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Camellia Sinensis as a Safe Neuroprotective Radiation Countermeasure Agent.

[1]Jyoti Sharma, [2]Raman Chawla, [3]Raj Kumar, [4]Ashok Sharma, [5]Rakesh K Sharma,[6]Rajesh Arora,
Institute of Nuclear Medicine and allied Sciences, Brig. SK. Mazumdar Road, Timarpur, Delhi-110054, India.

ABSTRACT: *The neurobehavioral protective efficacy of hydroalcoholic extract of Camellia sinensis (CS) was evaluated by studying its prophylactic ability to mitigate gamma (γ-) radiation (2Gy)-induced conditioned taste aversion (CTA) in Sprague-Dawley rats (300mg/kg b.w. i.p. -1hr.) was effective in eliciting extinction of saccharin avoidance response on 5th post-treatment day. A comparative analysis of changes in the levels of antioxidant enzymes and endogenous antioxidants revealed significant ($p<0.05$) resumption of catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity by 5th day. However, an increase in hepatic glutathione transferase (GST), with concomitant decrease in neural GST was observed, indicating activation of drug metabolising pathways in hepatic tissue. The ability of CS to maintain membrane integrity was significant in both neural and hepatic (2-fold protection in each case) tissues indicated by supressed level of malondialdehyde (MDA). Similar trend was also exhibited in terms of maintenance of endogenous levels of antioxidants like reduced glutathione (GSH) and Ascorbate. A shift in antioxidant balance upon administration of CS indicates its inherent potential to act as a radiation countermeasure (RCM) agent, particularly for its ability to provide neurobehavioral radiation protection against gamma radiation exposure. The non-toxic nature of CS in humans makes a potential RCM agent with its practical application while responding during nuclear and radiological emergencies (NREs).*

KEYWORDS: *Radioprotection; Camellia sinensis, radiation countermeasure agents, Conditioned-taste aversion.*

I. INTRODUCTION

Oxidative stress induced by ionising radiation (IR) in the physiological milieu can be attributed to the inherent ability of IR to initiate a cascade of reactions to that significantly build up the levels of reactive oxygen/nitrite species (ROS/RNS) in cells and tissues. This process leads to significant damage to DNA, lipids and proteins as the resistance offered by antioxidant enzymes, including superoxide dismutases (SODs), glutathione (GSH) peroxides (GPx), catalase (CAT) and a variety of thiols and free radicals scavenging agents, is often insufficient[1] [2] [3]. Oxidative stress leads to a disturbance in the delicate balance of oxidants and antioxidants under normal physiological conditions. Oxidative stress can lead to extensive biological damage, and can result in increased susceptibility to both short/ long-term effects as well as inflammatory insults [4] [5].This, in turn, also contributes toappearance of neurochemical and behavioural deficits alongwith progression of neurodegenerative diseases [6-8] [9] [10]. The deleterious effects are even more pronounced upon irradiation. Neurological deficits, induced by oxidative stress, have been widely studied in rat brain model. A plethora of antioxidant compounds of both synthetic and natural origin have been screened for their prophylactic and therapeutic efficacy using such models [11-12]. In addition, several compounds of natural origin can modulate radiation-induced oxidative stress.

The most widely accepted model for studying the neurobehavioral effect of ionizing radiations is conditioned taste aversion (CTA) [13]. CTA is manifested when a gustatory conditioned stimulus (CS) is paired with as aversive illness inductive agent (e.g. radiation/toxin)[14] [15-16]. As a result of pairing, the organism avoids intake of that taste on subsequent pairing [17] [16]. Radiation-induced CTA has been defined as a behavioral endpoint, which is mediated by the deleterious effects of gamma-radiation on peripheral systems, primarily the gastrointestinal system [17]. Development of a pharmacological agent that can mitigate the early damage produced in neural cells by ionizing radiation remains a major challenge. The widely used synthetic radioprotector (mainly sulfhydryl compounds; phosphorothioates) i.e. WR-2721(Amifostine), WR-1607, WR-3689 etc. [18], have been reported to render significant levels of protection. However, the beneficial effects of these synthetic radioprotectors are accompanied by side effects [19-20], which include nausea, vomiting, diarrhoea, hypotension and allergic reactions, implying behavioral toxicity and performance degradation [21-

22]. Amifostine (WR-2721) has been reported to induce CTA (conditioned taste aversion) on its own and even accentuate radiation-induced

CTA in rats at doses optimal for radioprotection [23]. Studies on cancer patients undergoing radiotherapy have shown WR-2721 to have poor bioavailability, mainly due to first-pass metabolism by intestinal mucosa during absorption and the drug gets hydrolyzed in the acidic environment of the stomach, a factor that is aggravated by its ability to slow gastric emptying [24]. The overall risk for WR-2721 is unfavourable, especially as a radiation prophylactic drug for its potential use at incident site during emergency scenarios.Combination modality (i.e. combining WR-2721with antiemetics, anti-inflammatories) at therapeutic sub-toxic doses possesses the ability to reduce the lethal effects of radiation; however, less attention has been paid towards the ability of drug to prevent radiation-induced behavioural disruption and performance decrement [25] [26] .

