physiology of all cells in an organism [1, 2]. Therefore, inadequate thyroid hormone has widespread consequences for the body. Some studies have shown that hypothyroidism may induce delayed skeletal development, cardiovascular diseases, secondary hypertension , deterioration of human reproductive health, and changes in the brain structure and function[3]. One of the common hallmarks of hypothyroidism is increased oxidative stress, which arises due to an imbalance between the production of reactive oxygen species (ROS) and the body's

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antioxidant defense mechanisms [4, 5]. Amiodarone (AMD) is a class III effective antiarrhythmic agent with non-competitive α- and β adrenergic blocker and multichannel blocker properties. Due to its little negative inotropic impact, it is frequently utilized, even in heart failure cases [6]. AMD has numerous impacts on physiology of the thyroid and peripheral metabolism of thyroid hormones. In fact, greater than 50% of patients receiving prolonged treatment of AMD have abnormal thyroid function test results. The pathogenesis of AMD is multifactorial, and may be related to its lipophilic nature, presence of 2 iodine atoms, a high propensity for deposition in various tissues, high bio-distribution potential, and long halflife in plasma (13–30 days) [7.[

Moreover, amiodarone enhances lipid peroxidation and promotes the formation of free radicals, leading to cellular and tissue damage, particularly in sensitive endocrine organs like the thyroid gland[8.[

Natural antioxidants derived from plant sources have garnered increasing attention as potential therapeutic agents to combat oxidative damage. Pumpkin (Cucurbit maxima), in particular, is rich in bioactive compounds such as flavonoids, phenolic acids, vitamins (notably vitamin E and beta-carotene), and essential minerals, all of which contribute to its potent antioxidant and anti-inflammatory properties[9]. These components make pumpkin extract a promising candidate for protecting against drug-induced oxidative stress and maintaining thyroid gland integrity[10, 11 .[

This study aims to evaluate the protective role of pumpkin extract against oxidative stress induced by amiodarone in a rat model of hypothyroidism. Histological changes in the thyroid gland were assessed alongside biochemical markers including serum levels of T3, T4, and TSH, in addition to malondialdehyde (MDA) as an indicator of lipid peroxidation. By investigating both tissue and biochemical parameters, this research provides a comprehensive view of the extent of damage induced by amiodarone and the ameliorative effects of pumpkin extract.

Material and Methods

Plant Material

The study was conducted at the Scientific Technology Center, University of Nahrain. The seeds of Cucurbita maxima were collected from local market of Erbil city, the plant has been classified in the Natural Museum of Plant Taxonomy.

Extract Preparation

The seeds were extracted from the residual plant matter and permitted to dry at room temperature. After drying, the seeds were processed in a mechanical grinder to obtain a consistent powder. Each 20 g sample was measured, and 200 ml of ethanol was added to it. Their maceration was permitted for 24 hours in a shaker at room temperature. The materials underwent further filtration utilizing grade 1 Whatman paper. The ethanol was removed via evaporation at room temperature beneath a fume hood. The resulting extracts were retained for future study [12 .[

Animal

A study was conducted on 40 male rats weighing between 180 and 220 gm at the Animal facility of the College of Education for women, Anbar University,. The temperature was maintained at a constant level of 24 ± 1 °C, and there was a consistent 12-hour cycle of light and darkness. In addition, rats were provided with complimentary access to water and regular food. The rats were housed in a laboratory setting for a period of one week in order to acclimatize them to the conditions of the laboratory.

Design of experiment

A total of 40 adult male white rats were randomly allocated into five categories, each one consisting of 8 pets, and were selected

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based on their similar weights. Following the conclusion of the acclimatization duration, which spanned one week, the rats had therapy dosed orally by tube feeding daily for 30 days. The therapies administered were tailored to the specific requirements of the experiment:

Group 1 (G1): (Negative group), healthy control group .

Group 2 (G2): (Positive group), this groups were treated with Amiodarone orally (5 mg/kg) of body weight daily using a rat gavage device for 30 days.

Group 3 (G3): This group was treated with pumpkin plant extract (200 mg/kg) and amiodarone (400 mg/kg) orally concurrently in a single dose daily for 30 days..

Group 4 (G4): This group was treated with pumpkin plant extract (300 mg/kg) and amiodarone (400 mg/kg) orally concurrently in a single dose daily for 30 days

Group 5 (G5): This group was treated with pumpkin plant extract (400 mg/kg) and amiodarone (400 mg/kg) orally concurrently in a single dose daily for 30 days

2.5Laboratory analyses

2.5.1Blood sample collection

After the end of the experiment, the animals were transferred to the College of Education for women, Anbar University, for the purpose of completing the procedures for withdrawing blood and dissecting the animals to obtain the organs required in the experiment. The transfer process was carried out is 30 days, which is the Orally dosing period. The animals were weighed after the experiment and then anesthetized by using of Chloroform anesthetic. After ensuring that the animals were anesthetized, blood was withdrawn directly from the heart by heart puncture using a sterile medical 5 ml syringe to obtain the largest amount of blood. The blood samples were placed directly in sterile test tubes free of anticoagulant Gel tubes, and then the tubes were transferred to a centrifuge at a speed of 3000 rpm for 15 minutes to obtain the serum is transferred to small Eppendorf tubes and clean, dry, labeled tubes. The serum is stored in the refrigerator at a low temperature of -20°C until the biochemical and histopathological tests are performed .

