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EVALUATION OF THE RADIOPROTECTIVE EFFECT OF TURMERIC EXTRACT AND VITAMIN E IN MICE EXPOSED TO THERAPEUTIC DOSE OF RADIOIODINE

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ABSTRACT

The aim of this study was to evaluate the radioprotective effect of turmeric extract (40 mg/kg body weight) and vitamin E (α- tocopherol acetate, 400 IU/kg body weight) supplementation on lipid peroxidation, reduced glutathione and antioxidant defense enzymes in various organs like liver, kidney and salivary glands at 24 h in adult Swiss mice. ^{131}Iodine exposure significantly increased lipid peroxidation in kidney and salivary glands in comparison to control animals. Pre supplementation with turmeric extract for 15 days showed significant lowering of lipid peroxidation in kidney. On the other hand vitamin E pre supplementation showed marked reduction in lipid peroxidation in salivary glands. Reduced glutathione levels decreased significantly in liver after radiation exposure. However, pre supplementation with turmeric extract and vitamin E did not improve glutathione levels in liver. In conclusion, we have observed differential radioprotective effect of turmeric extract and vitamin E in kidney and salivary glands. However, Vitamin E seems to offer better radioprotection for salivary glands which is known to be the major site of cellular destruction after radioiodine therapy in patients.

KEY WORDS

Turmeric extract, Vitamin E, ^{131}Iodine, Lipid peroxidation, Antioxidant enzymes.

INTRODUCTION

Radiotherapy uses ionizing radiation to produce cell death mainly through free radical formation. In thyroid cancer patients, oral radioiodine therapy after thyroidectomy is also known to induce oxidative stress thereby causing considerable damage to cellular components such as lipids, proteins and DNA (1,2). However, in addition to the intracellular antioxidants such as glutathione and enzymes like glutathione transferase, reductase, peroxidase and superoxide dismutase, certain chemical substances known as antioxidants scavenge reactive oxygen species to reduce the free radical mediated oxidative stress (3). Currently there is considerable interest in antioxidants of dietary origin such as vitamin E and C, carotenoids and plant polyphenols, epigallocatechin and flavonoids (3,4). However until now there is not a single radioprotective agent available which fulfills all the requirements of an ideal radioprotector (5).

Our earlier work has shown the radioprotection offered by oral supplementation of *O. Sanctum L* in organs like liver, kidney and the salivary glands of mice exposed to high dose of ^{131}Iodine (6). In continuation with our earlier study, we have tried to compare whether dietary antioxidant like turmeric extract and vitamin E (α-tocopherol acetate) can provide radioprotection in experimental animals following *in vivo* ^{131}Iodine exposure. Turmeric extract is one of the most popular natural antioxidant which is known to be non-toxic, easily available with various pharmacological actions (7). On the other hand, vitamin E is one of the most potent free radical scavengers, which is reported to significantly reduce damage to DNA and cell membrane peroxidation (8). We have selected oral route of administration of radioiodine as well as antioxidants as ^{131}Iodine is administered orally to patients with thyroid cancer and it is convenient to use oral radioprotector in human population.

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The present study reports the extent of cellular damage and the changes in antioxidant defense enzymes and the levels of reduced glutathione in various organs like liver, kidney and salivary glands in mice 24 h after high dose of ^{131}Iodine exposure. Further, the possible radioprotection observed by supplementing the animals with turmeric extract and vitamin E orally for 15 days preceding to radiation exposure is studied.

MATERIALS AND METHODS

Male adult Swiss mice of 8 to 10 week old, weighing 25 to 30 g obtained from Animal House Facility, Bhabha Atomic Research Centre, were used for the present study. Animals were maintained under controlled conditions of temperature and light in an animal house and with free access to water and standard mouse diet. Animal studies were performed in compliance with Bhabha Atomic Research Centre Institutional Animal Ethics Committee's guidelines.

Total 72 mice were divided into six different groups and treated as:- Group I – Control animals on normal diet; Group II - ^{131}Iodine orally (3.7 MBq, obtained from Board of Radiation & Isotope Technology, Vashi, India.) 24 h prior to sacrifice; Group III - Turmeric extract alone (40 mg/kg body weight, obtained from Saiba Industries Pvt. Ltd, Mumbai India) supplemented orally for 15 days preceding sacrifice; Group IV - Turmeric extract (40 mg/kg body weight) supplemented orally for 15 days and 3.7 MBq ^{131}Iodine orally 24 h after the last dose of turmeric extract; Group V – Vitamin E alone (400 IU/Kg body weight, obtained from Loba Chemicals Pvt. Ltd, Mumbai India) supplemented orally for 15 days preceding sacrifice; Group VI – Vitamin E (400 IU/Kg body weight) supplemented orally for 15 days and 3.7 MBq ^{131}Iodine orally 24 h after the last dose of vitamin E.

