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MORINGA OLEIFERA EXTRACT HINDERS THE CASCADES OF AMIODARONE MEDIATED THYROTOXICOSIS IN ALBINO RATS

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ABSTRACT

Amiodarone AMD, a drug used in management of angina and refractory ventricular arrhythmia induces thyrotoxicosis in rat. The objective was to evaluate the role of Moringa Oleifera leaf methanolic exteract (MOE) administration and exposure to low doses of gamma-irradiation (LDR) as adjunctive strategy to modulate AMD associated thyrotoxicosis. The experiment started by rats i.p administration of AMD (100 mg/kg, b.wt./day) and oral administration of MOE (250 mg/kg b. wt./day) for 10 and 15 consecutive days, respectively. While, the exposure to 0.5 Gy fractionated whole body gamma radiation was carried out among 2 fractions (0.25Gy on the 1st and 10th days of experiment). The thyroid profile (tri-iodothyonine; T3, thyroxine; T4 and Thyrotrophin; TSH) and the inflammatory indicator Interleukin 6 (IL6), in addition to the marker of thymus activity thymulin (TH) as well as, the hormones sensitive to thyroid secretion (Prolactin; PLR, Growth hormone; GH and Cortisol; COR) were determined in serum.The administration of MO extract and/or LDR to AMD rats significantly ameliorated the changes induced in Serum concentration of, thyroxine (T4), thyrotrophin (TSH), interleukin 6 (IL-6), thymulin (THY), prolactin (PLR), growth hormone(GH) and cortisol (COR). In conclusion, the administration of MO extract and/or LDR rats might provide substantial protection against amidorone-induced thyirotoxicosis and the subsequent undesirable bearing alterations.

KEY WORDS

Amiodarane, γ-irradiation, inflammation, Moringa Oleifera, thyroid

INTRODUCTION

Medications or environmental agents can affect the concentration of intracellular iodide or its regulatory mechanisms. Amiodarone (AMD) is an antiarrhytmic drug that contains two atoms of iodine in an inner benzene ring, similar to thyroid hormones [1]. Its administration was associated with variations in thyroid gland structure and function. Patients received AMD medications have developed either hypothyroidism or thyrotoxicosis [2]. Anu Bhalla [3] reported that chronic treatment with AMD induced thyrotoxicosis. The pathogenesis of AMD was related to iodine overload or its direct toxic effect followed by release of iodothyronine into the circulation. The autonomy of thyroid gland is due to its autoregulatory mechanisms of hormone secretion. Excess iodine concentrations disrupt this mechanism of thyroid hormone synthesis; a Jod-Basedow effect [4]. Worthwhile, the thymus produces the humoral factors (inducers of T-cells proliferation and differentiation) responsible for cell-mediated immunity is affected particularly by thyroid status and GH [5]. A significant correlation was found

between circulating thymulin, (thymus hormone) and serum T4 and T3 levels [6].

Moringa oleifera (MO) is one of multipurpose "Miracle" plants which have received attention as "Natural nutrition of the tropics". The tree is highly valued as every part of the plant is edible and has established medicinal and folkloric significance in treating different diseases [7]. The pharmacological studies showed that its various parts possess a biologically active compounds which have hypotensive, antioxidant, anti-inflammatory, antinociceptive, wound healing, anthelmintic, hypolipidaemic, antiatherosclerotic, antiurolithic, antiulcerogenic, analgesic, anesthetic, anti-HIV and antimicrobial activities [8, 9]. However, identifying novel biological activities of *Moringa oleifera and* its mechanism of action remains to be determined.

Radiation is known to have significant effects on living organisms dependent on the dose received. Low radiation doses (LDR) are no longer considered to be as harmful as once though. Hormesis and adaptive response of cells or tissues in response to LDR were extensively documented [10, 11, 12, 13, 14]. There is some evidence that a little radiation can be effective in stimulating the body's natural defenses against free radical formation that damages cells. Bushing states that "the prevailing explanation is that a little radiation stimulates hormonal and immune responses to other toxic environmental agents [15].

The objective of this study was to elucidate the role of *Moringa Oleifera* leaf ethanolic extract (MOE) and/or by exposure to low doses of gamma irradiation (LDR) against toxicity induced by AMD in female albino rats.

MATERIALS AND METHODS

Materials: Amiodarone was obtained from Sanofi-Aventis, Montpellier, France (Commercially found as Cordarone®). MO leaves was obtained from Egyptian Society of *Moringa*, National Research Center (NRC), Giza, Egypt. All chemicals used in these studies were obtained from Sigma Chemical (St. Louis, MO).

Experimental Animals: Young Female albino rats 6 weeks old weighed 120-150g purchased from the breeding unit of Egyptian Holding Company for Biological Products and Vaccines were used in this study. The animals were housed in standard cages and food and water were provided *ad libitum*.

