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Zingiber officinale exhibits behavioral radioprotection against radiation-induced CTA in a gender-specific manner

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Abstract

At the organismic level, exposure to radiation can produce taste aversion (CTA) learning and emesis, which have been proposed as behavioral endpoints that are mediated by harmful effects of radiations on peripheral systems, primarily the gastrointestinal system. Thus, the aim of the present investigation was to study the gastroprotective action of hydroalcoholic extract of zingiber rhizome (*Zingiber officinale* Rosc.) against radiation-induced conditioned taste aversion (CTA) in both male and female species of animals, for testing its potential as a behavioral radioprotector. Administration of zingiber extract 1 h before 2-Gy gamma-radiation was significantly effective in blocking the saccharin avoidance response, with 200 and 250 mg/kg b.wt. i.p., being the most effective doses for male and female rats, respectively. A comparison of the efficacy of zingiber extract with two antiemetic drugs, ondansteron and dexamethasone, revealed that the extract rendered comparable protection against radiation-induced CTA. Our experiments also confirmed the existence of sex dichotomy (i.e., the sex of animal greatly influenced response towards radiation exposure) in relation to behavioral responses (CTA) or differential metabolism. The observed gender variations were hypothesized to be a result of hormonal fluctuations and differences in pharmacological parameters in male and female rats. To correlate the mechanism of action, the free-radical-scavenging potential of zingiber extract to scavenge hydroxyl ion and nitric oxide was also tested, in cell-free system and a concentration of 1000 μg/ml, was found to be the most potent, which has been proposed as one the many activities assisting in its overall ability to modulate radiation-induced taste aversion. The results demonstrate that *Z. officinale* possesses antioxidant, radioprotective and neuromodulatory properties that can be effectively utilized for behavioral radioprotection and for efficiently mitigating radiation-induced CTA in both males and females species.

Keywords: Conditioned taste aversion; Behavioral radioprotection; *Zingiber officinale*; Free radical scavenger; 5-HT antagonist; Gender differences

1. Introduction

In addition to their effect on nervous system, exposure to ionizing radiations/toxins also produce behavioral effects and can serve as unconditioned stimuli, leading to the development of a conditioned taste aversion (CTA) (Smith et al., 1984). A CTA is thought to be produced when a toxic unconditioned stimulus (radiation/toxin) is paired with a novel tastant (saccharin/sucrose) (Rabin and Hunt, 1986). As a result of that pairing, the organism avoids intake of that tastant on subsequent pairing (Rabin, 1996). Radiation-induced CTA has been defined as a behavioral endpoint that is mediated by the toxic effects of gamma-radiations on peripheral systems, primarily the gastrointestinal system (Rabin et al., 1998). As such, the CTA procedure has been proposed as a standard procedure for assessing the behavioral toxicity of a stimulus (Riley and Tuck, 1985).

One of the cherished and most elusive goals in radiobiology has been the development of a pharmacological agent that can mitigate the early damage produced in cells and tissues by ionizing radiation. Historically, the development of radioprotective

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agents has been dominated by the study of sulfhydryl compounds, particularly the aminothiols, i.e., WR-2721, WR-1607, WR-3689, etc. (Giambarresi and Jacobs, 1987). High levels of protection have been achieved in phosphorothioates only at doses that are accompanied by unacceptable side effects (Spence et al., 1988), which include nausea, vomiting, diarrhea, hypotension and allergic reactions, implying behavioral consequences (Weiss, 1997). Studies on one of the most effective radioprotectors *S*-2 (3-aminopropylamino)ethylphosphorothioic acid (WR-2721) in a variety of animal species have shown significant behavioral toxicity and performance degradation (Bogo et al., 1985). It has been even found to cause CTA (conditioned taste aversion) on its own and even accentuate radiation-induced CTA in rats at doses optimal for radioprotection (Cairnie, 1983). Studies on patients undergoing cancer radiotherapy have shown WR-2721 to have poor bioavailability, due to first-pass metabolism by intestinal mucosa during absorption. In addition, the drug is hydrolyzed in the acidic environment of the stomach, a factor that is aggravated by its ability to slow gastric emptying (Brizel et al., 2000). Thus, the overall risk versus benefit profile for the drug becomes unfavorable.

While measures to reduce the lethal effects of radiation have been extensively studied, relatively little attention has been paid towards the ability of drugs to prevent radiation-induced behavioral disruption and performance decrement (Bogo et al., 1988). An ideal radioprotector should not only afford protection from radiation-induced lethality, but also should possess the ability to mitigate performance and behavioral deficiencies (Walker, 1988). Thus researchers are seeking to control or minimize the side effects of WR-2721 by combining it with antiemetics (WR-2721+ondansteron) (Belkac et al., 1996) or by using sub-toxic amounts of drugs in combination with other agents that act synergistically or additively (WR-2721+anti-inflammatory agent) (Bump and Malaker, 1997). As such 'antiemetics' on their own have been grouped as the third category of behavioral radioprotectors after 'direct attenuators' and 'incidental attenuators' (Bogo, 1988; Harding, 1988). However, several side effects, like drowsiness, sluggishness, headache, stomach cramp, disturbed vision, etc., have also been documented with the usage of antiemetics (Benline and French, 1997).

