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RESEARCH ARTICLE

EVALUATION OF IRRADIATION INDUCED OXIDATIVE INJURY OF SWISS ALBINO MICE USING *GARCINIA INDICA* OF METHANOL EXTRACT

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ABSTRACT

The current research is to investigate the antioxidant activity of different doses of *Garcina Indica* against gamma irradiation oxidative damage in the liver tissue with caused single dose of 6Gy. The Malondialdehyde (MDA), Cu,Zn-superoxide dismutase (Cu,Zn-SOD) and selenium-dependent glutathione peroxidase (GSH-Px) are currently considered to be basic markers of oxidative stress. MDA is one of the end-products of the peroxidation of membrane lipids, whereas enzymes Cu,Zn-SOD and GSH-Px belong to the natural antioxidants. The mice were divided into five group with twenty mice each, the group one was with 5mg/kg and group two with 10mg/kg of *Garcina Indica* extract, and the third group was injected with an isotonic Nacl solution, the fourth group was injected only with *Garcina Indica* and the fifth was observed as control. Following with a time span of 30 minutes, 6Gy total body irradiation was given to groups one to three in a single dose. The activities were measured in all groups for MDA, SOD and GSH-Px, total body irradiation results in increase in the liver tissue MDA levels and a decrease of SOD and GSH-Px activities and it shows that liver tissue MDA levels in irradiated mice that were pre treated with *Garcina Indica* were decreased significantly, where SOD and GSH-Px activities were significantly increased. Thus the reported result suggests that *Garcina Indica* administration prior to irradiation may prevent liver damage by irradiation.

INTRODUCTION

A search for the chemical agents that are able to protect human beings from ionizing radiation is a key issue in radiation biology (Nair *et al*., 2001). Radiation produces various pathological changes in living systems and these changes were reduced with the help of certain synthetic chemicals such as cys-teine (Patt *et al*., 1949), cysteamine (Luning *et al*., 1961), lipoic acid (Ramakrishnan *et al*., 1992) and deoxyspergualin (Nemato *et al*., 1995). But clinical applications of these compounds are very few owing to their high toxicity at optimum dose level. Recently, the interest has been developed in search for potential drugs, especially, of herbal origins, which are capable of modifying immune and radiation responses without their side effects. Several studies concerning radioprotection have been conducted on vitamins (Sarma and Kesavan, 1993; Felemovicius *et al*., 1995). Among diverse markers of oxidative stress, malondialdehyde (MDA) and the natural antioxidants, metalo enzymes Cu, Zn-superoxide dismutase (Cu, ZnSOD) and selenium-dependent glutathione peroxidise (GSH-Px), are currently considered to be the most important.

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Malondialdehyde (MDA) is a three-carbon compound formed from peroxidized polyunsaturated fatty acids, mainly arachidonic acid. It is one of the end products of membrane lipid peroxidation. Since MDA levels are increased in various diseases with excess of oxygen free radicals, many relationships with free radical damage were observed (Ohkawa *et al*., 1979; Guichardant *et al*., 1994) Cu, Zn-SOD is widespread in nature. It is present in all oxygen-metabolizing cells. Cu, Zn-SOD is an intracellular enzyme, which dismutates the extremely toxic superoxide radical into potentially less toxic hydrogen peroxide. GSH-Px, an intracellular enzyme, belongs to several proteins in mammalian cells that can metabolize hydrogen peroxide and lipid hydroperoxides.

Ayurveda, the most ancient system of medicine, provides the classical textures viz. Vedas, Upanishads serving today as a source of potential radio protective herbal medicines. Herbal drugs offer an alternative to the synthetic drugs as they are considered as effective and less toxic. Based on the properties and significance of *Garcinia indica*, the present study has been undertaken to investigate the antioxidant effect of different doses in prevention of oxidative damage caused by gamma irradiation in mice liver tissue after total body irradiation at 6Gy.