Animal experimentation were consistent with the guidelines of Ethics by the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) in accordance with the recommendations for the proper care and use of laboratory animals approved by animal care committee of the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt.

Animals were divided into eight groups as follows: (Group 1) Control (C): rats received oral supplementation of distilled water (0.5 ml/day for 15 days), (Group 2) Amidarone (AMD): rats received doses of amiodarone (100 mg/kg b.wt. /day)via intraperitoneal injection for 10 consecutive days [16], (Group 3) *Moringa (MO*) : rats received oral doses of MO extract (0.5 ml ≈250 mg/kg b.wt./day) for successive 15 days [17], (Group 4) Low dose irradiation (LDR): rats exposed to 0.5 Gy of whole body gamma radiation fractionated into 2 equal doses (2x 0.25 on the 1st and 10th days of the experiment) and received 0.5 ml distilled water via oral tube for 15 days, (Group 5) MO + LDR: rats 0.5 ml of MO for 15 days as routed in group 3 and exposed to gamma radiation as routed in group4, (Group 6) AMD+MO : rats received amiodarone and treated with MO extract as routed in group 2 and 3, respectively (Group 7) AMD + LDR : rats received amiodarone as routed in group 2 while, exposed to gamma radiation as routed in group 4 , (group 8) AMD + LDR + *MO*: rats received amiodarone,

exposed to gamma radiation and treated with MO as routed in groups 2, 3 and4, respectively.

Radiation Facility:

Whole body gamma irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, using (^{137}cesium) Gamma Cell-40 biological irradiator. The dose rate of the gamma source was 0.49 Gy min^{-1}. Animals were not anesthetized before irradiation.

Preparation of Methanolic Extract of *Moringa Oleifera* (MO) Leaves: The leaves of *M. oleifera* harvested from different trees cultivated in Egypt were rinsed firstly with distilled water, dried in shade and completely extracted with methanol (70%) using Soxhlet apparatus for 3 days. The percolated extract was then dried in vacuum using Rotary Evaporator apparatus (Model RE52A, China), weighed and dissolved in double-distilled water to give the final concentration of 250 mg extract /kg body weight with the help of a cyclomixer just before oral administration [18].

Biochemical Assay: Animals were sacrificed 24 h after the last injection. Blood samples were taken just prior to dissection 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 analyzed for thyroid hormones. Thyroxine (T4), Triiodoth-yronine (T3) and thyroid stimulating hormone (TSH) and Thymulin (THY), concentration in the serum samples were determined by enzyme immunoassay (ELISA) test kit according to [19,20] respectively. Serum Interleukin 6 (IL-6), Growth hormone (GH), Cortisol (COR) and Prolactin (PRL) were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit for rats (Cusabio Biotech Co., Ltd., Wuhan, China) according to the manufacturer's instructions.

Statistical Analysis: Data subjected to statistical analysis and performed tests of significance using the statistical package SPSS (Statistical Program for Social Science) version 15.0 by applying one-way ANOVA test to determine variations between mean values of different groups. All data are expressed as mean of 6 values ±S.E and difference between means are considered significant at $P<0.05$ [21].

RESULTS

Table (I): Changes in serum IL-6, T3, T4 and TSH levels of the different animal groups.

Groups	Parameters			
	IL-6 (pg/ml)	T3 (ng/dL)	T4 (ng/dL)	TSH (ug/ml)
C	29.80±0.597	1.21±0.004	8.33±0.26	15.27±0.12
AMD	69.46±2.59 [a]	1.19±0.016	10.03±0.25 [a]	12.88±0.33 [a]
MO	24.70±0.745 [a,b]	1.22±0.01	8.27±0.28 [b]	15.19±0.13 [b]
LDR	32.66±0.796 [b]	1.22±0.02	8.25±0.23 [b]	15.16±0.1 [b]
MO+LDR	37.86±0.928 [a,b]	1.23±0.013 [b]	8.28±0.32 [b]	15.12±0.23 [b]
AMD +MO	45.40±0.657 [a,b]	1.25±0.009 [b]	8.41±0.19 [b]	15.23±0.24 [b]
AMD +LDR	34.10±0.632 [a,b]	1.22±0.02	8.38±0.27 [b]	15.46±0.14 [b]
AMD +MO+LDR	39.40±0.754 [a,b]	1.22±0.013	8.50±0.22 [b]	15.32±0.22 [b]

Each value represents the mean of 6 female rats ± SE. **a :** significantly different from that of the control. **b :** significantly different from that of rats treated with (AMD) treated group. **c:** significantly different from that of the (MO+AMD) group.

