Original Article

Dose – Related Amiodarone induced Thyrotoxicity in Adult Albino Rat and its Possible Reduction by Vitamin E: Histological and Immunohistochemical Study

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ABSTRACT

Introduction: Amiodarone (AMN) is one of the most common anti-arrhythmic drugs having many harmful effects on many organs such as the thyroid gland. The aim of the present study is to delineate the possible protective action of vitamin E to ameliorate the AMN induced thyrotoxicity.

Materials and Methods: 20 rats were utilized and divided into group I were given no medications, group II were given half the therapeutic dose of amiodarone, group III were given the therapeutic dose of amiodarone orally and group IV were given the therapeutic dose of amiodarone and vitamin E. after 3 weeks, all rats were sacrificed and the thyroids were examined histologically and histochrmically for Bax. The sera were analyzed for the inflammatory markers including CRP and P53.

Results: The thyroids showed amiodarone related histological and histochemical alterations with hormonal and inflammatory disturbances. It was concluded that vitamin E could reduce the amiodarone induced thyrotoxicity.

Conclusions: QU-CSNPs has prophylactic effects in an anosmia model than QU via its anti-inflammatory and antioxidant effects. This could pave the way for additional studies to investigate further mechanisms underlying the potential beneficial effects of QU-CSNPs and bring up a new line for anosmia therapy.

Key Words: Amiodarone, Bax, thyroid and vitamin E.

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INTRODUCTION

Amiodarone (AMN) is one of the most popular anti-arrhythmic drugs belonging to the iodinated class III drugs that could be used for the treatment of the tachyarrhythmias and also the ischaemic heart disease but unfortunely, its structure resembles the T4 causing thyroid dysfunction and histopathological alterations[1]. However, amiodarone, treated patients developed either hypothyroidism or thyrotoxicosis[2].

Martino *et al.,*[1] described the injurious effects of AMN on the thyroid gland as two different ways; disruption of thyroid hormone synthesis process and direct injury to the thyroid cells. Moreover, Bogazzi *et al.,*[3] added that there are many risk factors that predisposes the AMN- induced thyrotoxicity including the dietary iodine status and the thyroid health status.

Anu Bhalla[4] reported the AMN induced thyrotoxicity with long term treatment only and they explained it by inceased iodine level or the injurious effect of the AMN on the gland releasing iodothyronine into the blood.

Rios-Prego *et al.,*[5] postulated that many medications such as interferon and AMN could damage the thyroid cells but the outcome depends on the administration rate of the drug as a slow long term administration induced hypothyroidism but the patients exposed to acute administration develop thyrotoxicosis.

Ratini[6] reported another harmful alteration induced by AMN represented by the thyroid tumor that might be caused by a disturbance in the regulation of these hormones which can range from a minor goiter to life-threatening conditions as thyroid cancer.

Ramachandran *et al.,*[7] mentioned the oxidative stress as one of the mechanisms of the AMN induced thyrotoxicity by the production of the reactive oxygen species which are chemically reactive molecules containing oxygen and the overload of these chemicals might cause damage to cell structures and they added that the imbalance between oxidation and anti-oxidation can result in hazardous events such as lipid peroxidation (LPO) and oxidative DNA damage.

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Vitale *et al.,*[8] supposed another mechanism of AMN induced thyrotoxicity in the form of the apoptosis of the thyroid cells which was reported in some experimental trials and was declared by the iodine overload through a p53- mechanism resulting in oxidative stress which leads to the production of reactive oxygen species with an increase in lipid peroxide levels.

Zaki and Eid[9] had identified the high affinity of Vitamin E to the three main structural elements of the cell membrane, mitochondria and endoplasmic reticulum including phospholipids, cholesterol and triglycerides overbalancing vitamin E as the first line of defense against peroxidation of the polyunsaturated fatty acids of the cell membranes.

Accordingly, Israel *et al.,*[10] described vitamin E as playing a potent role in the protection against the AMN induced thyrotoxicity by reducing the excess production of oxygen free radicals at cell membranes especially mitochondrial membranes. Moreover, Bolt *et al.,*[11] suggested other physiological mechanisms of vitamin E including restoration of Fas-dependent apoptosis signaling in cancer cells, reduction of superoxide generation in neutrophils and decrease in fibroblast collagenase expression.