The animals were sacrificed by cervical dislocation 24 h after the oral administration of ^{131}Iodine. Each group consisted of 12 animals. The liver, kidneys and salivary glands were dissected out, thoroughly washed in ice cold saline (0.9 %), dried and weighed. The tissues were homogenized with 9 volumes of cold phosphate buffer (*p*H 8) with 5 mM EDTA and 25mM sucrose. The homogenate was used to measure malondialdehyde (MDA) content as an index of lipid peroxidation (9), reduced glutathione (10) and protein (11). The remaining homogenate was treated with Triton X-100 (0.05%) and centrifuged at 10,000 g for 30 mins at 4ºC. The supernatant obtained was used to estimate glutathione peroxidase (GPx) (12) , Catalase (13) and superoxide dismutase (SOD) (14).

All values were expressed as mean ± SD. Data were tested for normal distribution and homogeneity of variance (Levence test). The ANOVA with Bonferroni correction has been used for the normally distributed data and Mann-Whitney U test has been used for non-normal data. *P* value < 0.05 was considered to be significant.

RESULTS

None of the experimental animals showed any noticeable toxic effect following supplementation of turmeric extract and

Table 1: Effect of turmeric extract and vitamin E supplementation on the various antioxidant defense enzymes in liver(A), kidney (B) and salivary gland (C) of experimental mice (Mean ± SD)

Parameters	Organs	Group I	Group II	Group III	Group IV	Group V	Group VI
GP_X (10^4x U/gT)	A	16.4 ± 3.8	27.2 ± 4.9 [a]	17.9 ± 0.9	27.5 ± 3.3 [b]	15.5 ± 2.8	12.6 ± 2.5 [c,d]
	B	17.8 ± 3.6	28.5 ± 4.1 [a]	20.4 ± 2. 4	26.7 ± 5.7 [e]	22.3 ± 4.9	26.7 ± 1.6 [f]
GP_X (10^3 x U/gT)	C *	95 ± 13	97 ± 13	93 ± 15	78 ± 18	99 ± 18	105 ± 15
Catalase (U/gT)	A	1373 ± 316	1268± 301	1641±369	1307 ± 220	1030 ± 201[g]	1122 ± 206
	B *	739 ± 125	568 ± 147	724 ± 140	643 ± 116	701 ± 166	629 ± 161
	C	135 ± 18	144 ± 30	158 ± 42	166 ± 22	124 ± 18	136 ± 52
SOD (U/gT)	A	363 ± 17	364 ± 10	365 ± 17	356 ± 13	360 ± 11	357 ± 23
	B *	301 ± 13	296 ± 18	292 ± 21	312 ± 26	290 ± 34	296 ± 21
	C	99 ± 28	102 ± 21	109 ± 15	94 ± 17	90 ± 6	95 ± 18

* Statistical analysis done by ANOVA, rest of the statistical analysis done by Mann-Whitney U test; Liver, Kidney n=12, Salivary gland n=9
p values: a- p = 0.0001 vs Gr I , b- p = 0.0001 vs Gr III , c- p = 0.0001 vs Gr II,
d- p = 0.024 vs Gr V, e- p = 0.014 vs Gr III , f- p = 0.012 vs Gr V, g- p = 0.009 vs Gr I

vitamin E for 15 days duration. In addition, 3.7 MBq oral dose of ^{131}Iodine did not give any morbidity or mortality in animals up to 24 h.

Figure 1 shows levels of lipid peroxidation in liver, kidney and salivary glands of animals from all the groups. Lipid peroxidation levels remained unaltered in liver tissue in all the six groups. On the other hand levels of lipid peroxidation increased significantly in kidney and salivary glands in animals exposed to ^{131}Iodine (Gr II) in comparison to controls. However, pretreatment with turmeric extract (Gr IV) in kidney and vitamin E (Gr VI) in salivary glands showed significant reduction in MDA levels.

Table 1 shows the activity of various defense enzymes such as GPx, catalase and SOD in all the six groups in liver, kidney and salivary glands. Significant elevation in liver GPx was observed after ^{131}Iodine exposure which remained elevated even after pretreatment with turmeric extract. On the other hand, pretreatment with vitamin E exhibited marked reduction in liver GP_X levels. However levels of GPx in kidney showed marked increased in all the three radioiodine exposed groups irrespective of their pretreatment with turmeric extract or vitamin E. Catalase levels showed significant lowering in liver tissue of vitamin E supplemented mice (Gr V).