An ideal neurobehavioral radiation countermeasure (RCM) agent should possess the ability to mitigate performance and behavioural deficiencies without being toxic [27]. In due course of time, 'antiemetics' on their own have been grouped as a third category of behavioral radioprotectors after 'direct attenuators' and 'incidental attenuators' [28] [29]. However, several side effects, like drowsiness, sluggishness, headache, stomach cramps, disturbed vision, etc., have also been documented with the usage of antiemetics limiting their for use in military setting utility [30]. The attention of researchers has shifted towards utilizing herbal products as RCM agents due to their low toxicity profile and holistic mode of action [31]. Considering the paucity of availability of safe, effective and efficient RCM agents for practical use during nuclear/radiological emergencies, the present study was undertaken. In this study we evaluated the potential of Green Tea extract against radiation-induced neurobehavioral impact using CTA. In addition, we have also estimated the antioxidant ability at in vitro level to assess its potential to act as a neuroprotective radiation countermeasure agent.

II. MATERIAL AND METHODS

Animals

The experiments were carried out with Sprague Dawley adult male rats (12-15 weeks old; 323± 25 gm) that were in-bred at animal house of the Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi. Animals were kept in the animal care facility of INMAS under standard laboratory conditions with photoperiod of 12h/day and temperature 25±2ºC. The rats were housed individually in polyvinyl cage and fed standard animal food pellet (Golden Feeds, Delhi, India) and were offered tap water ad libitum. All the procedures were carried out in strict compliance approved by an Institutional Review Committee for the use of Human or Animal Subjects followed in this institute 'Institute of Nuclear Medicine and Allied Science'.

Preparation of Extract

The herbal extract was prepared from fresh leaves of Camellia sinensis. The plant material was procured from Jammu and Kashmir region, India followed by in house identification by an experienced Botanist. A voucher specimen (specimen No RADI-JK-007) has been maintained. Leaves was shade dried and crushed, extracted in 50% ethanol at mild temperature (35-50ºC), filtered, concentrated in a rotary evaporator (Buchi, Switzerland) at 50±5°C, lyophilized and stored at −20°C in freezer until use. The yield on w/w basis was approximately 10±1%. The extract (CS) was suspended in 10% ethanol and filtered through Milipore 0.2 microns filter prior to use. CS was administered intraperitoneally to experimental animals 1h prior to irradiation (2Gy). For in vitro experimentation <5% ethanol was used. Hereafter, the code 'CS' will be used to describe extract being tested.

Maximum Tolerable Dose Estimation

Different doses of CS were administered to Sprague Dawley rats via intraperitoneal (i.p.) route and the non-toxic dose was determined in a study ranging over a period of 2-3 days, recorded in terms of gross changes in behaviour, fecal count and several distress [31] ,

Irradiation

Each rat was placed in a wire gauze container and exposed to 2Gy γ-radiation in the ^{60}Co Gamma irradiator (Model 220, Atomic Energy Commission, Canada) having a dose rate of 0.3 Rads/sec. Dosimetry was carried out with Baldwin Farmer secondary dosimeter and Fricke dosimeter.

Conditioned Taste Aversion (CTA) Test

All animals were habituated to the laboratory conditions at least 4 weeks prior to training. Rats were trained for 23.5 hours water deprivation scheduled for 10 days (conditioning period), wherein each animal was offered tap water only (i.e.,

one bottle paradigm) [32] for 30 min (10:00- 10:30 am) afterward in a complete cycle of 24 hrs. Water consumption of individual animal 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 minutes (two- bottle regimen) [33], 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 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 experiment [34]. Immediately following the conditioning session, the rats were divided into the following groups:

Group I: Vehicle + Sham Radiation (V+SR)
Group II: Vehicle +Radiation (V+R)
Group III: CS + Sham Radiation (D+SR)
Group IV: CS + Radiation (D+R)

Group I and II animals were administered with vehicle [saline (0.9%)] prior to exposure of sham radiation and 2Gy respectively. Group III and IV animals were administered CS (300mg/kg b.wt.) and after 1 hour 2Gy radiation was given to group IV only. After 20 hours of incubation, the animals were given choice between saccharin solution and tap water at regular interval and their respective intake was recorded. This procedure was repeated for 5 post-irradiation days. The measure of extinction of CTA are represented in the form of Saccharin Preference Ratio (SPR), where SPR = amount of Saccharin solution (0.1%) consumed /total fluid intake.