2.6Biochemical assays

Biochemical test T3, T4, TSH, and MDA measurement by ELISA kit manscripit (SunLong-China) based on Sandwich – ELISA kit.

2.7Histological preparations

After anaesthetizing the animals and taking blood samples, microscopic slides were prepared based on the method of [13], where the animal was placed in the dissecting tank, and its limbs were fixed with pins. An incision was made using sharp scissors in the shape of an inverted letter "T" from the beginning of the abdominal cavity to the end of the upper sternum. Using a scalpel, the internal organs were removed, which included (the liver and kidneys(. Then, the organs to be studied (Thyroid gland) were placed in a petri dish containing 0.9% physiological saline (NaCl) for the purpose of washing; then they were transferred to the fixative solution (10% formalin) for (24) days. The samples were then passed through a series of steps [13.[

2.8Ethics approval

The Experimental protocols and animal usage (protocol number CHREC/11/2022) have been authorized by the Institutional Animal Ethics Committee, and all procedures adhered strictly to the ethical standards established by Anbar University.

2.9Statistical analysis

Results are presented as Mean ± SD of twelve distinct samples, each duplicated. The

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Statistical Analysis Systems (SAS, 2019) software was employed for data analysis [14 .[

Results and Discussion

The study was conducted on five groups of rats to evaluate the effect of amiodarone and the effectiveness of pumpkin extract in mitigating amiodarone-induced disturbances in thyroid hormone levels. The experiment included a healthy group (Control-), a group treated with amiodarone alone (Control+), and three groups treated with a fixed dose of amiodarone (400 mg/kg) and pumpkin extract at different doses (200, 300, and 400 mg/kg.(

The results showed that the group treated with amiodarone alone (Control +) experienced a significant decrease in T3 levels compared to the control group (Control -), with the mean T3 level in the affected group being 0.742 ± 0.09 ng/dl compared to 0.949 ± 0.02 ng/dl in the control group. This decrease suggests that amiodarone inhibits the conversion of T4 to T3, an effect well-known and documented in previous studies[15, 16.[

In contrast, no significant change in T4 levels was observed between the two groups, supporting the hypothesis that the primary effect of amiodarone lies in inhibiting peripheral conversion rather than reducing thyroid hormone secretion. As for the TSH hormone, the affected group recorded an increase in its level compared to the healthy group (0.275 ± 0.04 versus 0.154 ± 0.01 µIU/ml), but this increase was not statistically significant, indicating that the response of the pituitary-thyroid axis had not reached the stage of full compensation.

The three groups treated with the combination of amiodarone and pumpkin extract showed gradual improvements in hormone levels compared to the control group. In the Plant200 group, T3 (0.850 ± 0.04 ng/dl) and TSH (0.209 ± 0.03 µIU/ml) showed slight improvements, while T4 decreased significantly (1.320 ± 0.31 µg/dl) compared to the other groups, indicating that the lower dose of the extract was not sufficient to restore hormonal balance.

In the Plant300 and Plant400 groups, T3 levels improved significantly, and T4 levels rose to near normal levels. In the Plant300 group, T4 levels were recorded at 2.125 ± 0.09 µg/dl, while in the Plant400 group, T4 levels were recorded at 2.316 ± 0.12 µg/dl, very close to the control group. This positive effect is attributed to the pumpkin extract containing natural antioxidants such as beta-carotene and flavonoids, which contribute to reducing oxidative stress caused by amiodarone and maintaining the integrity of thyroid cells [17, 18 .[

Statistical analyses showed significant differences between groups in T3 and T4 levels at a significance level ($P \leq 0.05$), while no significant differences were found in TSH levels, indicating that pumpkin extract had a direct effect on restoring T3 and T4 hormonal balance without significantly affecting the hypothalamic-pituitary-thyroid axis during the study period.

ISSN 2072-3857

Table 1: Comparison between difference groups in Thyroid hormones level

Group	Mean ±SE		
	T3 (ng/dl)	T4 (µg/dl)	TSH (µIU/ml)
G1	0.949 ±0.02 a	1.951 ±0.22 a	0.275 ±0.04
G2	0.742 ±0.09 b	2.412 ±0.10 a	0.154 ±0.01
G3	0.850 ±0.04 ab	1.320 ±0.31 b	0.209 ±0.03
G4	0.836 ±0.02 ab	2.125 ±0.09 a	0.245 ±0.05
G5	0.846 ±0.05 ab	2.316 ±0.12 a	0.268 ±0.06
L.S.D.	0.154 *	0.551 *	0.122 NS