The aim of the present study is to delineate the possible protective action of vitamin E to ameliorate the AMN induced thyrotoxicity functionally and structurally and also to spotlight its mechanism for this action.

MATERIALS AND METHODS

Materials:

A- Chemicals:

1. Amiodarone: was provided as tablets containing 200 mg of amiodarone hydrochloride by Global Napi Pharmaceutical Co., Egypt (under license of Sanofi Aventis, France).

2. Vitamin E: was given in the form of capsules containing 400 mg of vitamin E by El Kahira Pharmaceutical Co., Egypt.

B- Animals:

The present research utilized 20 adult male albino rats (Sprague-Dawley), weighing 140 - 180 g. incurred from the Animal house, Faculty of Medicine, Cairo University. All rats had lived in the laboratory for a period of three weeks from 7 April, 2022 to 28 April, 2022 before being sacrificed with free access to food and water ad libitum and had been cared in separate clean cages, five rats/cage under standard laboratory and environmental conditions approved by the Animal Ethics Committee, Cairo University.

All animals were randomly divided into four groups with 5 rats each as following:

1. **Group I (the Control group):** were given no medications and left to survive for 3 weeks.

2. **Group II (half dose Amiodarone treated goup):** were given half the therapeutic dose of amiodarone orally via gastric gavage in a daily dose of 15 mg/kg body weight for 3 consecutive weeks after dissolving the amiodarone tablet in 10 ml distilled water[12].

3. **Group III (full dose Amiodarone treated goup):** were given the therapeutic dose of amiodarone orally via gastric gavage in a daily dose of 30 mg/kg body weight for 3 consecutive weeks after dissolving the amiodarone tablet in 10 ml distilled water[12].

4. **Group IV (Amiodarone and vitamin E treated):** were given the therapeutic dose of amiodarone orally via gastric gavage in a daily dose of 30 mg/kg body weight for 3 consecutive weeks after dissolving the amiodarone tablet in 10 ml distilled water[12] and were given simultaneously vitamin E capsule orally by gastric tube in a daily dose of 100 mg/kg/day after being dissolved in 2 ml vegetable oil for 3 consecutive weeks[13].

Methods:

A- Histological assessment of rats' thyroid gland:

At the end of the experiment, all rats were sacrificed by cervical dislocation after being anesthetized with ether and the thyroid tissue of each rat were extracted and was fixed in 10 % formalin solution and embedded in paraffin wax. Sections of 5 µm thickness were cut on a microtome and stained with haematoxylin and eosin (H and E) for general histologic examination[14].

B- Biochemical study:

To detect the biochemical alterations, blood samples were taken just prior to sacrifaction from the venous plexus behind the orbit in a sterile centrifuge tube and allow to clot overnight (4□C) and serum was separated following centrifugation (1500 per minute for 30 min). Sera were stored at -70□C until

analysed for thyroid hormones. Thyroxine (T4), Triiodoth-yronine (T3) and thyroid stimulating hormone (TSH) measurements in the sera were detected by enzyme immunoassay (EIA) test kit according to Kaplan[15]. Tumour marker (P53) was checked by enzyme linked immunosorbent assay (ELISA)[16].The CRP level (reference range\3 mg/L) was determined with a highly sensitive latex based immunoassay (Dade Behring, Newark, DE, USA; sensitivity 0.05 mg/L)[17].

C- Immunohistochemistry analysis of Bax:

Thyroid tissue samples were fixed in formalin and embedded in paraffin, and section thickness was 4 μm. Endogenous peroxidase was inactivated with a methanol solution containing H2O2 (1:50) for 10 min and washed with PBS. The tissue sections were blocked with 1.5 % serum for 30 min, incubated with anti-rat BAX primary antibody (1:100) overnight, followed by incubation with anti-rat BAX secondary antibody for 30 min and rinsing with PBS. Subsequently, samples were incubated with AB enzymes for 30 min and rinsed in PBS. Positive signals were detected using peroxidase chromogenic substrates. The negative control included PBS instead of the secondary antibody[18].

D- Histomorphometric analysis:

Image analysis was performed using the software Leica Quin 500, Germany. The area percent of positive BAX was measured in a standard measuring frame using a magnification x 400 by light microscopy transferred to the monitor's screen. These areas were masked by a green color using the computer system. Area percent values for each group were obtained from 5 different fields from different slides. Values were presented as a mean and standard deviation and statistically analyzed[19].

