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Mitigation of ^{131}I induced oxidative stress by supplementation of turmeric and green cardamom in thyroid patients

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► ***Original article***

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ABSTRACT

Background: The use of ^{131}I in thyroid diseases is responsible for oxidative stress due to increased production of reactive oxygen species that may lead to multiple disorders in human. Purpose of this study was to mitigate the oxidative stress, generated by ^{131}I therapy, by supplementation of phytoprotectants. ***Materials and Methods:*** After eliminating absconders, 97 volunteers of benign and malignant hyperthyroid diseases viz. control (n=42), standard (n=23), turmeric (n=19) and green cardamom (n=13) groups participated in this study. Vitamins were administered orally in standard group while turmeric (*Curcuma longa*) and green cardamom (*Eletteria cardamomum*) as phytoprotectants were given orally in the respective groups, 5 days before ^{131}I therapy. The malondialdehyde (MDA) was measured in serum before (baseline), at 3-hours and after 2-weeks of ^{131}I administration. ***Results:*** After radioactive iodine therapy (RAIT), there was a continuous decline of serum MDA in vitamin group (3.57 to 2.64 µmol/L) in contrast to the control, where there was a ceaseless rise (3.01 to 3.69 µmol/L) in the oxidative stress in terms of MDA. In akin to the standard group of vitamins, there was incessant decrease in both the treatment groups of green cardamom (3.55 to 2.89 µmol/L) and turmeric (3.45 to 2.06 µmol/L). The turmeric was proved as better phytoprotectant. ***Conclusion:*** The turmeric and green cardamom are good scavengers of free radicals and can be used as supplements along with ^{131}I, in thyroid patients as radioprotective agents. The turmeric is more potent radioprotectant as compared to green cardamom.

INTRODUCTION

Thyroid diseases including benign hyperthyroidism and thyroid cancers are increasing day by day due to the presence of multiple carcinogens in the environment, adulterated human diet, use of junk and fast foods and also with excessive use of radiation based diagnostic and therapeutic procedures [1, 2]. ^{131}I is being used as therapeutic agent for thyroid patients in the nuclear medicine health centers throughout the world [3]. This mode of treatment is preferred and more acceptable by the patients due to a single or few oral doses of ^{131}I with high recovery rate. Simultaneously it is taking its toll in the form of immediate and delayed harmful effects in the human due to emission of radiations [4]. ^{131}I emits both beta and gamma rays [5] and generates reactive oxygen species (ROS) which builds oxidative stress in the biological systems. In this way radiations of ^{131}I impart damage in targeted and non-targeted tissues and also induce deleterious changes in the biomolecules including DNA, proteins and lipids [6]. The oxidative damages may lead to different degenerative diseases with their chronic manifestations [7]. Moreover, the superoxide and hydroxyl radicals, generated by the ^{131}I cause the lipid peroxidation in the plasma and other intracellular membranes, causing hyper production of malondialdehyde (MDA) [8]. MDA is highly reactive aldehyde and causes damage to the biomolecules, thus imposing the severe threat for the cellular survival. Excessive and prolonged generation of MDA may also lead to mutation in the genetic blueprint leading to the development of various carcinomas [9].

Oxidative stress of radiotherapy can be ameliorated by enforcing the body's antioxidant system by the administration of antioxidants like vitamin C and E and zinc (Zn) which are scavengers of superoxide and hydroxyl radicals [10]. These antioxidants are radioprotectors for the rapidly growing cells when administered prior to the radiotherapy [11]. Furthermore, Zn is a cofactor of more than 300 enzymes which take part in body's defense system and in many metabolic processes to maintain normal human physiology. In this way the vitamins and Zn support the antioxidant defense system of the body [12, 13]. Moreover, Zn deficiency increases the probability of mutations leading to

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different types of cancers, while its supplementation provides the barrier against genetic damage and also protects the DNA and other biomolecules from oxidative injury [14]. Zinc also maintains the normal cell homeostasis by controlling the over production of ROS and by stabilizing the normal activity of mitochondrion in stress full situations. Therefore, the therapeutic use of these antioxidants in combination of vitamin C and E with the addition of Zn provides the better radioprotection [15].