Figure 2 shows GSH levels in all the six groups in liver, kidney and salivary glands. Significant lowering of GSH was observed in ^{131}Iodine exposed groups irrespective of their supplementation with turmeric extract or vitamin E in liver (Gr II, IV & VI).

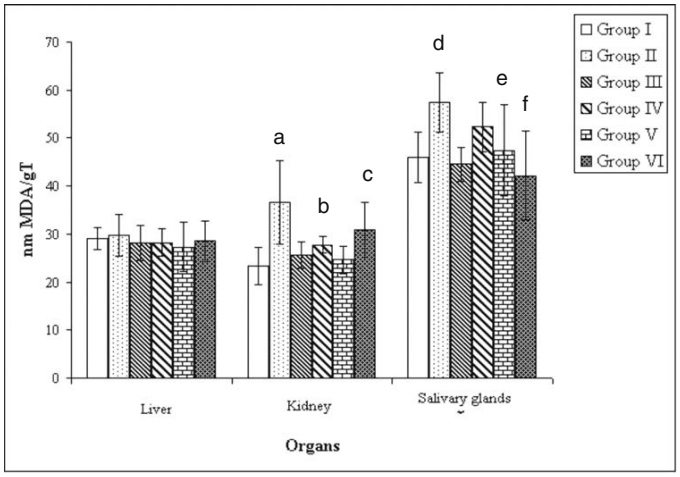

Figure 1: Effect of turmeric extract and vitamin E supplementation on lipid peroxidation in various organs in control and experimental mice.

a – p = 0.0001 vs Group I; b – p = 0.006 vs Group II
c – p = 0.013 vs Group V; d – p = 0.001 vs Group I
e – p = 0.0001 vsGroup III; f – p = 0.0001 vs Group II

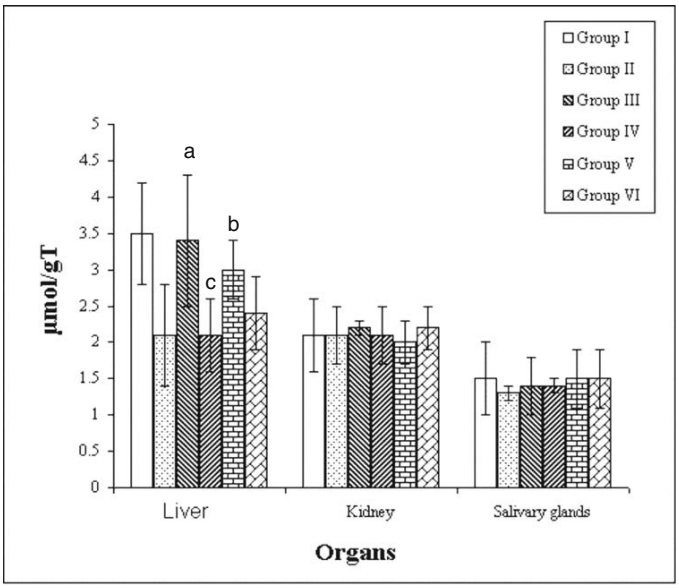

Figure 2: Effect of turmeric extract and vitamin E supplementation on tissue GSH Levels in control and experimental mice.

a - p = 0.0001 vs Group I; b - p = 0.045 vs Group I;
c - p = 0.0001 vs Group III

DISCUSSION

^{131}Iodine is a well-known radionuclide which emits beta as well as gamma rays and is extensively used *in vivo* particularly for the treatment of hyperthyroidism and thyroid cancer. Unlike external radiation, radionuclides *in-vivo* generally deliver a radiation dose over an extended period depending upon their physical and biological half life (15). Ionizing radiation causes cellular damage mainly by formation of reactive oxygen species. Therapeutic exposure to ^{131}Iodine is known to generate the oxidative stress and cause cellular damage in target organs involved in concentrating radioiodine (16). Due to the presence of sodium iodide symporter salivary glands are known to concentrate high amount of radioiodine causing irreversible damage and ultimately impairing the quality of life of the patients (16,17). Liver plays a major role in thyroid hormone metabolism and is likely to get exposed to radioiodine. Kidneys are known to excrete the major portion of radio iodine and thus get maximum exposure to radiation (18). Therefore, the present experiments were conducted so as to study the oxidative stress generated in organs like liver, kidney and salivary glands and the possible radioprotective effect of natural antioxidants like turmeric extract and vitamin E in these organs.