E- Determination of superoxide dismutase (SOD):

The enzymatic activity of SOD was assessed according to Marklund[20], SOD activity was expressed as units of activity per gram wet tissue. One unit of SOD activity is defined as the amount of the enzyme causing 50 % inhibition of auto-oxidation of pyrogallol.

F- Statistical analysis:

The data obtained were analysed using SPSS software version 13 (SPSS Inc., Chicago, IL, USA), then compared by one-way analysis of variance (ANOVA) test followed by Tukey's test to compare different groups with the control group. The results were expressed as mean ± standard deviation (SD). The differences were considered statistically significant if probability value $P < 0.05$ and highly significant if $P < 0.001$ and non-significant if $P > 0.05$[21].

RESULTS

Histological examination:

The thyroids of the rats of the control group showed normal appearance in the form of thyroid follicles of different sizes lined by cuboidal mononucleated follicular cells with pale abundant cytoplasm and central round homogenous nuclei. Their lumens showed homogenous acidophilic colloid material inside and the inter-follicular cells in between with clear cytoplasm and oval nuclei (Figure 1a).

The thyroids of the rats of the half therapeutic dose treated group with amiodarone (Group II) exhibited mild loss of normal thyroid gland structure as the follicles appeared irregular in shapes and sizes and looked vacuolated with a little colloid peripherally. The lining epithelial cells got flattened with little cytoplasm and heterogeneous nuclei. There were some shedded cells and inflammatory cell infiltrates. The clustered parafollicular cells with dark nuclei (arrow heads) were present (Figure 1b).

The thyroids of the rats of the full therapeutic dose treated group with amiodarone (Group III) showed hyperplastic follicles with columnar and flattened cells with atypical nuclei. The lumens of some follicles were obliterated. Moreover, the haemorrhage, necrosis and inflammatory cell infiltrations appeared in some areas (Figure 1c).

The thyroids of the rats of the full therapeutic dose treated group with am iodarone and vitamin E (Group IV) partially restored their partial normal architecture in the form of partially patent follicles lined by cuboidal cells with little colloid material. Less inflammatory cells and little necrosis were also observed. Some follicles were lined by flattened cells and some shedded cells appeared. There were some congested vessels (Figure 1d).

Immunohistochemically, the Bax protein was expressed in the areas of regenerating follicular cells in high percent in full therapeutic amiodarone treated rats (Figure 2c) more than half therapeutic amiodarone treated group (Figure 2b), but was not expressed in thyrocytes from control rats (Figure 2a) and less expressed in vitamin E treated group (Figure 2d).

Biochemical study:

There were alterations in the plasma thyroid hormones in the groups given full therapeutic dose of amiodarone as we reported a significant increase in tri-iodothyronine (T3) and tetra-iodothyronine (T4) ($p \leq 0.05$) and a significant decrease in the level of TSH ($p \geq 0.05$) when compared to the control group. The thyroid hormones were significantly ($p \leq 0.05$) improved in the rats given half therapeutic dose of amiodarone but non-significantly improved in the vitamin E treated group. Moreover, the measurements of inflammatory markers including P53 and C-reactive proteins in sera of showed that there were a significant increase in full therapeutic treated groups ($p \leq 0.05$) as compared to the control group. However, when we added vitamin E or reduced the amiodarone to the half, there was a significant ($p \leq 0.05$) amelioration in the inflammatory markers plasma level (Table 1).

BAX expression:

Bax protein expression was non-significantly higher in rats with full therapeutic dose of amiodarone ($p \geq 0.05$) with non-significant ($p \geq 0.05$) reduction of BAX protein expression in half therapeutic dose of amiodarone treated rats while significant ($p \leq 0.05$) reduction in the vitamin E treated rats (Figure 2 and Table 1).

SOD activity:

SOD activity in thyroids of control and full therapeutic amiodarone treated experimental animals was significantly increased ($p \leq 0.05$) with significant ($p \leq 0.05$) improvement in the half therapeutic amiodarone treated group and significant improvement ($p \leq 0.05$) in the vitamin E treated group (Table 1).

