

**OLTIPRAZ PROVIDES PROTECTION TO SWISS ALBINO MICE
AGAINST GAMMA RADIATION.**

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Summary

In the present study Radiation protection from Oltipraz (5-[2-pyrazinyl]-4-methyl-1, 2-dithiol-3-thione) has been evaluated in Swiss albino mice. The oral administration of Oltipraz (100 mg/kg/day dissolved in 10% Tween 80) was given to 6-8 week old Swiss albino mice (25±2gm) for 2 consecutive days prior to irradiation. A regression analysis of survival data yielded LD_{50/30} as 5.82 and 8.38 Gy for the control (radiation alone) and experimental (Oltipraz + radiation) groups, respectively, yielding a dose reduction factor of 1.44. The results of the present study suggest that Oltipraz provides protection against gamma radiation induced alterations in Swiss albino mice.

Keywords: *Oltipraz, Swiss albino mice, Radioprotection.*

Introduction

Radiation protection concepts and philosophy have been evolving over the past several decades. The inadvertent exposure of humans to various sources of radiation causes ionization of molecules, setting off potentially damaging reactions *via* free radical production. Free radicals are believed to play a major role in more than sixty different health conditions, including the ageing process, cancer, radiation damage, atherosclerosis, etc¹. These harmful effects were minimized with the help of certain synthetic chemicals such as cysteine, cysteamine, 2-MPG, lipoic acid and deoxyspergualine²⁻⁶. However, clinical applications of these compounds are very few, owing to their high toxicity at optimum dose levels. Cruciferous vegetables (*e.g.*, green leafy vegetables such as cabbage) contain several agents, including dithiolethiones, which appear to inhibit carcinogenesis. Oltipraz [5-(2-pyrazinyl)-4-methyl-1, 2-dithiole-3-thione] derived and synthesized from cruciferous plants, was originally developed for the treatment of schistosomiasis⁷. Administration of Oltipraz, at non toxic dose, in the diet, was found to protect rodents from the formation of carcinogen-induced tumors at various sites⁸⁻⁹. Kim *et al.* (1998) reported *in vivo* radioprotective effects of Oltipraz in gamma irradiated mice¹⁰. The present study has been undertaken to investigate the radioprotective efficacy of Oltipraz on Swiss albino mice.

Materials and Methods

Animals:

Healthy male Swiss albino mice, 6-8 weeks old, with an average body weight of 25 ± 2 gm were used for the present study. The mice were obtained from an inbred colony, procured from Indian Veterinary Research Institute, Izatnagar, Bareilly, UP. Animals were maintained under optimal conditions of temperature ($25 \pm 2^\circ\text{C}$) and light (14 hrs light and 10 hrs darkness) in an animal house in the University and were provided standard feed and water *ad libitum*.

Irradiation:

Animals selected for experiments were irradiated at the Cancer Treatment Centre, Radiotherapy Department, SMS Medical College and Hospital, Jaipur with the help of the Cobalt-teletherapy Unit (Theratron-780 C). Unanaesthetized mice were restrained in a well ventilated box (35 cm x 35 cm x 2.5 cm) and were exposed to whole-body gamma radiation at a surface distance (SSD) of 80 cm from the source to deliver the dose rate of 1.59 Gy/min.

Oltipraz:

Oltipraz [5-(2-pyrazinyl)-4-methyl-1, 2-dithiole-3-thione] is a synthetic compound (Fig. 1), substituted from 1, 2-dithiole-3-thione previously used in humans as an antischistosomal agent. For the present study Oltipraz was supplied by Canopus BioPharma Inc. Oltipraz (100 mg/kg/day dissolved in 10% Tween 80) administered through oral gavage at a volume of 0.1ml/kg to 6-8 week old Swiss albino mice for two consecutive days prior to radiation exposure.

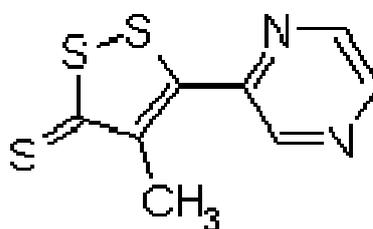


Fig. 1: Structure of Oltipraz

Experimental design:

- **The determination of optimum dose of Oltipraz against radiation:**

40 mice were divided into 4 groups of 10 animals each and given Oltipraz orally (0, 50, 100 and 200 mg/ kg /day) for 2 consecutive days. 30 minutes after the final administration, the animals were exposed to whole- body 8Gy gamma radiation. All animals were observed for 30 days for any signs of radiation sickness, morbidity, abnormal behavioral, toxicity or mortality.