Means having with the different letters in same column differed significantly. * (P≤0.05),

Malondialdehyde (MDA) is one of the most important biomarkers used to assess the degree of oxidative stress within cells, as it is an end product of lipid breakdown via lipid peroxidation[19]. Elevated levels of MDA indicate cellular damage caused by free radicals, making it an important tool for assessing the oxidative status of tissues. Table (2) showed a comparison between the different groups in terms of the average level of MDA in blood serum (µmol/l), the results showed that the group of rats treated with amiodarone alone (Control+) recorded the highest mean MDA levels (60.31 ± 4.53 µmol/l), with a significant difference (P≤0.05) compared to control group (Control -) (41.73 ±4.50 µmol/l). This indicates that amiodarone caused a significant increase in oxidative stress by stimulating lipid peroxidation in cell membranes. This negative effect is expected and scientifically known, as studies indicate that amiodarone, despite its antiarrhythmic properties, can cause excessive production of free radicals (ROS) and consequently oxidative damage in various tissues, including the liver and thyroid[20.[

The Plant 200 group (200 mg/kg pumpkin with amiodarone) recorded an average MDA level of 47.87 ± 6.28 µmol/l, which was lower than the (Control+) group treated with amiodarone alone, but still higher than the healthy group . This suggests that the 200 mg/kg pumpkin extract began to show an antioxidant effect and helped reduce oxidative damage to some extent, but not enough to reach normal levels. Presumably, the dose was relatively low and not effective enough. In the Plant 300 group (300 mg/kg pumpkin with 400 mg/kg amiodarone), the MDA level was 49.21 ± 8.67 µmol/l, indicating a slight improvement compared to the disease-only group. Increasing the pumpkin dose appears to have improved oxidative status to some extent, but the statistical difference was not significant between this group and the group receiving a lower dose, indicating a limited response with increasing dose in this group. Stage. In the Plant400 group (400 mg/kg pumpkin with 400 mg/kg amiodarone), this group recorded an MDA level of (48.46 ± 3.82 µmol/l), which is close to the results of Plant200 and Plant300. The lack of a significant difference between the three groups receiving pumpkin extract indicates that the protective effect of the antioxidants present in pumpkin has reached a stage of equilibrium or "saturation," where increasing the dose did not lead to a significant additional improvement in reducing oxidative stress.

ISSN 2072-3857

Table 2. Comparison between difference groups in MDA

Group	Mean ±SE of MDA (µmol/l)
G1	41.73 ±4.50 b
G2	60.31 ±4.53 a
G3	47.87 ±6.28 ab
G4	49.21 ±8.67 ab
G5	48.46 ±3.82 ab
L.S.D.	16.753 *

*Means having with the different letters in same column differed significantly. (P≤0.05),

Monitoring animal weights in experimental studies is an important biomarker used to evaluate the physiological effects of drugs or plant extracts, particularly with regard to toxicity, appetite, metabolism, and hormonal balance. In this study, the weights of rats were analyzed before and after treatment with amiodarone, with or without pumpkin extract, to determine the potential effects of these substances on body mass over the 30-day experimental period.

The results showed that the animals' weights before the start of treatment did not differ significantly between all groups (L.S.D. = 19.172 NS), indicating that the randomization was balanced and that there were no initial differences that might have affected the treatment outcomes. This observation enhances the accuracy of the comparison at the end of the experiment and supports the strength of the results.

When comparing weights after the treatment period, a significant difference was found between groups (L.S.D. = 21.065*, P≤0.05), indicating that the type of treatment had a real impact on weight changes. The group treated with amiodarone alone (Control+) recorded the greatest weight gain compared to the other groups, with the mean weight increasing from 206.25 to 233.13 grams, a significant increase. This increase may seem unexpected, as amiodarone is known to have potentially negative effects on certain vital functions, such as the liver and thyroid. One of the most prominent known effects of amiodarone is its effect on the thyroid gland [21]. From the results of our study, we note that the Control+ group recorded lower TSH (0.154 ± 0.01 µIU/ml) and T3

Higher T4 compared to some other groups. These changes suggest a condition similar to hyperthyroidism at first, which may later develop into mild hypothyroidism with continued use. In some cases, particularly with chronic amiodarone use, long-term inhibition of T4 to T3 deiodination occurs, disrupting hormonal balance and affecting metabolism. In cases of mild hypothyroidism, the basal metabolic rate decreases, leading to fat accumulation and gradual weight gain, even if food intake does not increase [22]. This may partly explain the weight gain in rats. It is noteworthy that some studies indicate that rats treated with amiodarone exhibit decreased motor activity due to general fatigue or central nervous system effects. Decreased movement means decreased energy expenditure, and therefore, even with the same food intake, the body begins to store excess energy as fat or fluid, leading to weight gain[23 .[

Although evidence is limited, some studies suggest that amiodarone may increase appetite