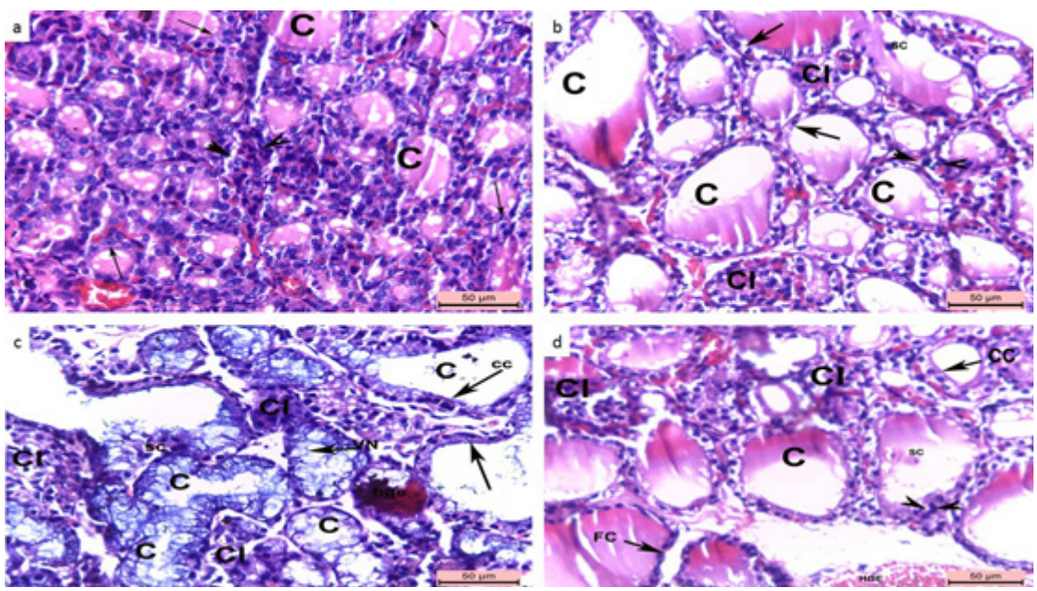

Figure 1: A photomicrograph of the thyroids of the rats of a) the control group (Group I) showing follicles of various sizes with its walls lined by cuboidal centrallized mononucleated follicular cells with pale abundant cytoplasm (arrows) surrounding homogenous acidophilic colloid (C) inside its lumina. Interfollicular cells with clear cytoplasm and oval nuclei are shown between the follicles (arrow heads).b) the half therapeutic dose treated group (Group II) showing mild loss of normal architecture. The acini showed irregular shapes and sizes with scanty amount of colloid (C) lined by flattened cells (arrows) with some shedded cells (sc). The clustered parafollicular cells with dark nuclei (arrow heads) were present and there were moderate inflammatory cell infiltrates (CI). c) The full therapeutic dose treated group (Group III) showing marked epithelial hyperplasia with atypical nuclear features and vacuolated cytoplasm (VN). The follicles were lined by columnar cells (CC). Some follicles showed obliterated lumen with absence of colloid (C). The shedded cells (sc) and congested vessels (hge) appeared in some follicles. d) The full therapeutic dose with vit.E treated group (Group IV) showing partial restoration of normal architecture of the acini lined by columnar cells (cc) and flattened cells (FC) with some shedded cells (sc) and scanty amount of colloid (C). The clustered parafollicular cells (arrow heads) were present and there were less inflammatory cell infiltrates (CI) and some congested vessels (hge). (Hematoxylen and eosin × 400).