Preparation of tissue homogenate and protein estimation

Prior to biochemical analysis, neural and hepatic tissues (100mg tissue/ml buffer) were homogenized in 50mM phosphate buffer (pH 7.2); the homogenate was then centrifuged at 10,000×g for 30min and the supernatant was used for biochemical analysis. The protein concentration in each fraction was determined by the Bradford method [35].

Catalase activity determination

Tissue homogenates were centrifuged at 10,000 rpm for 10 min. An aliquot collected from supernatant was used to determine neural catalase activity. Catalase activity was assayed spectrophotometerically by measuring the decrease in absorbance of Hydrogen peroxide (H_2O_2) at 240nm [36]. Specific activity was expressed as nmol/mg/proteins/ min. Protein levels were determined in the supernatant.

Superoxide dismutase (SOD) activity determination

SOD activity was measured at 560 nm according to standard protocol, [37] based on the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT). The reaction mixture contained 33 mM NBT, 10mM L-methionine, 0.66mM EDTA and 3.3 mM riboflavin in 50 mM phosphate buffer, pH 7.8. Reactions were carried out at 25 °C for 10 min, in light and were kept in dark for 20 min to stop the reaction. One unit of enzyme activity was defined as the quantity of SOD required for 50% inhibition of NBT reduction.

Peroxidase activity determination

The activity of peroxidase was determined essentially as described [38] . The principle of this method is that the rate of guaicol oxidation by hydrogen peroxide (H_2O_2) as catalysed by the peroxidase present in the supernatant is determined by comparing the absorbance of the test against a reagent blank at 412 nm on a microplate reader (Power Wave, Biotek, USA). The activity of peroxidase was expressed in terms of nmol/mg protein.

Glutathione-S-transferase (GST) activity determination

The activity of GST was determined by the method of Habig [39]. The conjugation of GSH with 1 chloro, 2-4 dinitrobenzenes (CDNB), a hydrophilic substrate, was observed spectrophotometrically at 340nm to measure the activity of GST. The enzyme activity was expressed nmoles/mg protein.

Glutathione Reductase (GR) activity determination

The assay was performed using standard method [40]. The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0), 250 mM NADPH and tissue homogenate. The reaction was initiated by the addition of GSSG (50 mM). The oxidation of NADPH was followed at 340 nm and the activity of glutathione reductase was expressed nmoles/mg protein.

Lipid peroxidation assay

Lipid peroxidation was evaluated by measurement of thiobarbituric acid-reactive substances (TBARS). Malondialdehyde (MDA) has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give red light absorbancy at 534nm. Protection index was measured on the basis of decrement in MDA formation. Results are expressed in nanomoles of MDA/mg protein [41].

Reduced glutathione determination

The GSH content was estimated by the method of Okhawa [42]. To the supernatant of the tissue homogenate, 0.5 ml of 10% trichloroacetic acid was added followed by re-centrifugation. To the protein-free supernatant, 4ml of 0.3M Na_2HPO_4 (pH 8.0) and 0.5 ml of 0.04% (w/v) 5,5-dithiobis- 2-nitrobenzoic acid were added. The absorbance of the resulting yellow colour was read spectrophotometrically at 412 nm. The results are expressed as nmol/mg protein.

Ascorbate (vitamin C) level determination

Ascorbic acid was measured by the method of Moron [43] Ascorbic acid is oxidized by copper to form dehydroascorbic acid which reacts with 2,4-dinitrophenyl hydrazine to form bis-2,4-dinitrophenyl hydrazine; this undergoes further rearrangement to form a product with an absorption maxima at 520 nm. The results are expressed as nmol/mg protein.

Statistical Analyses

All the data are expressed as mean ± SE. Inter-group comparisons were made using ANOVA, followed by post-hoc Tukey's test. All analysis was done using SPSS (trial version) Tukey's post hoc analysis and homogenous subsets, as required. 95% confidence level was considered significant i.e., $p < 0.05$.