Furtherance, the medicinal plants are rich in diverse nature of antioxidants which possess a righteous free radical scavenging potential. Turmeric (*Curcuma longa*) and green cardamom (*Eletteria cardamomum*) are astounding medicinal plants enriched with polyphenolics and other botanicals that are potent scavengers of free radicals [16, 17]. Curcumin, the main ingredient of turmeric, plays an important role in the prevention of the side effects of anticancer drugs and also effective in combating the oxidative stress induced by ionizing radiations when given prior to or in conjunction with radioiodine therapy [18, 19]. Turmeric is extremely safe and non-toxic to the human with highly negligible chances of adverse effects [20]. It is an amazing radioprotector for the normal cells and even radiosensitizer for the malignant tissues when used in conjunction with the radiotherapy [21, 22]. Therefore, the study was planned to mitigate the radiation damage caused by ^{131}I in thyroid patients with the supplementation of phytoprotectants.

MATERIALS AND METHODS

Consequent to the approval of Ethical Review Committee for Human (ERCH) of Punjab Medical College, Faisalabad (Registration No. 702/2016), the study was conducted on the adult volunteers suffering from thyroid diseases being treated with ^{131}I, at Pakistan Institute of Nuclear Medicine (PINUM) Cancer Hospital, Faisalabad.

Exclusion criteria was the pregnant ladies and the patients suffering from any infection, inflammation, autoimmune disease, renal, cardiac, hepatic, pulmonary or parathyroid diseases that may alter the level of MDA [23].

A total of 125 volunteers (50 in control group and 25 in each standard and treatments groups) suffering from benign (n=80) and malignant (n=45) thyroid disorders were registered for this study. Eventually the study was ended with 97 participants viz. 42, 23, 19 and 13 in control, standard, turmeric and green cardamom groups respectively, after eliminating the count of non responsive patients due to multiple reasons. To the benign patients low dose of ^{131}I (555–1073 MBq) was given and called as low RAIT (radioactive iodine therapy) group, while to the malignant patients, the high activity of radioiodine (3700–9250 MBq) was administered and termed as high RAIT group. Both low and high RAIT groups were subdivided gender wise, as "*male low RAIT*" "*female low RAIT*", "*male high RAIT*" and "*female high RAIT*" groups. The ^{131}I, of required activity, to the selected patients was administered by the expert staff of the hospital and supplementary treatments to the patients were given by the researcher, under the supervision of physician of nuclear medicine according to the following schedule.

Control group; the patients got only the ^{131}I therapy and no supplementary treatments were given.

Vitamin group; to these patients, in addition to ^{131}I, a combination of vitamin E (Capsule Evion, in a dose of 800 mg/day, in two divided doses), vitamin C (Tablet Cecon 500 mg, in a dose of 1500 mg/day, in three divided doses) and zinc sulphate (Syrup Zincday, in a dose of 40mg/day, in two divided doses) were administered.

Turmeric group; to these individuals, in addition to ^{131}I administration, a combination of turmeric rhizome powder and black pepper powder, in a ratio of 100:2 were administered, in an amount of 1500 mg/day, in three divided doses.

Green cardamom group; to these volunteers, in addition to ^{131}I administration, green cardamom in the form of grinded seeds and pods was administered in the dose of 1500 mg/day, in three divided doses.

Supplementations of phytoprotectants to the patients were started five days before the administration of ^{131}I and continued up to two weeks after RAIT, i.e. for a total period of nineteen days. Blood samples (3 mL) were collected by venipuncture 5 days before, 3 hours after and 2 weeks after RAIT (during routine visits of patients to their physicians). The blood was allowed to clot and centrifuged at 3000 rpm for 10 min at room temperature; serum was removed and stored in Eppendorf in refrigerator till biochemical analysis.

Measurement of MDA

The MDA concentration (umol/L) was measured by thiobarbituric acid reaction (TBAR) method [24]. The estimation of MDA was performed in the sera of volunteers at three different occasions, before RAIT (as a baseline), 3 hours after RAIT and 2 weeks after RAIT, in all the four groups. Before RAIT estimation of MDA and its comparison with 3 hours after RAIT and 2 weeks after RAIT created a separate control in each group. The MDA concentration in serum "Before RAIT" was compared with that of at 3 hours after RAIT and at 2 weeks after RAIT.