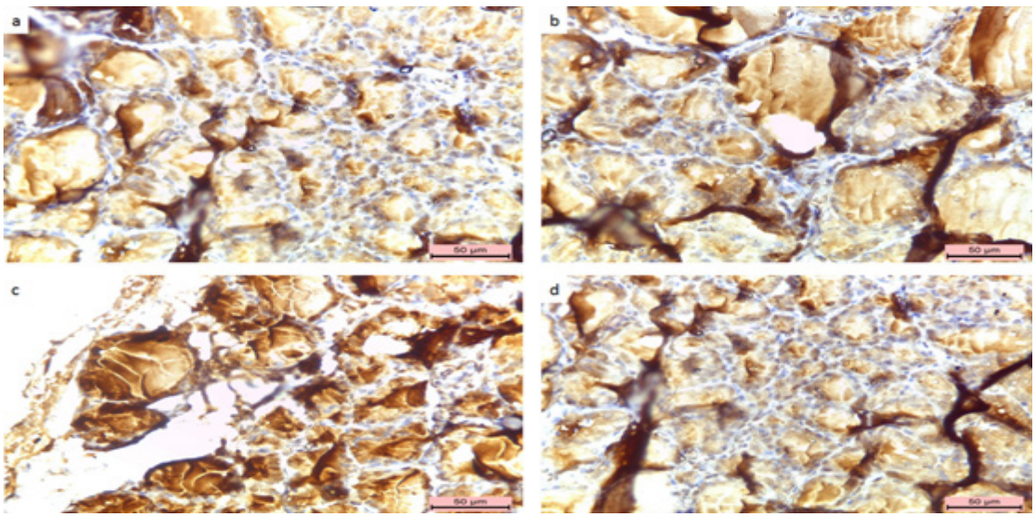

Figure 2: A Bax gene expression at a) normal untreated thyroid gland (group I). b) Thyroid gland after administration of half therapeutic dose of amiodarone (group II). c) Thyroid gland after administration of full therapeutic dose of amiodarone (group III). d) Thyroid gland after administration of full therapeutic dose of amiodarone with vit.E (group IV). (Bax × 400).

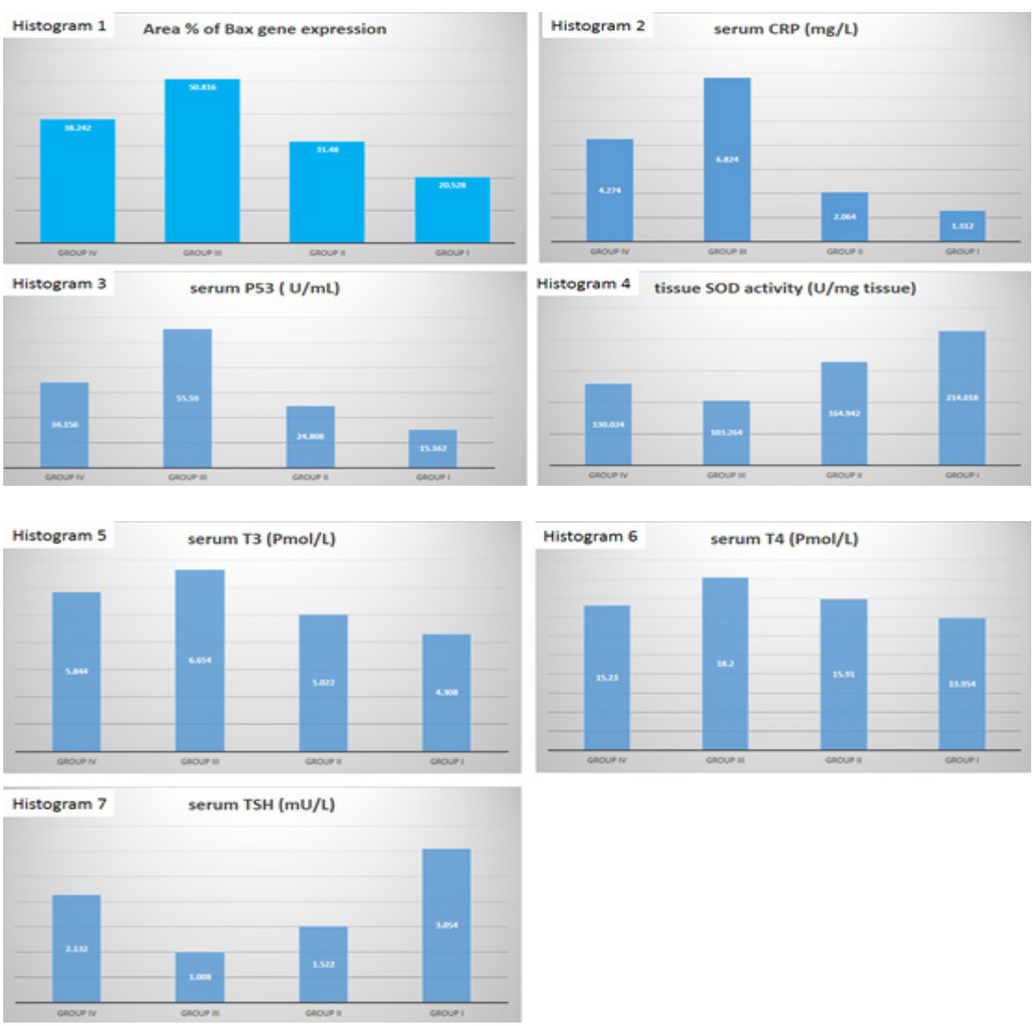

Table 1: ***P***-values > 0.05 were considered significant:

