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RADIOPROTECTIVE EFFECTS OF OCIMUM FLAVONOIDS ON LEUKOCYTE OXIDANTS AND ANTIOXIDANTS IN ORAL CANCER

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ABSTRACT

Oxidants (NADPH oxidase and myeloperoxidase) and antioxidants (GSH,GSH peroxidase, SOD and glucose 6 phosphate dehydrogenase , that provides NADPH for antioxidants) were assayed in the neutrophils from oral cancer patients, in three stages viz , baseline samples,15 days after radiation and 30 days following radiation. These samples were obtained from 2 groups of patients. Group A that received radiation alone and Group B that received radiation and ocimum flavonoids,a radioprotector. The results showed a significant fall in the SOD levels in the second follow up of group B.Glucose 6 phosphate dehydrogenase showed significant increase only in the first follow up of patients who received Ocimum flavonoids. Except for these findings all other parameters remained statistically nonsignificant.

KEY WORDS

Oxidants,Antioxidants,Radiotherapy,Radioprotector, Ocimum flavonoids

INTRODUCTION

Radiotherapy as a measure of treatment generates superoxide to bring about the killing of tumor cells. Certain reports indicate increased levels of O_2^- in the granulocytes of mice subjected to total body irradiation(1). A wide range of compounds have been investigated in the recent past for their efficacy to protect against radiation damage(2). The aqueous leaf extracts of ocimum sanctum have been shown to have antitumor activity(3), antiulcerogenic activity(4), chemopreventive activity(5) and also radiomodulating effect that protects the mice against radiation lethality(6). Flavonoids that offered radioprotection in mice was identified to be orientin and vicenin (7) which spared the tumor tissues(8). However references for relevant studies in human subjects are scarce. Since the mechanism of action attributed to Ocimum flavonoids (OF) is mainly by free radical scavenging, we sought to determine the levels of oxidants and antioxidants in the neutrophils from oral cancer patients subjected to radiation and treatment with OF in order to study the effect of OF as a radioprotector in human cancers.

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MATERIALS AND METHODS

Patients suffering from squamous cell carcinoma of oral cavity and oropharynx(n=34) were considered for the study. All patients were treated with radiation at Kasturba Medical college, Hospital, Mangalore, India. Inclusion criteria-All patients selected belonged to both sex and were aged between 30 and 70 years. Stage III and stage IV (TNM staging) of cancers were considered for the study. All cancer patients were selected based on the karnofsky's performance scale KPS>70% (Cares for self but unable to carry out normal activity:shows some signs or symptoms of the disease). The patients had no previous history of treatment. All patients were subjected to thorough clinical examination and those with severe systemic illness like diabetes mellitus, coronary artery disease and tuberculosis were excluded. Age and sex matched healthy non hospitalized controls(n=30) were considered for the comparative study with the patients. After selecting the patients , the study group were divided randomly into 2 groups - *GroupA:* Patients(n=17) received radiotherapy at a dose of 60 Gy in 30 fractions over 6 weeks. *GroupB;* Patients(n=17) received radiotherapy at the same dosage and

a radioprotector, ocimum flavonoids capsules. These capsules were prepared according to the reported protocol (9). Each capsule consisting of 60 mg (1.32mg/ kg body wt) of the drug, was given to the patients orally, half an hour prior to each sitting, during all sittings. Institutional Ethics committee had approved the drug trials

5ml of venous blood was collected from the patients ,in heparinised vacutainers in 3 stages : Baseline sample before radiations and then after 15 days (I follow up sample) & 30 days (II follow up sample) of radiation. Only seven patients were available for II follow up sampling. Likewise 5ml of blood was collected from controls.