The reagents used for this analysis were of analytical grade, sodium dodecyl sulfate (SDS) was purchased from Sigma Aldrich (Germany) while acetic acid and thiobarbituric acid (TBA) were obtained from *BDH chemicals* (England). Serum samples (0.2 mL) were added to 18×150 mm Pyrex

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test tubes (TT) containing the reaction mixture; SDS (8.1%) 0.2 mL, 20% acetic acid solution 1.5 mL (pH 3.5) and aqueous solution of TBA (0.8%) 1.5 mL. By adding 0.6 mL of distilled water to each TT, the mixture was made up to 4.0 mL and heated at 95°C in the water bath for 30 minutes. After cooling with tap water, 1.0 mL of distilled water and a 5 mL mixture of n-butanol and pyridine (15:1, v/v) were added and shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, the organic layer was taken in a cuvette and its absorbance was measured by Cecil Aquarius CE 7200 Double Beam Spectrophotometer (UK) at 532 nm, after adjusting its zero at blank. A stanadard graph (figure 1) was drawn by using 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 µM/L concentraions of stanadrd MDA, instead of serum sample in the obove procedure. The specification of MDA standard reagent was "malondialdehyde bis (diethyl acetal); molar mass, 220.31 g/mol; Density, 0.92 g/cm3 at 20 °C" (Merck KGaA, Germany CAS-No 122-31-6).

The concentration of MDA was calculated using equation of regression (y = 0.022x + 0.005) obtained by the standard graph.

Statistical analysis

Microsoft office Excel 2010 was the tool to calculate the means ± SD of MDA levels and to draw the MDA's standard curve. Graph Pad 7 software was used to generate the figures and to perform ANOVA at significant level of 5%. The data was presented in tabulated form.

RESULTS

Control group

In control group, there was a continuous rise in serum malondialdehyde (MDA) levels from baseline time (before ^{131}I therapy) to 2 weeks after RAIT (radioactive iodine therapy). At 3 hours after RAIT, there was a 9% increase in MDA level and after 2 weeks of RAIT its rise reached upto 23% (p=0.0021) (table 1, part-A).

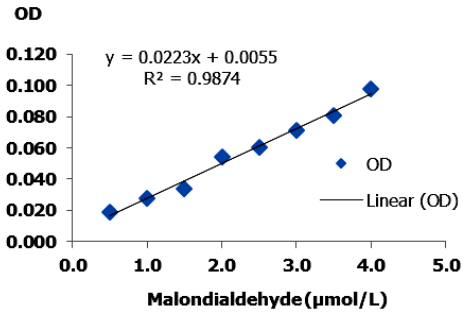


Figure 1. Standard graph representing MDA concentration (µmol/L) on x-axis vs absorbance (OD) at 532 nm, at y-axis.

Table 1. Comparison of serum MDA concentrations, in control and treatment groups; in high and low RAIT groups, in both genders.