The groups / The markers	Group I (the Control group) (mean ± SD)	Group II (half Amiodarone treated goup) (mean ± SD)	Group III (full Amiodarone treated goup) (mean ± SD)	Group IV (Amiodarone and vitamin E treated) (mean ± SD)
Area % of BAX gene expression	20.53 ± 1.10****	31.48 ± 1.45	50.82 ± 2.73	38.24 ± 1.73*
Serum CRP (mg/L)	1.31 ± 0.38	2.06 ± 0.15****	6.82 ± 0.42****	4.27 ± 0.41***,**
Serum P53 (U/mL)	15.16 ± 1.63	24.81 ± 1.53****	55.59 ± 0.62	34.16 ± 0.68**
Tissue SOD activity (U/mg tissue)	214.02 ± 7.63****	194.64 ± 4.7***	103.26 ± 2.8**	130.02 ± 1.89*
Serum T3 (Pmol/L)	4.30 ± 0.68**,****	5.02 ± 0.56*,***	6.65 ± 0.54*,**	5.48 ± 5.84
Serum T4 (Pmol/L)	13.95 ± 0.88	15.91 ± 0.79	18.2 ± 0.46****	15.23 ± 0.72***
Serum TSH (mU/L)	3.05 ± 0.57**,****	1.52 ± 0.26*,***	1.01 ± 0.19*,**	2.13 ± 0.25

*Statisti cally significant as compared to control group (group I). ** Statistically significant as compared to group II. *** Statistically significant as compared to group III. **** Statistically significant as compared to group IV.

DISCUSSION

Amiodarone (AMN) is an antiarrhythmic chemical belonging to the iodinated class III drugs that is very effective against many life-threatening cardiac rhythm irregularities[22]. Kodama *et al.,*[23] explained the therapeutic effect of amiodarone by its ability to prolong the action potential duration of atrial and ventricular muscles without changing the resting membrane potential.

In our experiment, we found that the administration of amiodarone to the rats led to histological damage to the thyroid gland in the form of hyperplastic follicles with stratified columnar cells and atypical nuclei. The lumens of some follicles were obliterated with fibrous tissue in between. Moreover, the haemorrhage, necrosis and inflammatory cell infiltrations appeared in some areas. These histological alterations were dose related as they were reduced by decreasing the dose of AMN to the half.

The previous histological picture was in agreement with Alan *et al.,*[24] who described the damage induced by amiodarone as degenerative and destructive follicular alterations with diffuse lymphocytic infiltration. Moreover, Santangeli *et al.,*[25] reported follicular damage that causes thyroid dysfunction by many mechanisms including high iodine intake, oxidative stress, inflammatory effects, direct drug effect or a combination of these mechanisms.

On contrary, Stoykov *et al.,*[26] mentioned that the AMN induced thyroid injury is only functional as the reduction of type 2 deiodinase activity caused thyroid hormones disturbances. In addition, Gutowski and Kowalczyk[27] described the amiodarone induced toxicity in other organs including the liver and the testis that are vulnerable to oxidative stress.

Our histological findings indicated that amiodarone induced thyroid injury were dose related as these histological alterations became more obvious in group III than group II. These findings were confirmed by Ola *et al.,*[28] who increased the dose gradually reaching toxic doses and they reported thyroid follicular cell damage that was more apparent with increasing the dose of AMN.

Apotosis was detected in the samples obtained from the thyroids of the rats of our experiment which was confirmed by tracing the Bax protein expression that was non-significantly higher in rats with full therapeutic dose of amiodarone ($p \leq 0.05$) with non-significant ($p \geq 0.05$) reduction in half therapeutic dose of amiodarone treated rats and a significant reduction in the vitamin E treated rats.