Leukocyte suspension was prepared according to the reported protocol (10). NADPH oxidase was determined by monitoring the reduction of cytochrome c by the superoxide generated by the enzyme in the presence and absence of superoxide dismutase (11*)*. Myeloperoxidase (MPO) was estimated by the method of Matheson(12).Glucose6 phosphate dehydrogenase (G6PD) was estimated by recording the rate of change of absorbance at 340nm due to the production of NADPH by this enzyme (13).The method for estimating glutathione (GSH) was based on the method of Beutler(14). Glutathione peroxidase (GSH-Px) activity was determined by recording the decrease in absorbance due to depletion of NADPH at 340nm for 5 min (15). Superoxide dismutase (SOD) was determined according to the method of Beauchamp and Fridovich(16*)*, based on inhibition of nitroblue tetrazolium reduction. Protein content in the leukocyte suspension was determined by Lowry's method(17).All enzyme activities were expressed as Units / mg protein in the leukocyte suspension.

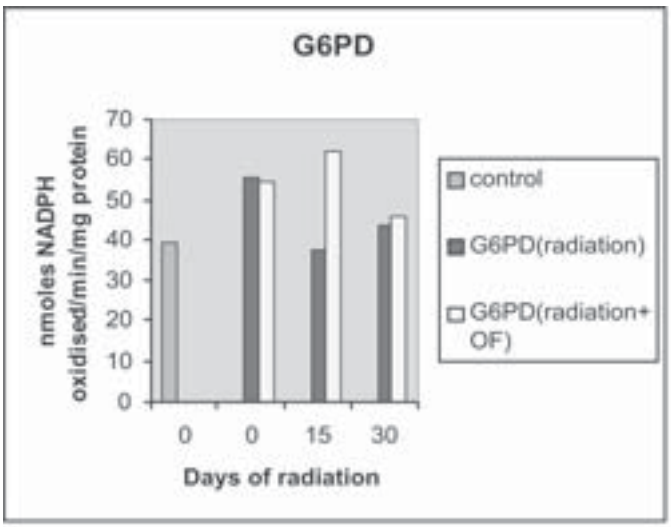
Data obtained were analysed using Mann Whitney 'U' test for comparison between independent groups. Wilcoxon's rank sign test was used for comparing the follow up cases. Whenever the baseline data for certain parameters showed wide variations in 2 groups, mean difference for the paired values in baseline and follow up cases were analysed by student's 't'test. The differences were considered significant when the probability was $p<0.05$.

RESULTS

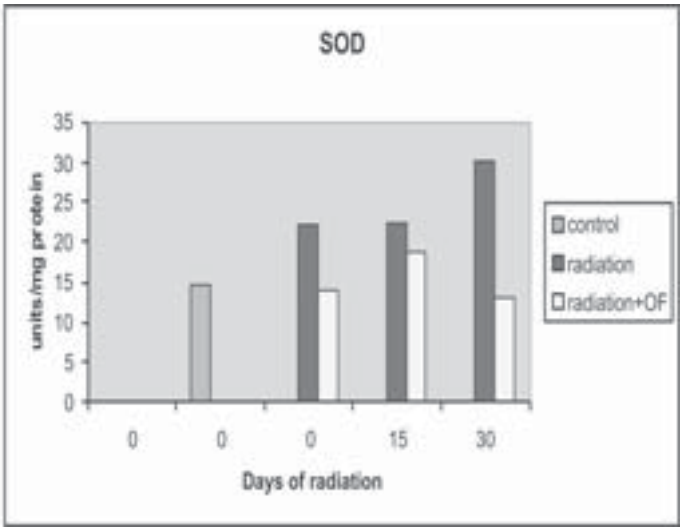
Results of the study are displayed in Tables 1 and 2. Results indicated an apparent decrease in NADPH oxidase activity in the cancer patients compared to controls. There was a progressive and consistent decrease in its activity following RT. But these observations were not significant. No significant changes were observed with respect to other enzymes studied namely MPO,GSHPX,G6PD and GSH when comparison were made between control and patients, so also in the baseline and follow up samples. A significant increase in SOD was observed in the second follow up samples compared to baseline,in the radiation treated group. Following treatment with ocimum flavonoids,again no significant changes were observed in the various parameters studied except for a significant rise in G6PD in the first follow up samples and a significant fall in SOD in the second follow up samples compared to first followup.