		Mean MDA level (µmol/L)			Decrease/increase (%) in MDA level	
	Groups	Before RAIT	3 hours after RAIT	2 weeks after RAIT	3 hours after RAIT	2weeks after RAIT
A	Control	3.01±0.83	3.27±0.80	3.69±1.07	9↑	23↑
	Vitamin	3.57±0.47	3.21±0.56	2.64±0.76	10↓	26↓
	G. Cardamom	3.55±0.69	3.11±0.84	2.89±0.87	12↓	18↓
	Turmeric	3.45±0.60	2.59±0.60	2.06±0.56	25↓	40↓
B	Control, Male (High RAIT)	1.71±0.28	2.32±0.44	2.86±0.51	36↑	68↑
	Control, Female (High RAIT)	3.59±1.24	4.02±1.49	4.58±1.60	12↑	28↑
	Control, Male (Low RAIT)	2.55±0.37	3.10±0.40	3.63±0.55	22↑	42↑
	Control, Female (Low RAIT)	3.14±0.65	3.25±0.53	3.60±1.00	3↑	15↑
C	Control (High RAIT)	3.03±1.34	3.45±1.47	4.01±1.46	14↑	32↑
	Control (Low RAIT)	3.02±0.64	3.22±0.51	3.60±0.91	7↑	19↑
D	Vitamins (High RAIT)	3.57±0.37	3.25±0.20	2.55±0.93	9↓	28↓
	Vitamins (Low RAIT)	3.57±0.51	3.12±0.60	2.67±0.73	13↓	25↓
E	Male (Vit+High RAIT)	3.45±0.19	3.19±0.21	1.64±0.71	8↓	53↓
	Female (Vit+High RAIT)	3.65±0.49	3.29±0.22	3.17±0.26	10↓	13↓
	Male (Vit+Low RAIT)	3.35±0.59	3.25±0.80	2.45±0.73	3↓	27↓
	Female (Vit+Low RAIT)	3.66±0.47	3.18±0.58	2.75±0.74	13↓	25↓
F	G. Cardamom (High RAIT)	3.91±0.09	3.55±0.63	3.32±0.41	9↓	15↓
	G. Cardamom (Low RAIT)	3.32±0.81	2.97±0.69	2.88±0.84	11↓	13↓
G	Male (G.C+High RAIT)	3.83±0.03	3.37±0.31	3.36±0.38	12↓	12↓
	Female (G.C+High RAIT)	3.97±0.07	3.67±0.83	3.29±0.51	8↓	17↓
	Male (G.C+Low RAIT)	3.03±0.90	2.74±0.62	2.47±0.93	10↓	19↓
	Female (G.C+Low RAIT)	3.50±0.80	3.10±0.77	2.93±0.96	11↓	16↓
H	Turmeric (High RAIT)	3.54±0.59	2.69±0.55	2.01±0.63	24↓	43↓
	Turmeric (Low RAIT)	3.41±0.62	2.54±0.64	2.08±0.55	25↓	39↓
I	Male (Turmeric+High RAIT)	4.02±0.03	3.09±0.58	1.89±1.00	23↓	53↓
	Female (Turmeric+High RAIT)	3.30±0.59	2.49±0.49	2.07±0.57	24↓	37↓
	Male (Turmeric+Low RAIT)	3.52±0.55	2.68±0.07	1.93±0.29	24↓	45↓
	Female (Turmeric+Low RAIT)	3.38±0.66	2.52±0.70	2.11±0.59	26↓	38↓

↑, increase (%) in serum MDA level; ↓, decrease (%) in serum MDA level; RAIT, Radioactive iodine therapy; MDA, malondialdehyde; vit, Vitamins; G.C, Green Cardamom.

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The gender and dose wise comparison of MDA levels was also performed. The higher increase in serum MDA concentration was in high RAIT groups in both genders, with maximum rise of 68% in MDA concentration in males followed by 28% rise in females. In low RAIT groups the males had 45% rise in MDA levels while the females had only 15% rise (table 1, part-B). Concluding that the rise in MDA levels in males, whether received low or high activity of ^{131}I, was high as compared to that of females (Tukey's multiple comparisons test, $p<0.023$), but there was a significant difference in the rise of MDA concentrations in both genders ($p<0.0001$) after RAIT, as compared to the base line (before RAIT).

The rise in MDA level was directly associated with the dose of RAIT, higher the ^{131}I activity; higher was the rise in MDA level. The increase in MDA level was 14–32% in high RAIT group, while in low RAIT group it was 7–19% (table 1, part-C). The rise was significantly high in both groups in contrast to the baseline level.

The calculated means of MDA levels in control and treatment groups (table 1, part-A) were statistically compared. There was a significant difference in the outcome of treatments as compared to that of control ($p<0.0001$). Moreover, the MDA levels "Before vs. after RAIT" in each group were also highly significant except in green cardamom group, where the decline in MDA was non-significant. The comparison of increase or decrease in MDA levels in control and treatment groups is presented in figure 2.