Lately, the attention of radiobiologists has shifted towards utilizing antioxidant properties of plant products as radioprotectants, due to their lower toxicity (Arora et al., 2005). Zingiber (Ginger/*Zingiber officinale*) is mentioned in Ayurvedic and Tibb system of medicine to be helpful in neurological disorders and has been successfully used against migraine headache (Mustafa and Shrivastava, 1990). A combination of *Z. officinale* and *Ginkgo biloba* has been reported to be neuroprotective in various behavioral tasks, e.g., antianxiety effects in elevated plus maze task (Hasenöhrl et al., 1998) as well as effects on learning and memory in an inhibitory avoidance and water maze task. Furthermore, it has been also shown that this combination preparation exerts memory enhancing effects in the hidden platform version of the water maze in aged animals (Topic et al., 2002a,b) and also blocks LiCl-induced conditioned place aversion (Frisch et al., 1995). *Z. officinale* alone is also known to have a strong anxiolytic and antiemetic activity (Vishwakarma et al., 2002). *Z. officinale* has been reported to have gastroprotective effects against: gastric ulcers (Yamahara et al., 1988), nausea and vomiting (Ernst and Pittler, 2000), and motion sickness and slow-wave dyrhythmias (Lien et al., 2003).

Perusal of published research reveals that there exist sex differences in response of an animal towards radiation damage, which have been attributed to the presence of hormonal variations in males and females (Mickley, 1980). Recently, it has even been shown that males and females vary in their antioxidant responses towards radiation-induced oxidative stress (Han et al., 2005). It has also been observed that taste preferences and ingestion behavior are influenced by sex and stage of estrous cycle (Curtis et al., 2004; Clarke and Ossenkopp, 1998). Several recent experiments have shown

Table 1
Behavioral-radioprotective properties of *Z. officinale*

S. no.	Radiation-induced perturbations	Side effects of synthetic radioprotectors (WR-2721)	Properties of *Z. officinale*
1	Nausea and vomiting in humans and CTA in rodents [emesis] (Mattsson and Yochmowitz, 1980)		Antiemetic (Sharma et al., 2005)
2	Behavioral degradation (toxicity) and performance retardation (Walter, 1987)		Behavioral protection and maintains performance (Li et al., 2003)
3	Changes in blood flow rate (Burghardt and Hunt, 1985)		Cardiotonic (Kobayashi et al., 1988)
4	–	Delay in gastric emptying (Dubois et al., 1987; Brizel et al., 2000)	Stops delay in Gastric Emptying (Sharma and Gupta, 1998)
5	–	Allergic reactions (Weiss, 1997; Brizel et al., 2000)	Antiallergic (Moon et al., 2005)
6	Motion sickness [space radiations] (Conklin and Hagan, 1987)	–	Anti-motion sickness (Holtmann et al., 1989)
7	Generation of free radicals (Gupta et al., 2003)	–	Antioxidant and free radical scavenger (Sharma et al., 2005)
8	Cause inflammation (Meeren et al., 2005)	–	Anti-inflammatory (Kiuchi et al., 1992)
9	Haemopoietic syndrome (Jagetia et al., 2003)	–	Immunostimulatory (Dugenci et al., 2003)
10	Gastrointestinal syndrome (Dublineau et al., 2000)	–	Gastroprotective (al-Yahya et al., 1989)
11	DNA damage (Weiss, 1997)	–	Protects DNA (Lu and Lai, 2003)
12	Lethality (Weiss, 1997)	–	Protects from radiation-induced lethality (Jagetia et al., 2003)

that gender differences also exist in various behavioral measures (Brotto et al., 2000), including the development of (CTA): by toxins, LiCl and even pulsed magnetic field (Foy and Foy, 2003; Choleris et al., 2000). But there is no specific study on the radiation-induced CTA effect in different sex of animals.

We have recently reported in our preliminary studies that Zingiber extract (*Z. officinale* rhizome) modulates radiation-induced CTA at 50–200 mg/kg b.wt. concentrations (dose-dependent manner), in male Sprague–Dawley rats (Sharma et al., 2005). Extending that work, in this present study, both male and female Sprague–Dawley rats were included for testing the extract at various further concentrations (150–300 mg/kg b.wt.) to extinguish radiation-induced CTA in terms of their 'saccharin preference ratios' (spr). The efficacy of zingiber extract was also compared with two commercially available antiemetic drugs ondansteron and dexamethasone in both male and female rats. This paper also reviews the properties of zingiber extract as a potential candidate for behavioral radioprotection, which can be used alone or in combination with synthetic radioprotectors as an adjunct/additive to mask their side effects (Table 1). The effect of the extract on nitric oxide and hydroxyl ion scavenging potential were also studied to further unravel some of the mechanistic aspects of the observed changes.