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in some animal models due to hormonal disturbances or effects on the appetite center in the brain, contributing to weight gain. However, some studies, such as Miller et al. (1982), suggest that amiodarone may cause weight gain due to its effect on fluid retention or modification of certain thyroid-related metabolic pathways[24, 25]. The control group (Control-) had a minor weight reduction, decreasing from 220.62 to 214.37 g, which was not statistically significant and is probably attributable to typical fluctuations in appetite or physical activity over the trial period. The cohorts with the combination of amiodarone and pumpkin extract exhibited notable enhancements in weight stability. The Plant200 group rose from 208.12 to 221.87 g, whilst the Plant400 group experienced a modest increase from 203.75 to 215.00 g. The results indicate that pumpkin extract mitigated the adverse metabolic effects of amiodarone, offered substantial nutritional support to the treated animals, and may have facilitated increased appetite or accelerated digestion. Conversely, the Plant300 group exhibited the lowest post-treatment weight (210.62 g), albeit its increase from the beginning weight (199.37 g .(

Nonetheless, this rise was not substantial in comparison to the other plant-treated groups. This outcome may be attributed to the preventive effect of pumpkin extract achieving equilibrium at this dosage, or to the body's response to the intermediate dosage being less effective than at previous levels. These results affirm that pumpkin extract positively contributes to the maintenance and stabilisation of body weight when administered alongside amiodarone, indicating its potential as a natural supplement that mitigates the metabolic adverse effects linked to some drugs with oxidative toxicity.

Table 3: Comparison between difference groups in animal weights

Group	Mean ±SE (gm)	
	Before treated	**After treated**
G1	206.25 ±6.66 ab	233.13 ±11.68 a
G2	220.62 ±6.43 a	214.37 ±9.84 ab
G3	208.12 ±6.81 ab	221.87 ±10.43 ab
G4	199.37 ±4.76 b	210.62 ±8.68 b
G5	203.75 ±5.73 ab	215.00 ±6.26 ab
L.S.D.	19.172 NS	21.065 *

*Means having with the different letters in same column differed significantly. (P≤0.05).

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Histopathological features

The results of histological examination of the thyroid gland of rat treated with five types of treatments showed that in G1, follicles appeared normal, filled with eosinophilic colloid (CO) and lined with epithelial cells (EC). While in G2, some follicles filled with eosinophilic colloid (SCO) and lined with hyperplastic epithelial cells (HEC), congestion (CO), necrosis (NC), degeneration (DE), and dilated irregular follicles (DF) appeared. In G3, some follicles filled with eosinophilic colloid (SCO) and lined with hyperplastic epithelial cells (HEC), and ectopic thymic tissue (ET) appeared. Furthermore, in G4, some follicles filled with eosinophilic colloid (SCO) and lined with hyperplastic epithelial cells (HEC) appeared. G5 showed some follicles filled with eosinophilic colloid (SCO) and lined with hyperplastic epithelial cells (HEC.(

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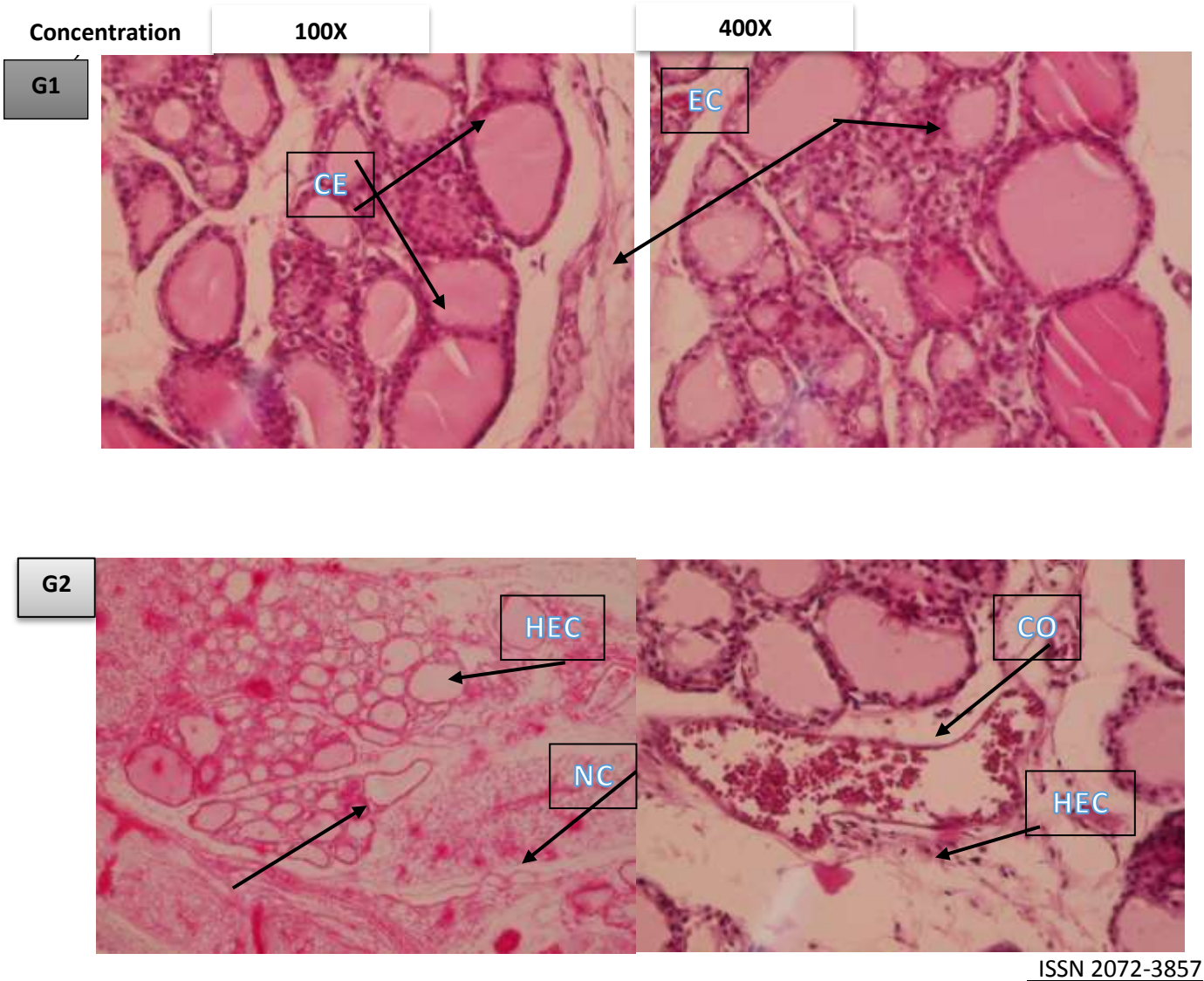