- **The determination of dose reduction factor (DRF):**

The animals selected for this study were divided into 2 groups (10 animals in each group). Those of one group were administered orally with optimum dose of Oltipraz for 2 consecutive days, and the control group received 10% Tween 80 (volume equal to Oltipraz). 30 minutes following administration of either the test article or control, the animals of both groups were exposed to different doses of gamma radiation (4, 6, 8, and 10 Gy). The percentage of mice surviving following exposure to each radiation dose was used to construct survival–dose response curves over a 30 day period.

- **Division of groups:**

Mice were selected from an inbred colony and divided into 3 groups.

Group 1: Normal group:

This group was administered 10% Tween 80 orally for 2 consecutive days; the volume provided was equal to that of Oltipraz administered in the experimental group (25 animals).

Group 2: Control group:

This group received 10% Tween 80 (volume equal to Oltipraz) and later exposed to 8 Gy gamma radiation (25 animals).

Group 3: Experimental group:

This group was administrated with optimum dose of Oltipraz orally for 2 consecutive days before radiation exposure (25 animals).

Results

Oltipraz did not show any toxic effect in animals that were treated for 2 consecutive days in the experimental group. Symptoms of radiation sickness such as lethargy, diarrhoea, loss of body weight, hair loss, facial edema, and loss of appetite were observed in the animals exposed to varying doses of gamma radiation in the control group.

The optimum dose of Oltipraz exhibiting maximum radioprotection was found to be **100 mg/kg/day** for 2 consecutive days before irradiation (8 Gy). The animals pretreated with 50, 100 and 200 (mg/kg/day) of Oltipraz showed 63.6, 86.66 and 50% survival (Fig. 2) respectively when exposed to 8 Gy gamma radiation. Therefore 100 mg/kg/day Oltipraz drug concentration was used for further experiments.

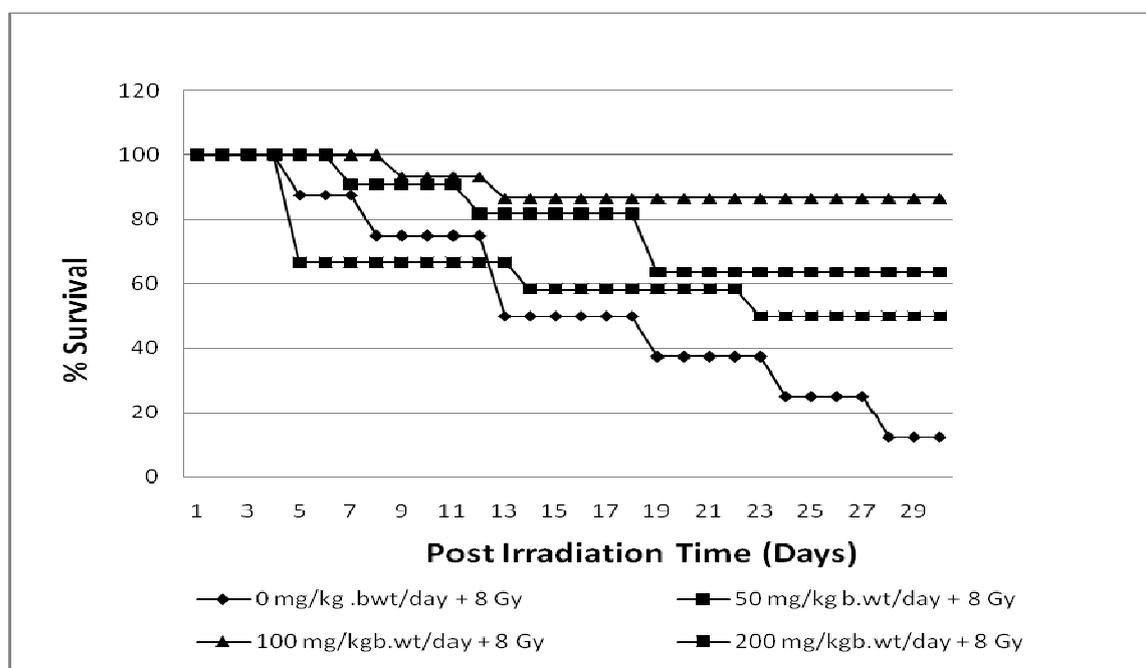


Fig. 2: Survival percentage of Swiss albino mice pretreated with different doses of Oltipraz and exposed to 8 Gy gamma radiation.