DISCUSSION

Earlier works have observed that there was no remarkable increase in O_2^- generation in cancer patients treated with



Effect of Radiation and Ocmium flavonoids on G6PD activity



Effect of Radiation and Ocmium flavonoids on SOD activity

Table 1 : Leukocyte parameters in controls and patients treated with radiation (Mean±SEM)

	Controls	Patients		
Parameters	n=30	Baseline n=17	I followup n=17	II follow up n=7
NADPH oxidase nmoles of O_2^-produced/mg protein	40.61±7.81	33.89±11.31	22.5 ±8.06	11.90±9.36
Myeloperoxidase U/mg protein	3.22 ± 0.43	2.56± 0.42	3.09±0.67	2.86±1.34
Glutathione nmol/mg protein	348.31 ± 106.44	347.66 ± 108.80	268.73 ± 105.5	296±156.69
Glutathione peroxidase nmol of NADP reduced /min/mg protein	98.32 ± 21.39	58.25 ±13.78	96.49±25.48	375.4±186.15
Glucose 6 phosphate dehydrogenase nmol of NADPH oxidised/min/ mg protein	39.54±4.25	55.75±9.24	37.79 ± 6.11	43.75±11.97
Superoxide dismutase U/mg protein	14.66± 2.54	22.30±3.14	22.35 ± 2.55	30.26± 3.41[a]

a = *P*<0.05 significant between 1st and 2nd follow up

Table 2 : Leukocyte parameters in controls and patients treated with radiation and Ocimum flavonoids (Mean±SEM)

	Controls	Patients		
Parameters	n=30	Baseline n=17	I followup n=17	II follow up n=7
NADPH oxidase nmoles of O_2^- produced/ mg protein	40.61±7.81	16.13±5.72	9.86±3.76	20.60±13.17
Myeloperoxidase U/mg protein	3.22±0.43	4.910±0.60	5.20±0.87	8.61±2.99
Glutathione nmol/mg protein	348.31±106.44	707.90±321.98	675.84±132.1	1337.14±589.50
Glutathione peroxidase nmol of NADP reduced /min/mg protein	98.32±21.39	104.19±26.67	99.46±24.19	118.85±27.20
Glucose 6 phosphate dehydrogenase nmol of NADPH oxidised/min/ mg protein	39.54±4.25	54.44±8.20	61.76±8.00[b]	45.83±7.80
Superoxide dismutase U/mg protein oxidised/min/ mg protein	14.66±2.54	14.04±1.81	18.83±2.09	13.08±3.17[a]

a=*P*<0.05 comparison between first and second follow up: b=P<0.05 comparison between baseline and first follow up of GroupA and Group B (mean difference)

radiation(18).Lower etal(19), have reported a decrease in H_2O_2 production by monocytes of untreated breast cancer patients .An apparent decrease in NADPH oxidase activity was observed in the present work before and after radiation which were not significant. Although earlier studies have reported a decrease in MPO activity in cancers in general(20) and an increase in activity in lung cancer in particular(21) the present work observes no change in this enzyme activity. Non significant results with respect to G6PD in cancer patients is in agreement with earlier works (22). A significant increase in G6PD in the first follow up samples of the group that received ocimum compared to radiation treated group, was observed probably due to an induction of this enzyme by ocimum flavonoids. SOD is found to increase significantly after radiotherapy. This enzyme may be induced by RT as an adaptive response, to scavenge the increased O_2^- generated by radiation. This could be supported by the fact that SOD was being used as a radioprotector to provide protection against secondary injuries caused by RT(23).OF treatment has resulted in decrease in SOD in the second follow up samples.Probably OF would have acted as an antioxidant by itself, sparing the effect of SOD, because free radical scavenging is a likely mechanism of action of OF as stated by Devi PU etal(24). Besides these , the other enzymes were neither influenced significantly by radiation nor by OF.

An increase in enzyme activities (GSHPx,SOD,GSH reductase) in the liver of mouse treated with radiation and OF have been reported(9). In addition of proved to be a good radioprotector in mice as it protected mouse bone marrow against radiation induced chromosome damage and stem cell death (25).Therefore it could be concluded that effect of radiation/radioprotector could be dependant on factors like differences in species,duration of exposure to radiation and dosage of radioprotector used in the studies.

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