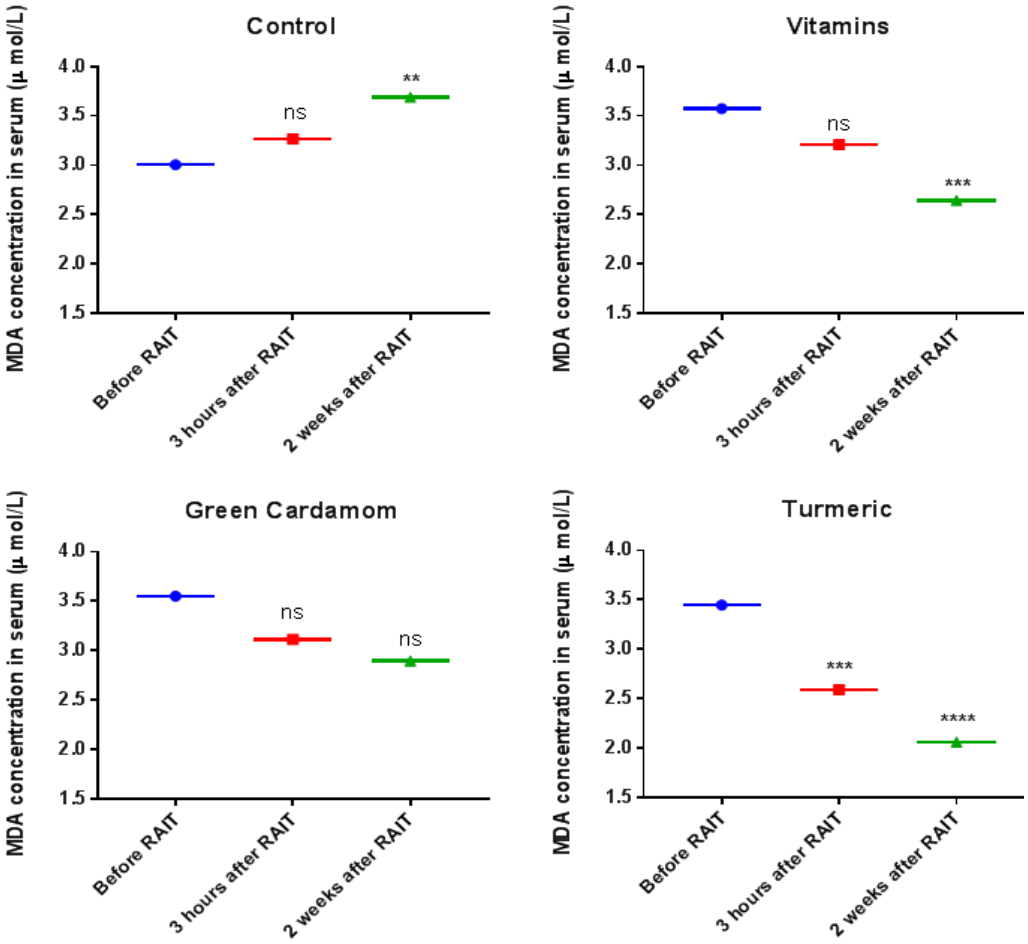


Figure 2. Comparison of serum MDA levels in control and treatment groups, ns stands for non-significant while symbols **, ***, **** show levels of significant difference from baseline (Before RAIT) in the same group, in ascending order.

Vitamin group

In vitamin group there was 10% and 26% decline in MDA levels at 3 hours and at 2 weeks after RAIT, respectively (table-1, part-A). The reduction in MDA levels (28% and 25%), was almost equal in both high and low RAIT groups (table 1, part-D). Gender wise analysis revealed that there was 53% and 27% reduction in MDA levels in males treated with high and low activity of RAIT respectively, while 13% and 25% reduction in MDA levels was in females receiving high and low activity of ^{131}I respectively, at 2 weeks after RAIT (table 1, part-E). Both genders were equally benefitted by the supplementation of vitamins irrespective of the dose of ^{131}I.