MATERIALS AND METHODS

Animals and experiments

For the current experiment we used hundred albino mice. The animals were cared and handled according to the guidelines. They were fed with laboratory feed and water before the experiment. Mice were selected from an inbred colony maintained under controlled conditions of temperature (28±2˚C), humidity (50±5%) and light (12hr, each of light and dark cycle). We divided the mice into five equal groups and were placed in different cages. Twenty-four hours before the experiment, the mice were fasted and allowed access to water ad libitum. On the day of experiment, group 1 and 2 were injected with 5 and 10 mg/kg of *Garcina Indica*, group 3 was injected with an isotonic solution. The *Garcina Indica* was dissolved in ethanol at the beginning, and further diluted in isotonic NaCl. NaCl was added with an equal volume of *Garcina Indica*, dissolved in ethanol. *Garcina Indica* was initially dissolved in ethanol diluted with isotonic. The drugs and isotonic NaCl were administrated intraperitoneally. The group 4 was injected with only 5 mg/kg *Garcina Indica*, and group 5 was reserved as control. After a time span of 30 minutes, group 1, 2 and 3 were anesthetized with ketamine HCl 50 mg/kg and irradiated 6.0 Gy to the total body with a single dose fraction. Irradiation was performed at 80 cm source skin distance. The dose was calculated along the central axis at 2 cm. The only *Garcina Indica* and control group animals were anaesthetized, but it was not irradiated. Animals in all groups were sacrificed after two hours of irradiation.

Biochemical analysis

Two hours after irradiation animals were anesthetized by 50 mg/kg of thiopental sodium. The mices livers were homogenized in a physiological saline solution using a homogenizer at 10,000 rpm for 1 h to remove any debris. A supernatant was taken, and enzymatic assays were carried out in the fraction. All the process was formed at lower optimum temperature throughout the experiments. With reference to MDA levels, the method published by Okawa[9] was used. In this method, samples less than 0.2 ml of 10% tissue homogenate were added by 0.2 ml of 8% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid solution with ph adjusted 3.5 and 1.5 ml of 0.8% aqueous solutions thiobarbituric acid. The solution was made up to 4 ml with distilled water, and heated at 90^0C for 50 min. After cooling 1 ml of distilled water and 5 ml of mixture of n-butanol and pyridine mixture were added and shook briskly. After centrifugation at 4.000 rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured. Total thiobarbituric acid reactive substances were expressed as MDA, using a molar extinction coefficient for MDA OF 1.5 X 10^5 /cm, M. The MDA level was expressed as nmol mg-1 protein. The SOD activity was detected according to Sun and co-workers (Sun *et al*., 1988). This method, xanthie-xanthine oxidase complex produces super oxide radicals, which react with nitrobluetetrazolium (NBT) to form the farmasone compound. The SOD activity was measured at 560 nm by detecting the inhibition of this reaction. By using a blank study in which all reagents except supernatant sample were present and by determining the absorbance of sample and blank, the activity was calculated by difference, and given below. One SOD unit is defined as the enzyme amount causing 50% inhibition hi the $NBTH_2$ reduction rate. The SOD activity was also expressed as U mg-1 protein of liver tissue sediment. The GSH-Px activity was measured according to the Paglia and Valentina method (Paglia and Valentina, 1967). In this method, GSH-Px catalyses the oxidation of glutathione in the presence of hydrogen peroxide. Oxidized glutathione is converted into the reduced form in the presence of glutathione reductase and NADPH, while NADPH is oxidized to $NADP^+$. Reduction in the absorbance of NADPH at 340 nm was measured. By measuring the absorbance change per minute and by using the molar extinction coefficient of NADPH, the GSH-Px activity of liver tissue was calculated. The GSH-Px activities were expressed as U mg-1 protein of liver tissue sediment. The protein content was determined by using the Bradford (Bradford, 1976). Biochemical measurements were carried out at room temperature using a spectrophotometer

Statistical analysis

The findings were expressed as the mean Standard Deviation. All parameters were analysed by variance analysis test. Least significant difference multiple range tests were used to compare the mean values A p value <0.05 was accepted as statistically significant. Statistical analysis was performed with SPSS25 (SPSS, 2000).

RESULTS AND DISCUSSION

All the parameters are tabulated in table 1. In the irradiation-only group, the liver tissue MDA levels were significantly higher compared with the *Garcina Indica*-only and sham control group ($p < 0.001$). The MDA levels in the irradiation-plus 5 and 10 mg kg-1 of *Garcina Indica* group were significantly decreased when compared with the irradiation only group ($p < 0.001$). The MDA levels were also significantly different between the irradiation plus 5 and 10 mg/kg *Garcina Indica* groups ($p < 0.05$). The SOD activity was significantly lower in the irradiation only group compared with the sham control group ($p < 0.01$) and the *Garcina Indica* only group ($p < 0.001$). In the irradiation-plus 5 and 10 mg/kg *Garcina Indica* groups, the SOD activity was significantly higher compared with that of the irradiation only group ($p < 0.01$).