The severity of radiation sickness was found to be dose dependent. After 30 days observation control group (irradiation alone) animals were exhibited 90, 30, 12.5 and 0% survival for 4, 6, 8 and 10 Gy gamma radiations respectively (Fig. 3). However the severity of radiation sickness was much less in experimental set animals' comparison to their respective controls when exposed to 4, 6, 8 and 10 Gy gamma radiations. No mortality was found in experimental group animals when exposed to 4 and 6 Gy radiation doses.

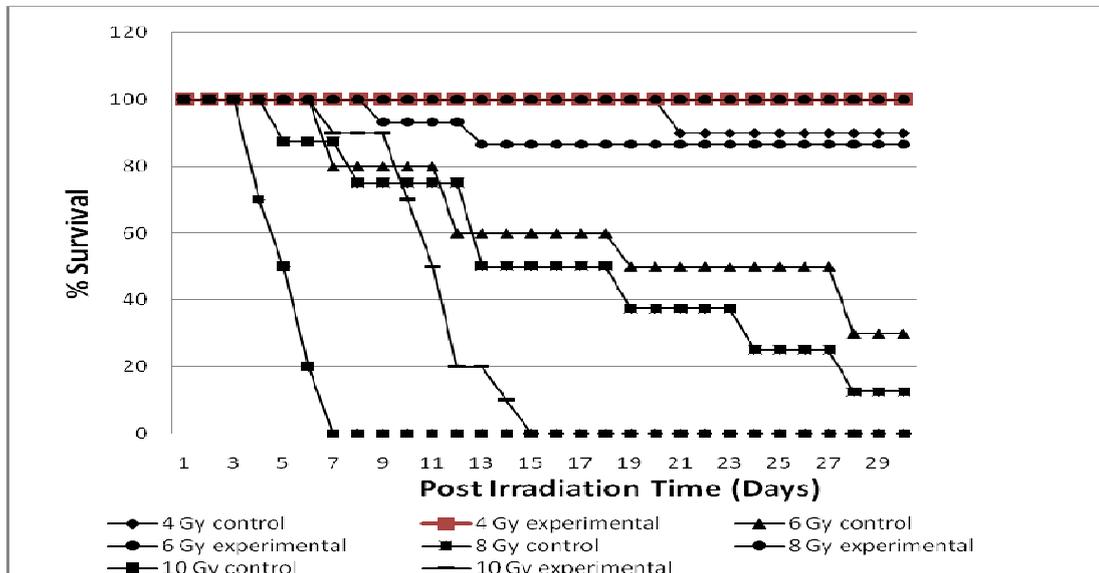


Fig. 3: Percent survival of Swiss albino mice with and without pretreatment of Oltipraz and exposed to 4, 6, 8 and 10 Gy gamma radiation.

Survival data were fit on regression line equation; LD_{50/30} values for control and experimental group were computed as 5.82 and 8.38 Gy, respectively. On the basis of LD_{50/30} values DRF was calculated as 1.44 (Fig. 4).