Green cardamom group

In individuals supplemented with green cardamom (G.C) there was no rise, even there was

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drop in serum MDA level and this decline in MDA concentration was 12% and 18% at 3 hours and 2 weeks after RAIT respectively (table 1, part-A). The pre-supplementation of G.C provided almost of same level of radioprotection in both high (9–15%) and low RAIT (11–13%) groups (table 1, part-F). Similarly both genders were almost equally benefited with supplementation of G.C in reducing the MDA levels after RAIT (p=0.0749) (table 1, part-G).

Turmeric group

In turmeric group there was significant reduction in serum MDA level at both the occasions viz. at 3 hours (p=0.0001) and at 2 weeks (p<0.0001) after RAIT (table 1, part-A). The radioprotection of pretreatment of turmeric was found excellent in both malignant and benign thyroid patients, showing 43% and 39% reduction of MDA levels respectively, in these patients (table 1, part-H). Maximum reduction of 53% in serum MDA concentration followed by 45% decline in its level was observed in males, treated with high and low activity of ^{131}I, respectively at 2 weeks after RAIT (table 1, part-I). Both males and females suffering from benign or malignant thyroid disorder were almost equally (p=0.5641) benefited by pre-supplementation of turmeric before RAIT. The gender wise response of supplementary treatments viz. vitamins, green cardamom and turmeric in cancerous thyroid patients receiving high activity of ^{131}I is compared in figure 3.

Vitamins exhibited good response in male thyroid patients, but turmeric supplementation presented better radioprotective response in both genders treated with low activity of ^{131}I (figure 4).

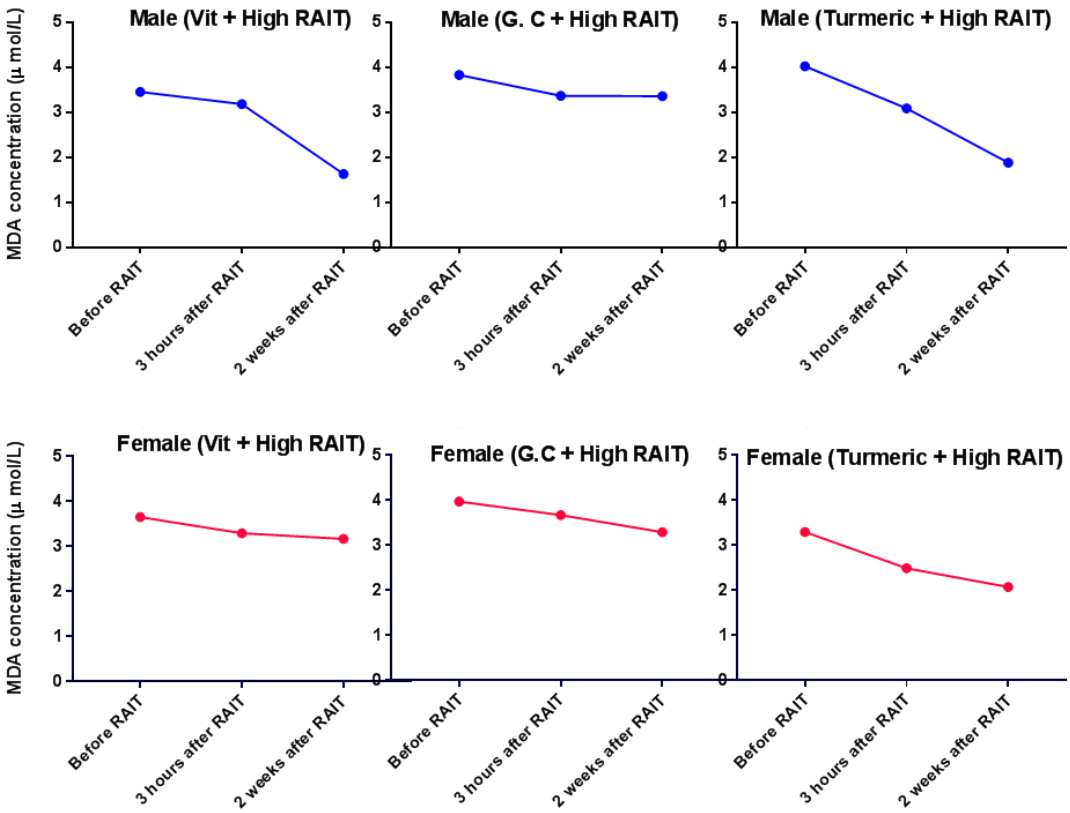


Figure 3. Effects of supplementary treatments on MDA levels in males and females suffering from thyroid cancer treated with high activity of 131-I; Where Vit stands for vitamin group, G. C for Green Cardamom, RAIT for radioactive iodine therapy.