Turmeric is known to consist of water soluble turmerin and lipid soluble curcumin with potent antioxidant properties (19,20). Turmeric and its constituents have shown cellular

protection against H_2O_2 induced renal epithelial cell injury (19). In the present study we have also observed membrane protective effect of turmeric extract as shown by the significant reduction in lipid peroxidation levels in kidneys (Gr. IV) along with raised levels of GPx. This may be a cumulative effect of $OH^{\cdot}$ radical quenching and GPx related detoxification of the reactive oxygen species. Kidneys are known to receive substantial radiation exposure after ^{131}Iodine therapy in thyroid cancer patients (18). Similar could be the cause of observed increase in lipid peroxidation within 24 h in our experimental animals. However, permanent kidney damage has not been observed in the patients receiving ^{131}Iodine therapy, which suggests the transient nature of the damage in kidney (21). Long term exposure to radioiodine may prove this experimentally.

The most active form of vitamin E, α-tocopherol, prevents lipid peroxidation chain reaction in the cell membrane (8). Our study shows the protective effect of vitamin E in salivary gland as demonstrated by pronounced decrease in lipid peroxidation. From the above observation it is clear that vitamin E is effective in ameliorating the salivary gland membrane damage.

Our experimental results have shown pronounced lowering of MDA levels in kidney by turmeric extract and significant reduction in salivary gland MDA levels by vitamin E pre supplementation. It is clear from the present observations that turmeric extract and vitamin E shows differential protective effect on these two organs of the same animal receiving equal amount of radioiodine exposure. Baydas *et al.* in their study on protective effect of melatonin and vitamin E in animal model of diabetes mellitus have also noted such differential nature of protection offered by vitamin E and melatonin (22). Avti *et al* have observed the diverse responses of antioxidant defense system in liver and lung of mice after low dose (<50cGy) whole body gamma irradiation (23). They have attributed the observed discrepancy to the difference in sensitivities of various organs to the ionizing radiation (23). It is possible that in the present study the differential responses of antioxidant defense system in the liver, kidney and salivary gland of mice after radioiodine exposure *in vivo* might be due to the variable sensitivities in these organs as well as difference in their extent of radioiodine exposure.

Reduced glutathione is the main intracellular non- protein thiol, which is a direct radical scavenger, and also acts as an essential cofactor for enzymes reducing oxidative stress (24). Our study demonstrated lowering of GSH in liver in animals exposed to radiation (Gr. II, IV and VI). Radiation exposure is known to lower the tissue GSH levels (3). Decreased level of GSH is generally considered as an index of increased oxidative stress. It may indicate inability of the cells to generate enough GSH, due to severe cellular damage or due to greater utility in combating the oxidative stress (3). In the present study liver GSH remained low in all the radioiodine exposed groups irrespective of their antioxidant pre supplementation. Lowering of GSH levels can lead to increased lipid peroxidation with concomitant changes in membrane permeability and cellular damage (23). However in the present study liver lipid peroxidation levels remain unaltered at 24 hour of ^{131}Iodine exposure. Avti *et al* have observed increase in liver lipid peroxidation at 12 h which returned back to normal at 24 h after low dose gamma radiation exposure (23). Uma Devi *et al*. have noted significant rise in liver lipid peroxidation after 2 h of ^{60}Co gamma radiation exposure which normalized by 24 h (3), which is in accordance with our observation at 24 hrs.

Major aim of our study is to assess the potential of turmeric extract and vitamin E as a radioprotective agent against ^{131}Iodine induced *in vivo* radiation damage. Salivary gland dysfunction is the major long-term side effect after high dose ^{131}Iodine therapy in thyroid cancer patients. So far Amifostine is the only drug available for reducing the side effects of ionizing radiation in salivary glands (17). However, amifostine suffers from several side effects, which limits its use in thyroid cancer patients (17,25). In addition, exorbitant cost of the drug makes its use difficult in majority of the patients. Till today search for an ideal radioprotective agent is going on for salivary gland after radioiodine therapy. Herbal products and vitamins are being assessed for their potential to be an ideal radioprotectant which could be nontoxic, safe and easily available and cost effective. In our study we have made an effort to evaluate the radioprotection offered by turmeric extract and vitamin E against therapeutic ^{131}Iodine exposure for 24 h. In this regard we have observed the differential nature of radioprotection offered by turmeric extract and vitamin E in different tissues. In conclusion vitamin E seems to be a better radio protective agent as compared to turmeric extract for salivary gland damage in mice.

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