XU *et al.,*[29] also were in agreement with our study by recording an increased expression of Bax in rat thyroid cells with the administration of gradually increasing doses of iodine in a similar manner to our experiment as the AMN contains iodine in its chemical structure. In contrast, Arscott and Baker[30] reported mild level of apotosis in normal thyroid tissues as an indicator of the basal thyroid cell turnover. However, Stassi and De maria[31] approved by immunohistochemical and morphological analyses that apoptosis was increased in frequency in thyroids from patients with destructive thyroiditis. In addition, Andriokula and Tsatsoulis[32] revealed rising in the level of the apoptic bodies in Hashimoto's thyroiditis, especially on the periphery of infiltrating lymphocytes.

The measurements of the plasma thyroid hormones in the groups given full therapeutic dose of amiodarone showed a significant increase in tri-iodothyronine (T3) and tetra-iodothyronine (T4) ($p \leq 0.05$) and a significant decrease in the level of TSH ($p \geq 0.05$) when compared to the control group with significant improvement in the sera of the group given half therapeutic dose of amiodarone but non-significant in the rats of the vitamin E given group.

In agreement with our study, Ernst *et al.,*[33] reported AMN induced thyrotoxicosis in the form of increased T4, T3 levels and decreased TSH levels and they explained these alterations by the inhibition of the transformation of T4 into T3 reducing the number of T3 receptors in the pituitary glands resulting in a decline of TSH levels. Moreover, Van Beeren *et al.,*[34] described AMN induced thyroitoxicosis in the form of increased T3, T4 levels without decline in the TSH levels and they assumed these changes to the reduction of type 2 deiodinase activity in a noncompetitive manner and weakening of T4 feedback mechanisms, leading to increased TSH levels.

On contrary, Melmed *et al.,*[35] detected different changes in the thyroid hormones and they found them as early as 2 weeks after the administration of amiodarone. Moreover, Burger *et al.,*[36] demonstrated amiodarone-induced peripheral metabolism of thyroid hormones resulting in low T3 syndrome in patients treated chronically with the drug.

Our current study supported the oxidative stress as one of the mechanisms that caused thyroid damage by detecting the SOD activity in thyroids of control and full therapeutic amiodarone treated experimental animals which was significantly increased ($p \leq 0.05$) with non-significant ($p \geq 0.05$) improvement in the half therapeutic amiodarone treated group and significant improvement ($p \leq 0.05$) in the vitamin E treated group.

Ghosh *et al.,*[37] also supposed the oxidative stress as the amiodarone-induced injurious mechanism by measuring the SOD activity in the AMN treated animals which showed a higher level of SOD activity. Moreover, Many *et al.,*[38] proposed earlier that the free radicals produced after institution of high dose of AMN could overshadow the normal cellular defense mechanism against those free radicals causing oxidative stress and changes in SOD activities.

In the present experiment, the measurements of inflammatory markers including P53 and C-reactive proteins in sera of showed that there were a significant increase in full therapeutic treated groups ($p \leq 0.05$) as compared to the control group. However, when we added vitamin E or reduced the amiodarone to the half dose, there was a significant ($p \leq 0.05$) amelioration in the inflammatory markers plasma level.

Our results were in agreement with Capen[39] who reported elevated P53 level in amiodarone induced thyrotoxicosis which induced histopathological alterations in thyroid gland. In addition, Zhao *et al.,*[40] who illustrated increased level of C-reactive protein in patient treated by AMN suggesting the inflammation as one of the mechanisms of the amiodarone-induced thyroiditis.

In the current study, we reported amelioration in the AMN-induced thyroid harmful effects when vitamin E was given together with amiodarone. These findings were in agreement with Yu *et al.,*[41] who found that vitamin E may increase thyroid antioxidant defenses and inhibit iodine-induced thyroid cytotoxicity. Also, Zaki and Eid[9] who observed that α tocopherol could protect rat hepatocytes in culture from the damaging effect of amiodarone. Moreover, Murray *et al.,*[42] demonstrated the importance of vitamin E for the cellular health by describing the high affinities of the phospholipids of mitochondria, endoplasmic reticulum and plasma membranes for vitamin E increasing their antioxidant defense mechanism.

CONCLUSION

It was concluded that amiodarone induced histopathological alterations in the thyroid gland which were dose related with mild hormonal disturbances and an ameliorating effect of vitamin E.

Depending on our results, it was recommended to use vitamin E in patients utilizing amiodarone as a treatment of the cardiac arrhythmias especially tachyarrhythmias to ameliorate the drug related thyroid alterations.

CONFLICT OF INTEREST

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