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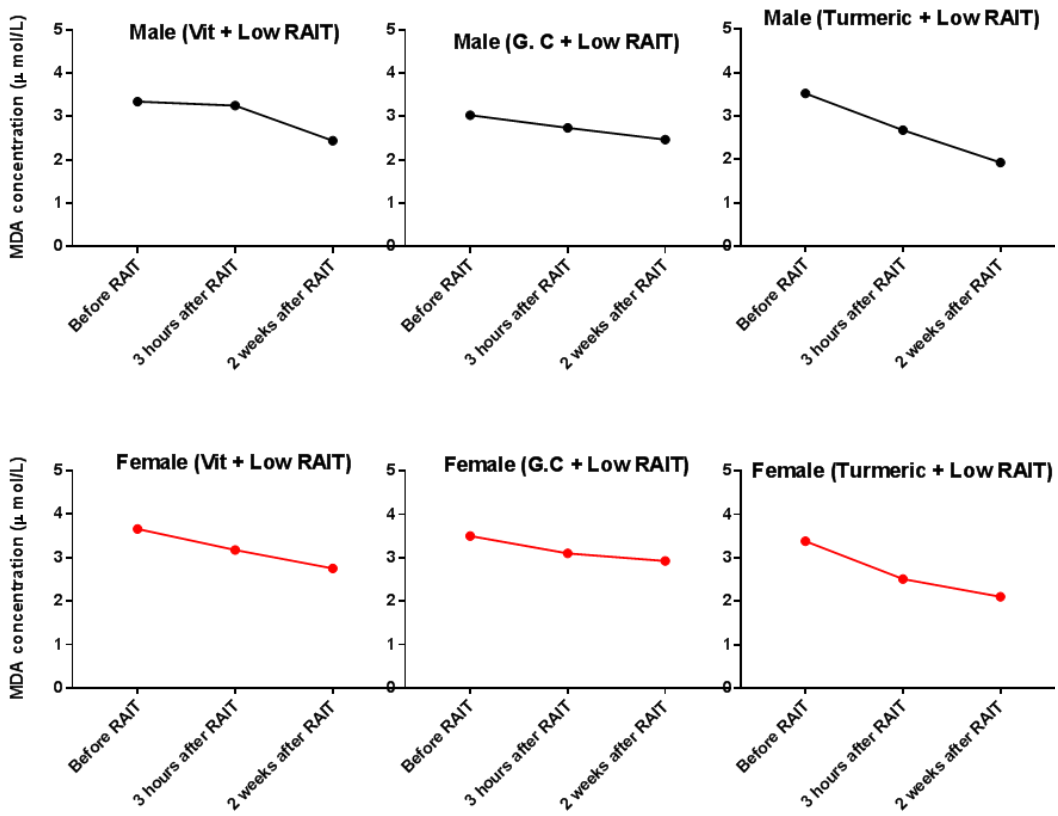


Figure 4. Effects of supplementary treatments on MDA levels in males and females suffering from benign hyperthyroid disorders treated with 131-I; Where Vit stands for vitamin group, G. C for Green Cardamom, RAIT for radioactive iodine therapy.