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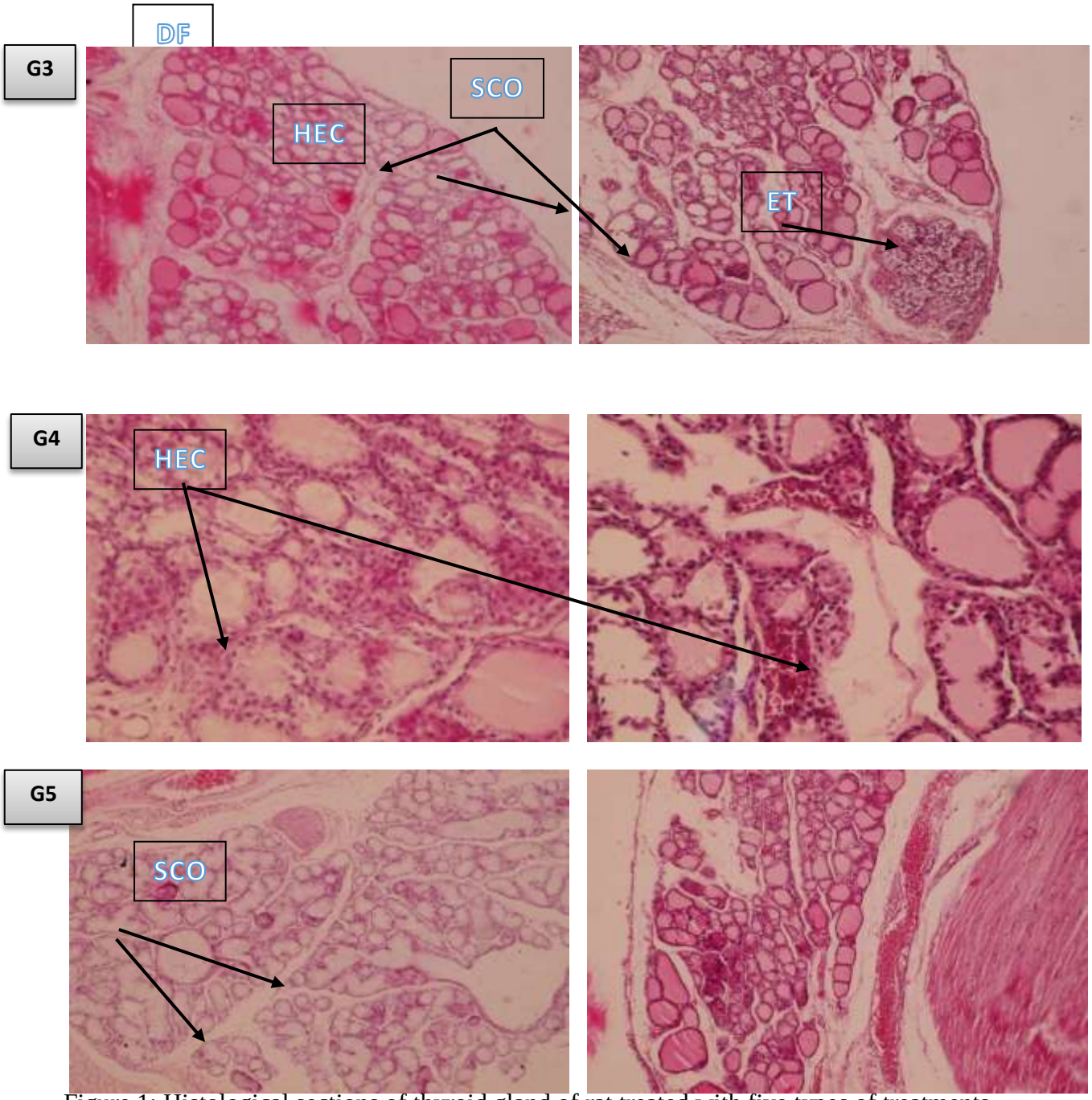