2. Materials and methods

2.1. Animals

Sprague–Dawley adult male and female rats (12–15 weeks old, 323±25 g for males and 223±25 g for females) that were in-bred at the Animal House of the Institute of Nuclear Medicine and Allied Sciences, Delhi were used for the experiments. Animals were kept under standard laboratory conditions with photoperiod of 12 h/day and temperature of 25 ±2 °C. The rats were housed individually in polyvinyl cage and fed standard animal foods pellets (Golden Feeds, Delhi, India) and were offered tap water ad libitum. All the procedures were carried out in strict compliance with the Animal Ethics Committee rules and regulations followed in this institute.

2.2. Irradiation

Each rat was placed in a wire gauze container and put in the Co^{60} gamma-irradiator (Model 220, Atomic Energy Commission, Canada), having a dose rate of 37.2 rads/min and exposed to 2-Gy of radiation, during the course of the study. Dosimetry was carried out with Baldwin Farmer secondary dosimeter and Fricke dosimeter.

2.3. Zingiber extract

Briefly, zingiber extract was prepared as described earlier in Sharma et al. (2005); *Z. officinale* rhizomes were collected and identified by Dr. Rajesh Arora (Medicinal Plant Scientist), at the Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi, India. The fresh rhizomes were crushed, and extracted in 50% ethanol at mild temperature, filtered, concentrated in a rotary evaporator (Buchi, Switzerland) at 50±5 °C, lyophilized and stored at −80 °C in a deep freezer (New Brunswick, USA) until use. The yield on w/w basis was approximately 8±1%. The extract was suspended in 10% ethanol and filtered through Millipore 0.2 microns prior to use. This extract was administered intraperitoneally to experimental animals 1 h before exposure to radiation (2 Gy). For in-vitro studies, <5% ethanol was used.

2.4. Standard antiemetic drugs

The commercially available antiemetic drugs, which were used in the experiment, were ondansteron (Emset-8, CIPLA Ltd., Goa, India) and dexamethasone (Dexona, Zydus Cadila, Cadila Health Care Ltd., Gujarat, India) dissolved in distilled water and injected intraperitoneally 1 h before radiation exposure. The dose used was 1 mg/kg b.wt. for both the drugs (Mele et al., 1992; Yamamoto et al., 2002).

2.5. Conditioning procedure

All animals were habituated to the laboratory conditions at least 4 weeks prior to training. Rats were trained for 23.5 h water deprivation schedule for 10 days (conditioning period), wherein each animal was offered tap water only (i.e., one-bottle paradigm) (Moron and Ballesteros, 2002) for 30 min (10:00–10:30 a.m.). Water consumption of the individual animal per day was recorded. On the 10th day of conditioning period, all the rats were given a choice between 0.1% saccharin solution and tap water for 30 min (two-bottle regime) (Miranda and Hong, 2001), and their respective intake of 0.1% saccharin solution and tap water was recorded. Only those animals, which exhibited saccharin solution intake of more than 50% of their total fluid intake, were selected for participation in the experiment. Body weight of the animals was recorded daily before beginning the experiments (Shobi and Goel, 2001).

Immediately following the conditioning session, the rats were divided into the following groups:

Group I: vehicle (−1 h/n=6)/sham radiation
Group II: vehicle (−1 h/n=6)/radiation
Group III: zingiber extract (−1 h/n=6)/sham radiation
Group IV: zingiber extract (−1 h/n=6)/radiation
Group V: ondansteron (−1 h/n=6)/sham radiation
Group VI: ondansteron (−1 h/n=6)/radiation
Group VII: dexamethasone (−1 h/n=6)/sham radiation
Group VIII: dexamethasone (−1 h/n=6)/radiation

The animals of different groups were injected with vehicle (10% ethanol, *i.p.*) or zingiber extract (*i.p.*) or the standard antiemetic drugs (ondansteron, dexamethasone, *i.p.*), and after 1 h, were sham irradiated or given 2 Gy of gamma-irradiation. Twenty-four hours after the experiment, the animals were again given the choice between saccharin solution and tap water and their respective intake was recorded. This procedure was repeated for 5 post-irradiation days (Mukerjee et al., 1997).

Note: The experimental procedure was used for testing four different concentrations, i.e., 150, 200, 250 and 300 mg/kg b.

wt., for the zingiber extract and one fixed concentration, i.e., 1 mg/kg b.wt. for standard antiemetic drugs, with six animals per group.

The measure of development and extinction of CTA are represented in the form of saccharin preference ratios (spr), where spr = amount of saccharin consumed / amount of saccharin consumed + amount of water consumed.