DISCUSSION

The rise in serum MDA concentration after RAIT was due to generation of free radicals by ^{131}I, resulting in lipid peroxidation in the membranes of the cell, especially those of red blood cells being rich in poly un-saturated fatty acids and in low density lipoprotein (LDL) circulating in blood [25, 26]. Due to high reactivity, MDA is the molecule of great attention in biomedical research and its estimation is an important marker of oxidative stress [27]. After ^{131}I therapy, oxidative stress in the form of lipid peroxidation was induced within three days of its administration and in the sera of these patients; the concentration of MDA was significantly raised upto 2 weeks [28]. Radioiodine therapy usually reduces the antioxidants and antioxidative enzymes in the body that leads to increased MDA level [29]. Vrndic *et al.* [30] reported significant increase in serum MDA level from 2 days up to a week after RAIT, but Alavi *et al.* [28] observed a significant increase in MDA level on 3rd day after RAIT. Males were found more susceptible to oxidative stress induced by RAIT as compared to females. The resistance in women is better against oxidative stress than men [31], particularly concerning the MDA biomarker. Gender specific dissimilarities in the predisposition of lipids to peroxidation may be ascribed to the higher levels of antioxidant sex hormone "oestradiol" in females [32].

The lipid peroxidation is minimized by the supplementation of natural antioxidants or phytoprotectants prior to administration of ^{131}I. Pre-treatment with phytoprotectants exhibited significant reduction through scavenging of ROS upto the extent of 40% in the serum MDA levels [33]. The radioprotection in the terms of reduced MDA levels in supplementary groups was greater in high RAIT patients as compared to that in low RAIT ones, at 2 weeks after RAIT. It might be explained that high activity of ^{131}I was administered to the thyroid cancer patient after thyroidectomy and there was lesser stay of ^{131}I in thyroidectomized patients having minimum thyroidal tissue to retain iodine.

Vitamin C and E along with zinc are potent antioxidants to provide safeguard against oxidative stress induced by ROS/free radicals, therefore their supplementation can inverse the hostile effects of radiation therapy [34]. Vitamin C and E administered in combination, proved to be good hunters of free radicals and offered radioprotection during radiotherapy [35]. Vitamins exhibited good response in male thyroid patients. Prior treatment with vitamin C can minimize the ^{131}I induced oxidative stress and leads to a significant decrease in MDA, indicating a relatively good radiation mitigative effect [36].

Green cardamom proved to be a notable phytoprotectant, by inhibiting the rise in MDA levels and even by reducing its concentrations in patients who were treated with ^{131}I. Green cardamom is rich in antioxidant polyphenolics that retain antioxidant

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potential and cause stimulation of the antioxidant defense system in the body [37, 38].

The decline in serum MDA levels due to turmeric supplementation was greater than vitamins and green cardamom administration. Turmeric has the potential to increase the antioxidative capability of the body and significantly reduces the serum MDA levels in oxidative stress [39]. The turmeric was the most effective antioxidant/radioprotective nutraceutical in respect to inhibition of lipid peroxidation in ^{131}I treated patients. Relatively better response of turmeric supplementation was in males than in females. The turmeric group showed prominent reduction in MDA as compared to that in control and green cardamom groups and even was better than the vitamin group. Japanese scientists came to the conclusion in a study in which rats undergoing whole body high dose irradiation (9.6 Gray), resembling the natural disaster of Chernobyl, produced lesser amount of oxidative metabolites such as MDA and 4-hydroxynonenal (4-HNE) if curcumin was fed as pre and post radiation therapy, as compared to that in control group [40].

In short, the administration of both phytoprotectants, green cardamom and turmeric, to the patients of thyroid diseases before administration of ^{131}I, was helpful in mitigating the harmful effects of radioactive iodine. The supplementary treatments provided the radioprotection to the body in the form of significant reduction in the generation of toxic aldehyde called malondialdehyde, the levels of which were raised by RAIT. Conclusively the turmeric was proved as a nutraceutical radioprotectant which was found equally effective in malignant and benign thyroid patients being treated with ^{131}I, in both genders.

CONCLUSION

The therapeutic use of ^{131}I generates ROS and causes a significant rise in MDA levels in serum within 3 hours to 2 weeks. The supplementation of phytoprotectants viz. green cardamom and turmeric being antioxidants can counter the ROS and protect the normal tissues against the oxidative stress. The turmeric (*Curcuma longa*) was found better radioprotectant as compared to green cardamom (*Eletteria cardamomum*) and even more effective than the combination of vitamin C and E and zinc, in both malignant and benign thyroid patients, when used in conjunction with ^{131}I therapy.

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