Figure 1: Histological sections of thyroid gland of rat treated with five types of treatments

The results show that the thyroid gland in the negative group showed a completely normal structure, with follicles filled with natural eosinophil colloid (CO) and lined with regular epithelial cells (EC). In the positive group treated with Amiodarone, we observed clear tissue damage represented by tumors, necrosis, and cell expansion. These changes are attributed to the toxic effect of Amiodarone on the thyroid gland, which is expected because it contains a high percentage of iodine, and it may lead to a disruption in the production of thyroid hormones, and drug-induced thyroiditis[26-28]. According to the findings of El-Sayed et al. (2007), a therapeutic dose of amiodarone causes thyroid acini to have an

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uneven shape and size, as well as small cystic follicles that contain a negligible amount of colloid. Columnar epithelium makes up the lining, and there is only a small quantity of cytoplasm and vesicular nuclei here and there [29]. On the other hand, when the thyroid follicles were exposed to a hazardous dose of amiodarone, a significantly elevated level of epithelial hyperplasia was seen. This was because the thyroid follicles were lined by stratified columnar epithelium. The lumen of several of the follicles was closed, and there was no evidence of the presence of colloid. Fibrous in nature. The results of this study are also consistent with the study Rasheed et al., 2018 which indicated a toxic effect of amiodarone on the thyroid gland of rats[30.[

In the G3 treatment group (Amiodarone + Pumpkin extract 200 mg/kg), we observed the same congestion and histological changes as in the positive group, but they were less severe due to the protective effect of the extract used. In the G4 (Amiodarone + Pumpkin extract 300 mg/kg), as the extract concentration increased, necrosis began to decrease, with the absence of congestion due to the extract's effect. This indicates that the higher dose of pumpkin extract began to exhibit an antioxidant and anti-inflammatory effect that prevented tissue deterioration. In the G5 group (Amiodarone + Pumpkin extract 400 mg/kg), the changes were mild and similar to those in G4: SCO and HEC only, with no necrotic manifestations, damage, or abnormal expansion. This suggests that the higher dose of pumpkin extract was more effective in protecting the thyroid from the toxic effects of the drug, perhaps due to its richness in compounds such as beta-carotene, flavonoids, and antioxidants, In a study Ekpono et al., 2024 it was indicated that pumpkin seed oil (C.maxima) has the ability to reduce oxidative stress caused by the drug tramadol in the liver, kidney, and brain of mice. This indicates that pumpkin seed oil may have a protective effect against oxidative damage that may be caused by some drugs [31.[

Conclusion

The results showed that treatment with amiodarone alone resulted in a significant decrease in T3 and T4 levels, and an increase in TSH and MDA, indicating a clear dysfunction of the thyroid gland accompanied by high oxidative stress. In contrast, the groups treated with pumpkin extract showed a significant improvement in thyroid hormone levels and a decrease in MDA.

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CONFLICTS OF INTEREST

The author declares no conflict of interest

References

[1] Al- Hassan, S., Attia, H., Alomar, H., Arafa, M., & Ali, R. A. (2021). The inhibitory mechanisms of losartan and vitamin D on amiodarone- induced lung inflammation in rats: Role of mitogen- activated protein kinases/activator protein- 1. Journal of biochemical and molecular toxicology, 35(12), e22923. https://doi.org/10.1002/jbt.22923

[2] Aldakhoul, M., Sleman, S., Alragheb, N., Alfarwan, M., Alallan, L., Shubber, Z. I., & Thallaj, N. (2024). Phytochemical Composition and Health Benefits of Pumpkin. Research Journal of Pharmacy and Technology, 17(10), 4915-4921. DOI:10.52711/0974-360X.2024.00756

[3] Alqahtani, N. K. (2025). Bioactive compounds and nutritional composition of butternut pumpkins (Cucurbita moschata) cultivated in Saudi Arabia (Hassawi Popper). Journal of Future Foods. https://doi.org/10.1016/j.jfutfo.2024.08.015

[4] Amirahmadi, S., Hosseini, M., Ahmadabady, S., Akbarian, M., Abrari, K., Vafaee, F., & Rajabian, A. (2021). Folic acid attenuated learning and memory impairment via inhibition of oxidative damage and acetylcholinesterase activity in hypothyroid rats. Metabolic brain disease, 36, 2393-2403. https://doi.org/10.1007/s11011-021-00815-3