2.6. Estimation of polyphenolic content

The polyphenolic content was estimated in ginger extract using the method of V.L. Singleton (Singleton and Rossi, 1965). To an aliquot (10 μl), taken from the stock solution (1 mg/ml) of ginger extract, 10 ml of water and 1.5 ml of folin ciocalteu reagent were added. The mixture was kept for 5 min at room temperature, and then 4 ml of 20% sodium carbonate solution was added and the volume made upto 25 ml with double distilled water. The mixture was kept for 30 min and absorbance of the color developed was recorded at 765 nm using UV visible spectrophotometer (Electronics Corporation of India Ltd., Hyderabad, India).

2.7. Estimation of nitric oxide ion scavenging potential

Varied concentrations of ginger extract were mixed with sodium nitroprusside (5 mM) (used as a generator of nitric oxide at physiological pH, which interacts with oxygen to generate nitrite ions), and the total assay volume was adjusted to 1 ml using phosphate-buffered saline. The reaction mixture was incubated at 25 °C for 150 min. The nitrite ions generated in the system were estimated by adding equivalent volume of Griess reagent (6% sulphanil amide in 3 M HCl + 0.3% naphthylethylenediamine dihydrochloride + 7.5% orthophosphoric acid in 1:1:1 ratio). The nitric oxide scavenging activity was evaluated as decrease in % absorbance of the complex formed by diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine readable at 546 nm in test samples with different concentrations of drug with respect to the control (Green et al., 1982).

2.8. Estimation of hydroxyl ion scavenging potential

The non-site-specific hydroxyl ion scavenging potential of ginger extract was measured using the deoxyribose degradation assay (Halliwell et al., 1987). Different concentrations of ginger extract (500 μl) were mixed with 100 μM each of ferric chloride solution, EDTA and L-ascorbic acid along with H_2O_2 (1 mM), deoxyribose (3.6 mM) in potassium phosphate buffer (pH 7.4) in a total assay volume of 1 ml. This was followed by incubation for 1 h at room temperature. 1 ml each of TCA (10% w/v) and thiobarbituric acid (0.5%w/v, TBA in 0.025 M NaOH) were added to each sample and re-incubated in a hot water bath (Yorco Instruments, India; 55 °C) for 15 min. The tubes were cooled to room temperature and the absorbance recorded at 532 nm against a blank containing phosphate buffer, while control contained no drug. The decrease in absorbance at a particular concentration indicated higher hydroxyl ion scavenging potential with respect to control. The percentage inhibition of degradation of deoxyribose or hydroxyl ion scavenging potential was evaluated as follows:

$$\% \text{ Inhibition} = \text{O.D. control} - \text{O.D. sample} / \text{O.D. control} \times 100$$

The procedure for evaluating site-specific hydroxyl ion scavenging potential was similar to the above-mentioned assay with a small change that in lieu of using EDTA, similar volume of buffer was used in a 1 ml reaction mixture.

2.9. Statistical analysis

Each parameter (sex, days, dose) was analyzed statistically by univariate analysis (factorial ANOVA) followed by multiple comparative analysis (using Tukey's HSD test) separately in male and female groups. Similarly, in order to study the triple interaction effect, three-way ANOVA analysis was performed. All these analysis were performed using SPSS software version-10.

3. Results

3.1. Effect of zingiber extract on basal saccharin intake

As shown in Figs. 1 and 2, zingiber extract was observed to considerably restore saccharin consumption of animals given 2 Gy of radiation, as compared to the radiation control group where animals did not receive the zingiber extract. The intake of saccharin was found to increase considerably over the period of 5 days reaching 5% level of significance ($P<0.05$), with the most effective extract concentrations being 200 mg/kg b.wt. for males and 250 mg/kg b.wt. for females, and the maximum saccharin intake being achieved on fifth post-irradiation day. Zingiber extract (at 200 mg/kg b.wt. for males and 250 mg/kg b. wt. for females) also scores significantly better in comparison to ondansteron and comparable to dexamethasone by the fifth observational day. In addition, the dexamethasone and ondansetron treatments have apparently been found to be more effective than some of the doses of zingiber in accelerating the extinction of CTA.

The increase in saccharine preference ratio (spr) over a period of 5 days is the direct indicative of extinction of CTA. In the present study, the administration of zingiber extract exhibited a significant extinction of CTA at all dosages in both male and female animals as compared to 2-Gy radiation control. The increase in mean values of spr (in male rats) in terms of time (days) showed significant change at $P<0.05$ ($F_{5,240}=72.948$) considering the 0 day as control. In addition, the analysis of different dosages (150, 200, 250 and 300 mg/kg b.wt.) of zingiber extract or administration of dexamethasone (1 mg/kg b.wt.) and ondansetron (1 mg/kg b.wt.) in male rats revealed that 200 mg/kg b.wt. followed by 150, 250 and 300 mg/kg b.wt. exhibited significant ($P<0.05$) extinction of CTA over a period of 5 post-irradiation days ($F_{7,240}=51.223$).