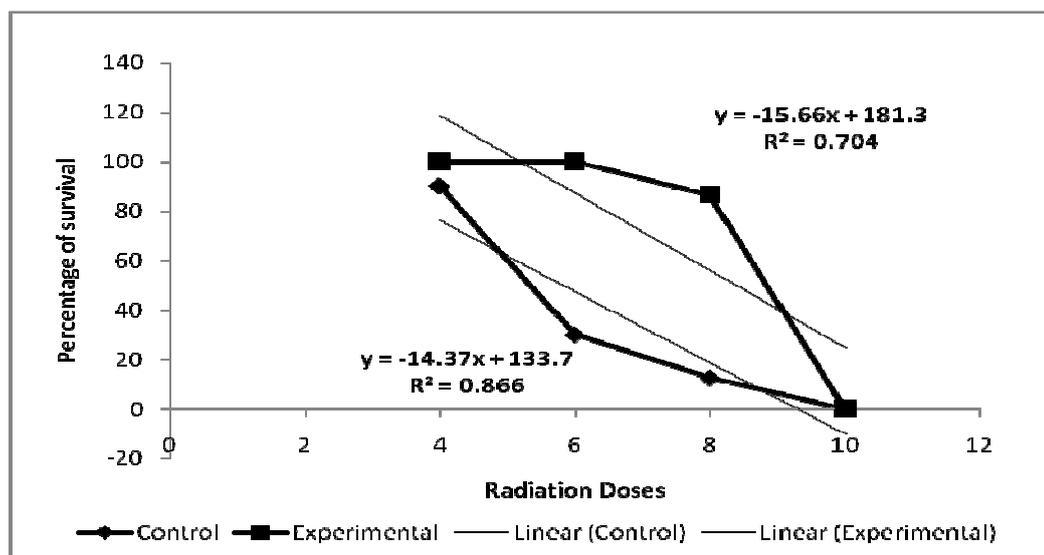


Fig. 4: Survival percentage (30 days post irradiation) of Swiss albino mice after regression analysis with or without pre treatment of Oltipraz and exposed to different doses of gamma radiation.

Discussion

In the present study, signs of radiation sickness and mortality were induced in animals exposed to varying doses of gamma radiation (4, 6, 8 and 10 Gy). These variations were less severe in animals treated with Oltipraz when compared to their respective control group animals. The reduced radiation sickness and mortality (DRF= 1.44) in Oltipraz pretreated animals indicates that Oltipraz is capable of providing protection from gamma radiation.

The results from the present study also confirm that maximum radioprotection (86.66% survival) was observed at 100 mg/kg/day Oltipraz for 2 consecutive days prior to irradiation. However, the degree of protection was reduced at higher (200 mg/kg/day) and lower concentrations (50 mg/kg/day) of Oltipraz.

Treatment of animals with Oltipraz increases cellular thiol levels in mice. The dithiolethione compound, Oltipraz, appeared to be a potent inducer of GST¹¹. It has been postulated that an increase in nonprotein sulfhydryl within cells might be responsible for the radioprotective effects of dithiolesters.

It has been shown that Oltipraz's chemopreventive effect is caused by the selective induction of phase II drug-metabolizing enzymes and/or the inhibition of some phase I cytochrome P450 enzyme activities¹². As a major molecular mechanism, the induction of the phase II drug metabolism enzymes helps to detoxify various types of carcinogens. Up-regulation of phase II enzymes by Oltipraz is caused by the direct activation of the antioxidant-responsive element (ARE), a *cis*-acting regulatory element that is widely present in the 5'-flanking regions of many detoxifying genes¹³. The ARE was first identified in rat glutathione *S*-transferase Ya¹³⁻¹⁴ and a mouse counterpart was found in the murine glutathione *S*-transferase Ya gene and has been named the electrophile responsive element (EpRE) because of its responsiveness to electrophilic compounds¹⁵⁻¹⁶. It has since been found that ARE/EpRE is composed of two copies of adjacent activator protein-1-like motifs (TGACNNNGC) and is required for maximum basal expression and induction by some xenobiotic compounds¹⁶⁻¹⁷. Recent studies implicated a critical involvement of a basic leucine zipper transcriptional factor, Nrf2 (NF-E2 p45-related factor 2), in the regulatory activation of the ARE¹⁸.

Oltipraz is thought to be a monofunctional inducer, selectively activating phase II genes¹⁹, however it was also found to affect P450 activity. There are several reports of inhibition of enzyme activities of CYP1A1, CYP3A4²⁰⁻²¹ and CYP1A2²², suggesting that inhibition of activation of procarcinogens by P450s could be a mechanism contributing to Oltipraz's antitumor effects.

Conclusion

The antioxidants, anticancerous and antiperoxidants properties of Oltipraz would have resulted in the reduction of radiation mediated generation of free radicals so we concluded that Oltipraz has a good radioprotective properties.

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