[5] Anfinsen, O.-G., & Lima, K. (2021). Amiodarone-induced thyrotoxicosis. Tidsskrift for Den norske legeforening. doi: 10.4045/tidsskr.21.0047

[6] Bozbek, Ç., & Şentürk, M. (2023). Effect of levothyroxine administration on oxidative stress and cytokine levels in rats with experimental hypothyroidism. Journal of the Hellenic Veterinary Medical Society, 74(1), 5227-5234. https://doi.org/10.12681/jhvms.28743

[7] Caili, F., Huan, S., & Quanhong, L. (2006). A review on pharmacological activities and utilization technologies of pumpkin. Plant Foods for Human Nutrition, 61, 70-77. https://doi.org/10.1007/s11130-006-0016-6

[8] Dammacco, R., Guerriero, S., Cardia, G., Alessio, G., Vacca, A., & Dammacco, F. (2025). Amiodarone-induced ocular and extra-ocular toxicity: a retrospective cohort study. Clinical and Experimental Medicine, 25(1), 1-11. https://doi.org/10.1007/s10238-025-01569-3

[9] de Lima Conceição, M. R., Teixeira-Fonseca, J. L., Marques, L. P., Souza, D. S., & Roman-Campos, D. (2023). Interaction of the antiarrhythmic drug Amiodarone with the sodium channel Nav1. 5 depends on the extracellular pH. European Journal of Pharmacology, 960, 176127. https://doi.org/10.1016/j.ejphar.2023.176127

[10] dos Anjos Cordeiro, J. M., Santos, L. C., de Oliveira, L. S., Santos, B. R., Santos, E. O., Barbosa, E. M., de Macêdo, I. O., de Freitas, G. J. C., de Assis Santos, D., & de Lavor, M. S. L. (2022). Maternal hypothyroidism causes oxidative stress and endoplasmic reticulum stress in the maternal-fetal interface of rats. Free Radical Biology and Medicine, 191, 24-39. https://doi.org/10.1016/j.freeradbiomed.2022.08.033

[11] Eid, R. A., Zaki, M. S. A., Al-Shraim, M., Eldeen, M. A., & Haidara, M. A. (2021). Grape seed extract protects against amiodarone-induced nephrotoxicity and ultrastructural alterations associated with the inhibition of biomarkers of inflammation and oxidative stress in rats. Ultrastructural pathology, 45(1), 49-58. https://doi.org/10.1080/01913123.2020.1864076

[12] Ekpono, E. U., Eze, E. D., Adam, A. M., Ibiam, U. A., Obasi, O. U., Ifie, J. E., Ekpono, E. U., Alum, E. U., Noreen, S., & Awuchi, C. G. (2024). Ameliorative potential of pumpkin seed oil (cucurbita pepo L.) against tramadol-induced oxidative stress. Dose-Response, 22(1), 15593258241226913. https://doi.org/10.1177/15593258241226913

[13] El-Sayed, N. M., Hamdy, K. N., Moustafa, A. Y., Sadek, A. S., & Rady, H. Y. (2024). Effect of Amiodarone on the Thyroid Gland of Adult Male Albino Rats: A Histological and Morphometric Study. QJM: An International Journal of Medicine, 117(Supplement_1), hcae070. 096. https://doi.org/10.1093/qjmed/hcae070.096

[14] El Sayed, O. A., Gawish, S. E., Aweida, G., & Auda, E. A. (2007). Histopathological and biochemical toxic effect of amiodarone on thyroid gland in albino rat. The Egyptian Journal of Hospital Medicine, 29(1), 463-474 .

[15] Gavril, R. N., Stoica, F., Lipșa, F. D., Constantin, O. E., Stănciuc, N., Aprodu, I., & Râpeanu, G. (2024). Pumpkin and pumpkin by-Products: A comprehensive overview of phytochemicals, extraction, health benefits, and food applications. Foods, 13(17), 2694. https://doi.org/10.3390/foods13172694

[16] Hosen, M., Rafii, M. Y., Mazlan, N., Jusoh, M., Oladosu, Y., Chowdhury, M. F. N., Muhammad, I., & Khan, M. M. H. (2021). Pumpkin (Cucurbita spp.): a crop to mitigate food and nutritional challenges. Horticulturae, 7(10), 352 https://doi.org/10.3390/horticulturae7100352 .

[17] Ibrahim Fouad, G., & R. Mousa, M. (2021). The protective potential of alpha lipoic acid on amiodarone-induced pulmonary fibrosis and hepatic injury in rats. Molecular and Cellular Biochemistry, 476(9), 3433-3448. https://doi.org/10.1007/s11010-021-04173-7

[18] Khorrami, M. B., Sadeghnia, H. R., Pasdar, A., Ghayour-Mobarhan, M., Riahi-Zanjani, B., Hashemzadeh, A., Zare, M., & Darroudi, M. (2019). Antioxidant and toxicity studies of biosynthesized cerium oxide nanoparticles in rats. International journal of nanomedicine, 2915-2926 .