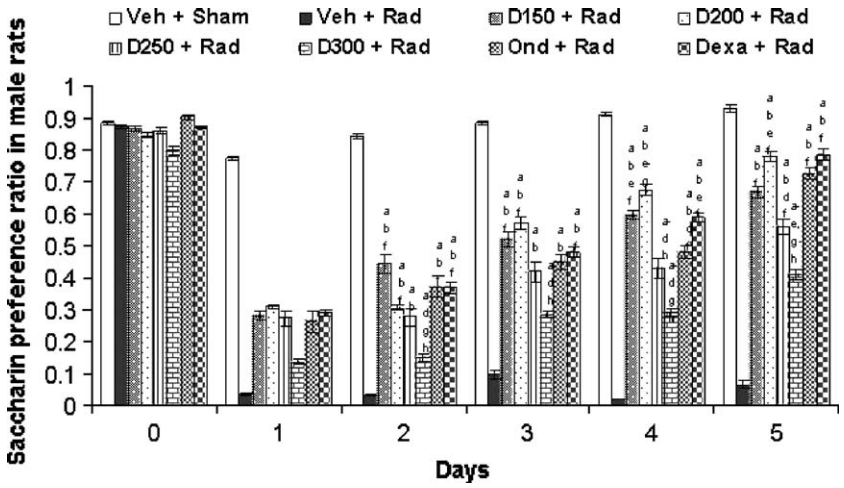

Fig. 1. Effect of different doses of zingiber extract on saccharin preference ratio (in CTA experimental setup, 2 Gy, over 5 days) and compared with standard antiemetics dexamethasone and ondansteron in male Sprague–Dawley rats. [a–h]Indicates ($P<0.05$) a change in saccharin preference ratio with respect to Veh+Sham[a], Veh+Rad[b], D150+Rad[c], D200+Rad[d], D250+Rad[e], D300+Rad[f], Ond+Rad[g] and Dexa+Rad[h] groups, respectively (based upon Tukey's multiple comparison analysis performed after factorial ANOVA). The extinction of CTA was observed between the period of 2–5 days.

The interaction effect between the days and dose in male rats was also found to be significant at $P<0.05$ ($F_{35,240}=2.864$). The administration of 200 mg/kg b.wt. of zingiber extract exhibited higher % extinction of CTA as compared to standard antiemetic ondansetron, specifically at third, fourth and fifth days of experimentation. Whereas with dexamethasone the % extinction of CTA is almost comparable with 200 mg/kg b.wt. concentration of extract, i.e., the saccharin consumption of dexamethasone-treated group reaches almost equal to the 200 mg/kg b.wt. extract-treated group on the fifth observational day. The overall mean differences in spr values between different treatment groups in males revealed a decrease in extinction of CTA in the following order: D200+R>Dexa+R>D150+R>Ond+R>D250+R>D300+R.

Similarly, the effects of zingiber extract on extinction of CTA in female animals were also investigated. The increase in mean spr values (in female rats) in terms of time (days) showed significant change at $P<0.05$ ($F_{5,240}=100.277$) considering the 0 day as control. In addition, the analysis of different dosages (150, 200, 250 and 300 mg/kg b.wt.) of zingiber extract or administration of dexamethasone (1 mg/kg b.wt.) and ondansteron (1 mg/kg b.wt.) in female rats revealed that 250 mg/kg b.wt. followed by 200, 300 and 150 mg/kg b.wt. exhibited significant ($P<0.05$) extinction of CTA over a period of 5 post-irradiation days ($F_{7,240}=62.923$). The interaction effect between the days and dose in female rats was also found to be significant at $P<0.05$ ($F_{35,240}=4.010$). The administration of 250 mg/kg b.wt. of zingiber extract exhibited higher % extinction of CTA as compared to standard antiemetics, i.e., dexamethasone and ondansteron, specifically at third, fourth and fifth days of experimentation. The overall mean differences in spr values between different treatment groups in females revealed a decrease in extinction of CTA in the following order: D250+R>Dexa+R>Ond+R>D200+R>D300+R>D150+R.