Table 1. Effect of *Garcina Indica* liver on the levels of MDA, CuZn-SOD and GSH-Px activities in irradiated mice

Group	MDA	CuZn-SOD	GSH-Px
Control	4.62±0.53[f]	4.08±0.33[e]	0.76±0.53[f]
Garcina Indica only	4.01±0.95[f]	5.98±0.98[e]	0.83±0.21[b]
Irradiation only group	8.36±1.02[e]	3.62±0.24[b]	0.42±0.11[e]
Garcina Indica 5mg + irradiation	6.54±0.84[e]	4.11±0.39[e]	0.61±0.04[d]
Garcina Indica 10mg + irradiation	5.12±0.74[g]	5.29±.084[e]	0.79±0.31[f]

[a]p<0.05, [b]p<0.01, [c]p < 0.001 vs Control, [d]p<0.05, [e]p<0.01, [f]p < 0.001 vs irradiation [g]p<0.05, vs *Garcina Indica* + irradiation time

The SOD activity was not different between the irradiation plus 5 and 10 mg/kg *Garcina Indica* groups (p> 0.05).The

activities of GSH-Px were significantly lower in the irradiation only group compared with the control and the *Garcina Indica* only group ($p < 0.001$). In the irradiation-plus 5 and 10mg *Garcina Indica* groups, the GSH-Px levels were significantly higher compared to that of the irradiation-only group, respectively ($p < 0.01$, $p < 0.001$). The activities of GSH-Px also were not different between the irradiation-plus 5 mg kg' group and the irradiation-plus 10 mg kg' *Garcina Indica* group ($p > 0.05$).

Conclusion

From the current study shows that *Garcinia Indica* extract, a plant based formulation provided fortification against the radiation induced mice of different groups. The extract may scavenge the free radicals produced by radiation and thus inhibit radiation induced damage. Thus the activities measured in all groups for MDA, SOD and GSH-Px, total body irradiation results in increase in the liver tissue MDA levels and a decrease of SOD and GSH-Px activities and it showed liver tissue MDA levels in irradiated mice that were pre treated with *Garcina Indica* were decreased significantly, where SOD and GSH-Px activities were significantly increased. Thus the reported result suggests that *Garcina Indica* administration prior to irradiation may prevent liver damage by irradiation.

REFERENCES

Nair, C. K., Parida, D. K. and Nomura, T. 2001. Radioprotectors in radiotherapy. *J. Radiat. Res*., 42: 21–37.

Patt, H. M., Tyree, E. B., Straube, R. L. and Smith, D. E. 1949. Cysteine against X-irradiation. *Science,* 110: 213.

Luning, K. G., Frelon, H. and Nelson, A. 1961. A protective effect of cysteamine against genetic damages by X–rays in spermatozoa from mice. *Radiat. Res*., 14: 813.

Ramakrishnan, N., Wolfe, W. W. and Catravas, G. N. 1992. Radioprotection hemopoietic tissues in mice by lipoic acid. *Radiat. Res*., 130: 360–365.

Nemato, K., Horiuohi. R. and Miyamoto, T. 1995. Deoxyspergualin is a new radioprotector in mice. *Radiat. Res*., 141: 223–225

Sarma, L. and Kesavan, P. C. 1993. Protective effects of vitamins C and E against gamma-ray-induced chromosomal dam-age in mouse. *Int. J. Radiat. Biol*., 63: 759–764.

Felemovicius, I., Bonsack, M. E., Baptista, M. L. and Delaney, J. P. 1995. Intestinal radioprotection by vitamin E (alpha-tocopherol). *Ann. Surg*., 222: 504–510.

Ohkawa h, Ohishi N, Yagi K, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.,* 95: 351-358.

Guichardant M, Vallete-talbi L, Cavadini C, Crozier G, Berger M. 1994. Malondialdehyde measurement in urine. *J Chromatogr B Biomed Appl.,* 655: 112-116.

Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem* 1988; 34: 497–500.

Paglia DE, Valentina WN. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*., 70: 158–169.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitaion of microorganism quantities of protein utilizing the principle of protein dye binding. *Anal Biochem.*, 72: 248-254

Statistical Package for the Social Sciences for Windows, version 20. SPSS: Chicago, IL, USA. 2000.

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