The data represented in Table (I) showed a cytotoxic effect of AMD manifested by significant (P<0.05) increased in the inflammatory marker IL-6 and the level of thyroid hormone T4 in serum in addition to the significant (P<0.05) decrease of TSH as compared with control rats.

Otherwise, the oral administration of MO and/or LDR to AMD rats significantly (P<0.05) improved the levels of IL-6 and T4 and elevates TSH when compared to AMD only treated rats.

Table (II): Changes in serum hormonal levels of PRL, GH, COR and THY in different animal groups.

Groups	Parameters			
	PRL (ng/mL)	GH (ng/mL)	COR (ng/mL)	THY (ng/ml)
C	26.50±0.286	2.06±0.078	7.67±0.32	0.857±0.061
AMD	18.89±0.753 [a,c]	1.31±0.239 [a,c]	15.82±0.31 [a]	0.396±0.073 [a]
MO	27.44±0.536 [b,c]	2.04±0.068 [b,c]	7.58±0.31 [b,c]	0.905±0.037 [b,c]
LDR	27.67±0.492 [b,c]	2.37±0.119 [b]	8.10±0.25 [b,c]	0.924±0.025 [b,c]
M+LDR	26.60±0.210 [b,c]	2.12±0.157 [b,c]	8.08±0.34 [b,c]	0.938±0.024 [b,c]
AMD+MO	37.12±1.149 [a,b]	2.59±0.097 [a,b]	9.15±0.42 [a,b]	0.607±0.024 [a,b]
AMD+LDR	31.79±0.529 [a,b,c]	3.17±0.247 [a,b,c]	9.53±0.42 [a,b]	0.65±0.11 [a,b]
AMD+MO+ LDR	29.57±0.443 [a,b,c]	3.06±0.071 [a,b,c]	9.78±0.34 [a,b]	0.675±0.019 [a,b]

Each value represents the mean of 6 female rat's ± SE. **a :** significantly different from that of the control. **b :** significantly different from that of rats treated with (AMD) treated group. **c:** significantly different from that of the (MO+AMD) group.

Scheduled data in Table (II) revealed that AMD significantly (P<0.05) decreased serum PRL, GH and COR concentrations as well as, a significant (P<0.05) decrease in THY level was observed when compared to control rats.

On the other hand, the oral administration of MO and/or LDR to AMD rats significantly (P<0.05) ameliorated the serum levels of PRL, GH, COR and THY compared to AMD only administrated rats.

DISCUSSION

Certain drugs could disturb physiological function and analytical structure of thyroid gland. Amiodarone is a uniquely effective antiarrhythmic drug, but it is also dangerously rich in iodine this feature effects from mild derangements in thyroid function to overt hypothyroidism or thyrotoxicosis [22]. The effects of amiodarone on thyroid function can be separated into two categories, iodine-induced effects and intrinsic drug effects. Outcomes can be further separated into two classes: disruption of thyroid hormone synthesis and direct damage to thyroid cells [2].

In the present study, administration of AMD to rats exerted significant increase in the level of T4 and a significant decrease in TSH which can be attributed to certain disturbances in thyroid function (Table1). It was claimed, that subclinical thyrotoxicosis is characterized by diminished serum TSH with normal level of T3 [23]. Daniels, [4] reported that, the disturbance of thyroid hormone synthesis auto regulatory mechanisms which lead to thyroid autonomy and thyrotoxicosis is the result of excess iodine concentration in AMD.

AMD altered thyroid hormone metabolism with a low incidence on thyroid state by inhibiting the outer ring monodiodination which block the

conversion of T4 to triiodothyronine (T3), which leads to significant increases in serum T4 and rT3 [24]. In addition, the decreases in TSH level might be the pituitary feedback response to increased serum T4 level. Al hendawi et al. [25] stated that, the alteration in the synthesis, transport and/ or metabolism of thyroid hormones is implicated on the thyroid stimulating hormone TSH synthesis and secretion.

Moreover, the experimental data reveals significant alterations in certain immune response parameters in AMD rats among them the significant elevation in serum interleukin (IL-6) and decreased thymulin concentrations (Table1 and 2, respectively). These results revealed the possibility of developing immune response alterations in association with thyroid alterations after AMD alterations. Wang and Klein et al. [26] demonstrated that the changes in thyroid hormone concentration are usually associated with immune alteration. IL-6, a cytokine synthesized in thyroid and other tissues is considered a good marker of the thyroid destruction and was appeared to be increased in AIT cases [27; 23]. The IL-6 elevation, greatly associated with thyroid damage, affects B-cell differentiation and T-cell activation [28] and could be responsible to the synergistic disturbances in immunity and alteration in thyroid hormones production and secretion.