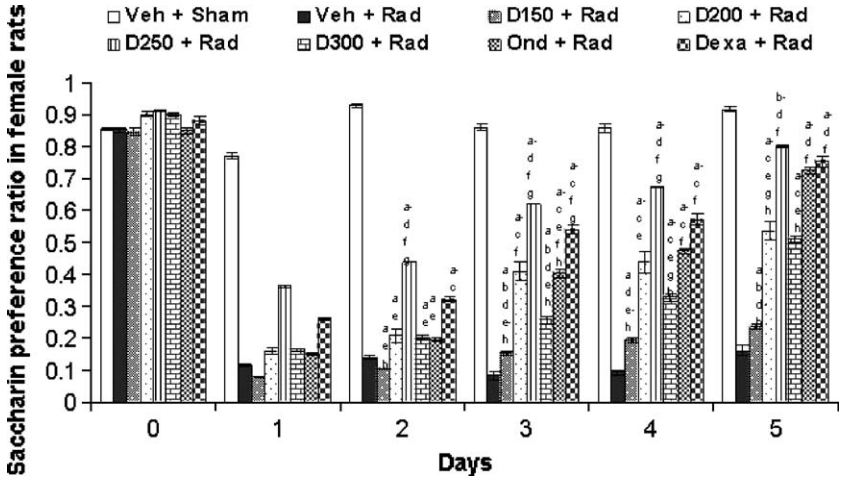

Fig. 2. Effect of different doses of zingiber extract on saccharin preference ratio (in CTA experimental setup, 2 Gy, over 5 days) and compared with standard antiemetics dexamethasone and ondansteron in female Sprague–Dawley rats. [a–h]Indicates ($P<0.05$) a change in saccharin preference ratio with respect to Veh+Sham[a], Veh+Rad[b], D150+Rad[c], D200+Rad[d], D250+Rad[e], D300+Rad[f], Ond+Rad[g] and Dexa+Rad[h] groups, respectively (based upon Tukey's multiple comparison analysis performed after factorial ANOVA). The extinction of CTA was observed between the period of 2–5 days.

The differences of the effects of radiation (2 Gy) with respect to the sex of the animal in all dosage groups over a period of 5 days were analyzed using three-way ANOVA analysis (saccharin preference ratio was considered to be a dependent variable). The analysis revealed that there is a significant ($P<0.05$) variation in saccharin intake over a period of 5 days ($F_{5,492}=170.29$) and with respect to different doses also showed significant change ($F_{7,492}=98.93$). The interaction effect between sex (male vs. female) and different dose groups revealed significant ($P<0.05$) change ($F_{7,492}=14.053$). The maximally significant ($P<0.05$) dose in males was found to be 200 mg/kg b.wt., while in case of females it was found to be 250 mg/kg b.wt. Such interaction between sex (male vs. female) vs. days revealed no significant change ($F_{5,492}=0.444$). On the other hand, the interaction effect between days vs. dose in both the sexes revealed significant ($P<0.05$) treatment effect ($F_{35,492}=5.845$). The triple interaction effect between sex, days and dose was found to be non-significant ($F_{35,492}=0.883$).

3.2. Polyphenolic content in ginger extract

The total phenolic content determined in zingiber extract was found to be 3.83±0.003 mg% quercetin (in which quercetin is used as standard polyphenolic compound).

3.3. Nitric oxide ion scavenging activity

As shown in Fig. 3, zingiber extract exhibited significant nitric oxide scavenging potential (IC_{50}: 710.17±5.05 mg/ml).

3.4. Hydroxyl ion scavenging potential

The hydroxyl ion scavenging of ginger extract was also measured using both non-site-specific and site-specific deoxyribose degradation assay. The hydroxyl ion scavenging potential was found to increase concomitantly with increase in concentration (0.05–1000 μg/ml) of zingiber extract (Fig. 4). Maximum hydroxyl ion scavenging potential was evaluated as percentage inhibition of deoxyribose degradation estimated using TBA. The maximum percent inhibition of ginger extract in case of non-site-specific and site-specific study was 32.47% (1000 μg/ml) and 31.51% (1000 μg/ml), respectively. The values that were found to be significant ($P<0.05$) vis-à-vis control (0% inhibition) are summarized in Fig. 4.

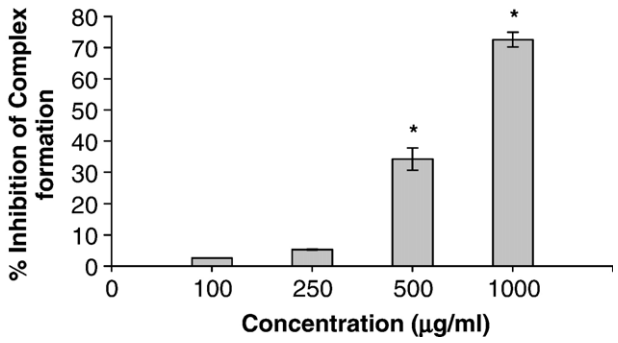

Fig. 3. Evaluation of nitric oxide scavenging ability of the extract expressed as percent inhibition of azodye vs. various concentrations of zingiber extract (*drug treated vs. control (0% inhibition); $p<0.05$).