[19] Leichtweis, M. G., Molina, A. K., Pires, T. C., Dias, M. I., Calhelha, R., Bachari, K., Ziani, B. E., Oliveira, M. B. P., Pereira, C., & Barros, L. (2022). Biological activity of pumpkin byproducts: Antimicrobial and antioxidant properties. Molecules, 27(23), 8366. https://doi.org/10.3390/molecules27238366

[20] Medić, F., Bakula, M., Alfirević, M., Bakula, M., Mucić, K., & Marić, N. (2022). Amiodarone and thyroid dysfunction. Acta clinica Croatica, 61(2), 327-341 .

[21] Motawea, A., Ahmed, D. A. M., Eladl, A. S., El-Mansy, A. A. E.-R., & Saleh, N. M. (2021). Appraisal of amiodarone-loaded PLGA nanoparticles for prospective safety and toxicity in a rat model. Life Sciences, 274, 119344. https://doi.org/10.1016/j.lfs.2021.119344

[22] Pattnaik, M., Pandey, P., Martin, G. J., Mishra, H. N., & Ashokkumar, M. (2021). Innovative technologies for extraction and microencapsulation of bioactives from plant-based food waste and their applications in functional food development. Foods, 10(2), 279. https://doi.org/10.3390/foods10020279

[23] Peixoto, M. S., e Souza, A. d. V., Andrade, I. S., El Giusbi, C. d. C., Faria, C. C., Hecht, F., Miranda-Alves, L., Ferreira, A. C. F., Carvalho, D. P., & Fortunato, R. S. (2021). Hypothyroidism induces oxidative stress and DNA damage in breast. Endocrine-Related Cancer, 28(7), 505-519. https://doi.org/10.1530/ERC-21-0010

[24] Rasheed, R., & Arsanyos, S. (2018). Vitamin E ameliorates the toxic effect of amiodarone on thyroid gland in rats: a histological and ultrastructural study. Journal of Medical Histology, 2(1), 57-68.

https://dx.doi.org/10.21608/jmh.2018.4614.1036

[25] Santos, T. P., da Silva Bastos, P. E., da Silva, J. F., de Medeiros Vieira, S. M., da Silva, M. C. G., de Andrade, A. L. C., Padilha, R. M. O., dos Santos Magnabosco, A. R., Cadena, M. R. S., & Cadena, P. G. (2023). Single and joint toxic effects of thyroid hormone, levothyroxine, and amiodarone on embryo-larval stages of zebrafish (Danio rerio). Ecotoxicology, 32(4), 525-535. https://doi.org/10.1007/s10646-023-02655-6

[26] Topcu, A., Ozturk, A., Deniz, E., Duman Ozturk, S., Arpa, M., & Atak, M. (2022). The effects of amiodarone in ovarian injury due to oxidative stress and inflammation caused by ischemia-reperfusion. Immunopharmacology and Immunotoxicology, 44(6), 1022-1031. https://doi.org/10.1080/08923973.2022.2102991

[27] Toto, A., Wild, P., Graille, M., Turcu, V., Crézé, C., Hemmendinger, M., Sauvain, J.-J., Bergamaschi, E., Guseva Canu, I., & Hopf, N. B. (2022). Urinary malondialdehyde (MDA) concentrations in the general population—a systematic literature review and meta-analysis. Toxics, 10(4), 160. https://doi.org/10.3390/toxics10040160

[28] Uğrak, U., Uzkar, Y., Düzen, İ., Acar, T., Karabey, E., & Durmaz, G. (2024). Evaluation of Procedure Doses and Staff Attitudes in Interventional Cardiology in Terms of Radiation Safety. Archives of the Turkish Society of Cardiology/Türk Kardiyoloji Derneği Arşivi, 52(4). DOI 10.5543/tkda.2024.18363

[29] Wu, S., Tan, G., Dong, X., Zhu, Z., Li, W., Lou, Z., & Chai, Y. (2013). Metabolic profiling provides a system understanding of hypothyroidism in rats and its application. PloS one, 8(2), e55599. https://doi.org/10.1371/journal.pone.0055599

[30] You, H.-S., Yoon, J. H., Cho, S. B., Choi, Y.-D., Kim, Y. H., Choi, W., Kang, H.-C., & Choi, S. K. (2022). Amiodarone-induced multi-systemic toxicity involving the liver, lungs, thyroid, and eyes: a case report. Frontiers in Cardiovascular Medicine, 9, 839441 .

[31] Zhao, H., Tan, Z., He, L., Zhu, S., Yan, R., Kou, H., & Peng, J. (2021). Amiodarone promotes heat-induced apoptosis, inflammation and oxidative stress in mouse HL1 atrial myocytes. Nan Fang yi ke da xue xue bao= Journal of Southern Medical University, 41(3), 406-411. https://doi.org/10.12122/j.issn.1673-4254.2021.03.13