Further, the reported decrease in thymulin concentration (Table 2) which is a thymic metallopeptide involved in several aspects of intra and extrathymic T-cell differentiation [29] emphasized the immune alteration accompanied thyroid disturbances. In the same context, Taub et al.[30] reported that, hyperthyroidism leads to a suppression of humeral and cell mediated immune responses is closely related to the bidirectional interaction between the neuro endocrine axis (hypothalamic pituitary thyroid) and immune responses. Moreover, the results obtained revealed significant decrease in serum growth hormone (GH) and prolactine (PRL) in addition to significant increase in cortisol of AMD rats (Table 2). The thymulin production and secretion is influenced directly or indirectly by the neuro endocrine system involved the growth hormone, prolactine and cortisol secretion [31]. The decreases in these hormones could be attributed to disturbance in thyroid and the increases of T4 secretion. TSH decreased in response to high T4 level in serum reduces the secretion of GH and PRL [32]. A signaling pathway involves membrane receptor-hormonal interaction is the authenticated mechanism for the influence of GH and PRL on thymulin synthesis and secretion. Human GH can stimulate thymulin release from thymic epithelial cells (TEC) in vitro that posses receptors for GH [31]. Similarly, Dardenne et al., [33] stated, the present of PRL receptors in TEC which stimulate the secretion of thymulin and so on, the increased thymulin could be the expected result of raised GH and PRL levels. The reported decrease in thymulin in response to AMD treatment (Table 2) could be referred to the action of increased cortisol in thymocyte that circumvented the stimulatory action of GH and PRL on TEC. Glucocorticoids (cortisol) induce thymocyte apoptosis, causing a profound reduction in thymic mass and volume and inducing hormonal thymectomy, which results in suppressed thymulin level [34].Worthwhile, hyperthyroidism associated with hypercorticosteronemia and hypothalamic pituitary adrenal (HPA) axis dysfunction is more pronounced as the duration of hyperthyroidism increases [35].

On the other hand, administration of Moringa ethanolic extract and /LDR concurrently with AMD treatment markedly improved the case of hyperthyroidism (T4 elevation and TSH decrease) in addition to amelioration of immune response parameters (IL-6 and thymulin) as well as

repairing of neuroendocrine axis represented by amelioration of GH, PRL and cortisol levels (Tables 1 and 2).

For decades, plant-based materials have been considered an important source of natural products used for health. For example *Moringa* has health promoting bioactive and nutritive components that increase its potential as a natural supplement in treating disease. Among the many positive benefits, it has immune modulating, antioxidant and anti-inflammatory properties [36].

The ameliorative effect of *Moringa* extract on the IL-6 could be interpreted in the view of its anti inflammatory activities that revoke cytokines production during the process of immune response. It was reported that pretreatment of human monocyte derived macrophages with varying concentrations of MOE abrogated TNF-a, IL-6 and IL-8 cytokine production upon Lipopolysaccharides (LPS) exposure [37]. *M. oleifera* pod, root, leaf and fruit extracts were reported to block inflammatory responses of a macrophage cell line [36, 38]. Further, the regulation of immune response and the inflammatory cascades (serum IL-6) by *Moringa* extract administration and /or LDR could contribute to the improvement of thyroid hormone T4 and TSH as the IL-6 is a thyroid destruction marker. *Moringa* extract has the capability to adjust the thyroid hormone metabolism in hyperthyroditis animals [39]. The low-dose whole-body gamma irradiation of rats might enhanced the immune response by augmenting the proliferative reactive response of T cells to mitogenic stimulation [40 ,41], altering cytokine release [42, 43] and immune cell populations [44], Chen et al. [45] found that LDR effectively inhibits cigarette-smoke carcinogen BPDE-induced secretion of proinflammatory cytokines such as IL-6 from human lung fibroblasts. According to Tsukimoto et al. [46], the increase in IL-6 levels in EAE mice was significantly reduced by LDR. Also, the serum thymulin significantly ameliorated in MO and/ LDR-AMD rats (Table 2) could be attributed to the protective action of extract against the AMD induce inflammation in thyroid gland. The recovery observed in serum thymulin of AMD rats by MO and /or LDR might be related to the correction in neuroendocrine axis function [47]. The results revealed improved cortisol secretion and also the level of GH and PRL by moringa ethanolic extract administration and /or LDR exposure which could participate in reduction of AMD apoptotic action on the thymocytes. These results are consisted with Tsukimoto et al. [46] and Mutiara et al. [48] who's reported that the administration of MO extracts, leads to modulation of GH and PRL release.

It could be suggested that the administration of MO extract and/or LDR protect thyroid against AMD induced thyrotoxicosis thought preventing IL-6 production and revoking inflammatory responses that disturb thyroid functions. The adjustment of immune system and neuroendocrine axis cascades by MO ethanolic extract and /or LDR might provide substantial protection against amidorone-induced toxicity in thyroid gland.

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