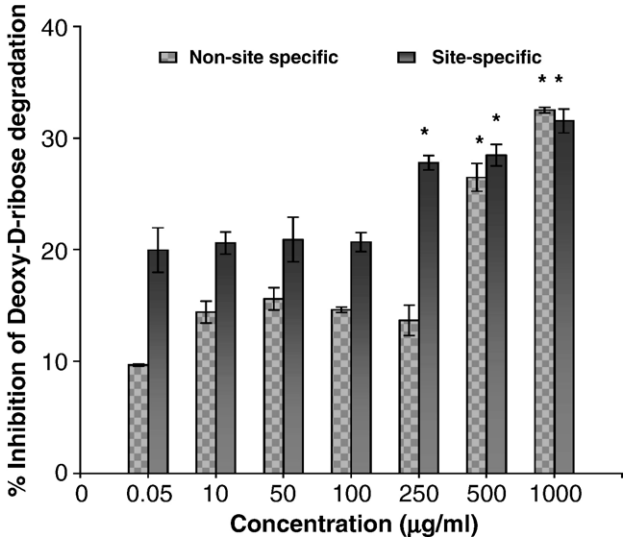

Fig. 4. Evaluation of site-specific/non-site-specific scavenging ability of zingiber extract (0.05–1000 μg/ml) (*$p<0.05$ significant with respect to control 0% inhibition).

4. Discussion

Ionizing radiations are known to have significant effects on a variety of neurobehavioral factors, of which the consumption behavior is relatively radiosensitive (Rabin et al., 1989). Exposure to ionizing radiation is also known to reduce food and water consumption and to induce nausea and vomiting (Spector et al., 1986). Instances of radiation-induced anorexia and adipsia have been noted (Morse and Mickley, 1988). Functional changes in the gastrointestinal tract occur very early in the radiation syndrome (GI syndrome) (Dublineau et al., 2000) with radiation-induced attenuation of ion transport in colon and changes in 5-HT-mediated pathways (Francois et al., 2000). It has been reported that post-irradiation release of serotonin (5-HT) from the gastric enterochromaffin cells could manifest nausea in man and taste aversion in rats (Lopez et al., 1999).

The present study indicated that zingiber extract was able to provide significant protection ($P<0.05$) against 2-Gy-induced CTA in male as well female rats between 3 and 5 post-irradiation days helping in extinction of CTA. Its efficacy was compared with two well-known commercially available antiemetic drugs; ondansteron (HT-antagonist) and dexamethasone (an anti-inflammatory corticosteroid). It was observed that zingiber, which possesses both 5-HT antagonistic (Holtmann et al., 1989) and anti-inflammatory (Bliddal et al., 2000) properties, was able to deliver comparable protection ($P<0.05$). A number of concentrations were tested and the dose of 200 mg/kg b.wt. for males and 250 mg/kg b.wt. for females were found to be the most effective in extinguishing radiation-induced aversion and maximally restoring the normal taste preference by the fifth post-irradiation day, as can be observed from Figs. 1 and 2.

It was also observed that, by the fifth day, anti-CTA efficacy of zingiber extract in case of males increased from 150 mg/kg b.wt. attaining its saturation level at 200 mg/kg b.wt. and beyond which it decreased from 250 to 300 mg/kg b.wt., whereas anti-CTA efficacy of zingiber extract in case of females increased from 150 to 200 mg/kg b.wt. attaining its saturation level at 250 mg/kg b.wt. and beyond which it decreased at 300 mg/kg b.wt. This could be attributed partially to multi-component nature of our zingiber extract and also to sex-dependent variations in various pharmacological parameters, although further studies are required to determine the exact mechanism for such a complex observation of reduced effect with higher doses.

These findings also indicate that male and female rats may respond differentially towards radiation exposure in relation to CTA and also the zingiber extract optimally acts at different concentrations in both sexes. Herbal extracts have been reported to elicit differential effects in male and females in the case of polyherbal antiobesity preparation OB-200G (Kaur and Kulkarni, 2001). Sex-based differences in pharmacological parameters like drug efficacy and toxicity profiles are now widely accepted (Gorski et al., 2004). Gender differences contribute to variations in pharmacokinetic parameters (absorption, distribution, metabolism and excretion) originating from various differences including body weight, BMR, plasma volume, gastric emptying time, plasma protein levels, cytochrome P450 activity, transporter function and elimination activity (Gandhi et al., 2004), which can partly explain difference in the dosage of zingiber extract for maximal efficacy in male and female rats against radiation-induced CTA . Further, it has been also observed that females are more sensitive towards oxidative stress as compared to male rats (Verma and Rana, 2004). It has been also noted that female sex hormones can lead to production of free radicals, lipid peroxidation and DNA damage (Liehr and Ricci, 1996; Ho and Roy, 1994; Ripple et al., 1997) and even cause conditioned taste avoidance (Curtis et al., 2004); thus, it supports our observations that more concentration of extract may be required in case of female than male rats. These very hormonal fluctuations have been also found to be responsible for differences in taste responses (differential sensitivity towards saccharin), feeding patterns (differential fluid intake) and aversive behavior (estrogen-induced avoidance) in relation to their sex, age and estrous cycle (Kanaka et al., 1979; Sharon et al., 1998). Gonadal hormone status has also been reported to regulate anorectic and non-anorectic effects of drugs through secretion or blockade of serotonin (Eckel et al., 2005), from which we can hypothesize that gonadal hormonal levels may also be modulating zingiber extracts (serotonin blocker) efficacy to restore feeding/drinking pattern (as radiation exposure causes secretion of serotonin, which suppresses food and water intake) differently, depending upon their sex and estrous cycle phase. All the above-stated factors could be contributing towards the observed sex-based variations in radiation/extract sensitivity, although precise mechanisms and pathways need to be elaborately worked out.

Free radicals have been implicated in induction of radiation and age related behavioral deficits (Shukitt-Hale et al., 2000). Nitric oxide is found to be a behaviorally toxic molecule and involved in induction of CTA as well (Wegener et al., 2001; Rabin, 1996). It has been reported that hydroxyl ion and reactive oxygen species damage gastrointestinal mucosa, causing ulcers and disturbing intestinal motility (Repetto and Llesuy, 2002), which could lead to vomiting/emesis.

Zingiber extracts hydroxyl ion scavenging potential and nitric oxide scavenging activities were evaluated at in-vitro level in a cell-free system to assess the inherent ability of drug to act at different fronts in case of radiation-induced oxidative stress. The antioxidant ability of zingiber extract in aqueous media was studied using deoxyribose assay in which non-site-specific ($Fe^{2+}+H_2O_2+EDTA$) as well as site-specific ($Fe^{2+}+H_2O_2$) hydroxyl ion scavenging potential was evaluated. In a site-specific assay, Fe^{2+} induces hydroxyl generation but maximally attack results directly to deoxyribose prior to hydroxyl generation (Aruouma et al., 1987). Zingiber extract exhibited both the activities, i.e., non-site-specific as well as site-specific maximally at 1000 μg/ml exhibiting its ability to chelate transition metal ions by filling its aqua coordination sites as well as direct scavenging ability of hydroxyl ions (Fig. 4) indicating that ginger extract could boost the natural defence system, which includes secondary antioxidants like transferrin and ceruloplasmin (Gutteridge, 1985; Kumar and Goel, 2000). Nitric oxide, an essential bioregulatory lipophilic molecule, readily diffuses across the cells and is induced during radiation-induced oxidative stress (Gupta et al., 2003) and zingiber extract exhibited significant nitric oxide scavenging potential (IC_{50}: 710.17±5.05 μg/ml) (Fig. 3). A chemically induced nitric oxide system was used and the effective quenching of NO by the bioactive constituents of ginger extract indicated its possible role in protection of the tissue against nitric oxide-mediated degradation. These antioxidant abilities of ginger extract could be attributed to its greater concentration of polyphenols (3.83 ±0.003 mg% quercetin), which are composed of one (or more) aromatic rings bearing one (or more) hydroxyl groups and are capable of scavenging free radicals by forming resonance stabilized phenoxy radicals (Arora et al., 2005).

The behavioral radioprotective effect of zingiber extract on radiation-induced CTA can be partly attributed to the above-mentioned properties. It can be stated that the extract exerts its effect at multiple points. By virtue of its 5-HT antagonistic (diterpenoid galanolactone, zingerones) (Huang et al., 1991; Yamahara et al., 1989) and hydroxyl radical scavenging activities, it may decrease gastric motility, blocking GI reactions and subsequent nausea feedback. The antioxidant and nitric oxide scavenging abilities of zingiber extract (thymol, carvacrol, 6-gingerol, zingerone, hydroxytyrosol, 6-dehydrogingerdione) exert their effect at the gastrointestinal as well as humoral route. Also the anti-cyclooxygenase activity of the extract (6-gingerol, 6-shogaol, labdane type diterpene dialdehydes, diarylheptanoids) may be acting at the visceral level suppressing inflammatory prostaglandins and thus inhibiting 5-HT release. (Tjendraputra et al., 2001; Kudo et al., 2001). Zingiber extract, through its neuromodulatory effect, may be acting at the brain level protecting it from radiation-induced activation of sensory receptors and mitigating the disturbances in neural activity (Hasenöhrl et al., 1996).

Thus the present study demonstrates that zingiber extract offers good behavioral radioprotection against CTA in rats by multitargeted action (Table 1). From which it seems that *Z. officinale* has immense potential for being used as a behavioral radioprotector, with promising applications particularly in cancer radiotherapy, planned radiation exposure, space explorations, etc. Though further biochemical, molecular and clinical studies are needed to delineate all the possible factors and pathways involved in such a complex response.

Z. officinale with its antiemetic-gastroprotective (al-Yahya et al., 1989), cardiotonic (Kobayashi et al., 1988), anti-inflammatory (Kiuchi et al., 1992) and neuromodulatory (Topic et al., 2002a,b) properties can be the answer to most of the problems associated with the use of synthetic radioprotectors (Table 1), which render it as the potential candidate for being used as an adjunct, negating most of their side effects.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pbb.2006.04.008.

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