III. RESULT

In Table 1 the maximum tolerable dose (MTD) was found to be 1500mg/Kg bwt., showing non-toxic nature of the extract. The various scoring was given based on observations of table indicating of lethargy, fecal counts and anxiety in the dose range from 100-1500mg/Kg bwt. Based on the observations, it was found that change in status with respect to anxiety, lethargy and fecal counts was non-significant upto 400mg/Kg bwt indicating that the dose range below this dose is appropriate. Accordingly,300 mg/Kg bwt. was selected as test dose (5 times lesser than maximally tested dose) for further experimentation. **Figure1 and figure 2.** As shown in Figs. 1 and 2, CS was observed to considerably restore saccharin consumption of animals exposed to 2Gy, as compared to the radiation control group. The intake of saccharin was found to increase considerably over the period of 5 days at 300 mg/kg b.wt. A comparison of average saccharin consumption over a period of 5 days is given in figure 1. The maximum saccharin intake was observed on fifth post-irradiation day and saccharin preference was restored by fifth post-irradiation day. Such an increase in saccharin preference indicated extinction of CTA as compared to 2 Gy radiation control maximally on 3rd, 4th and 5th post irradiation day. A significant increase in mean value of spr with respect to change in of time ($F_{5,72}$= 77.504) and treatment ($F_{3,72}$ =13.053) variable at $p<0.05$ level considering 0 day as control. The interaction effect between the days and treatment in male rats was also found to be significant ($F_{15,72}$ = 13.053) at $p<0.05$ level.

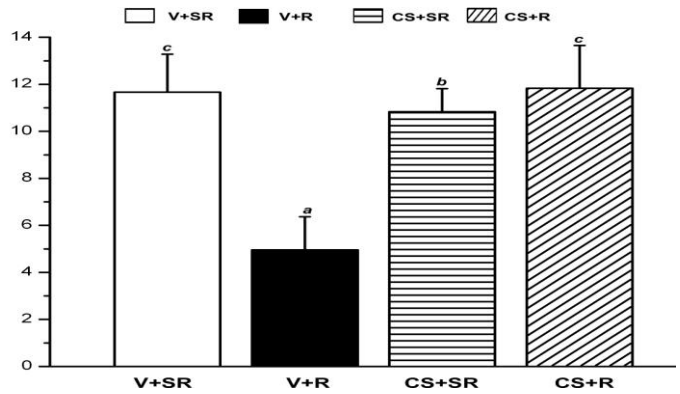

Fig 1: Effect of CS on Conditioned Taste Aversion (CTA) over a period of 5 days amongst four groups [V= vehicle, R= Radiation (2Gy), SR=Sham Radiation CS - Extract {300mg/Kg bwt/}, n=6 per group] . Values expressed as mean ± standard error. Bars with same letters are not significantly different. The average saccharin consumption has been estimated as Total Saccharin consumption over 5 days. Analysis is based on ANOVA followed by Tukey post HSD Hoc Test followed by homogenous subset analysis.

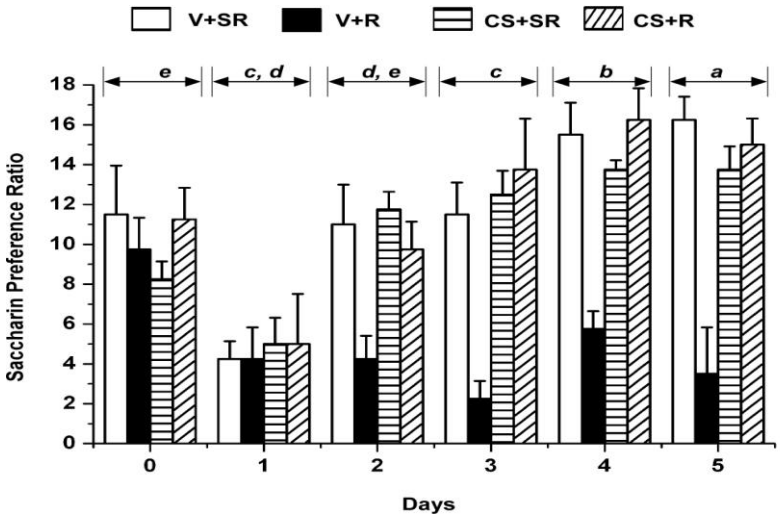

Table 2 and Table 3. Table 2 shows the mean specific activities of antioxidant enzymes i.e., CAT, GPx, GR, GSR and SOD estimated in the neural tissues of Sprague Dawley rats sacrificed on 5th day. The analysis revealed that in RADI−007 +2Gy group, CAT and GR levels achieved control levels or 1.2 times higher than 2Gy (negative control) by 5th day. Similarly, GPx, GST and SOD also exhibited resumption of control level specific activity as compared to 2Gy. A significant decrease ($P<0.005$) in CAT activity was observed in RADI−007 only group while other enzyme doesn't exhibit significant change as ($P<0.005$) compared to positive control. In case of hepatic tissue, increase in levels of all antioxidant enzymes was observed except SOD in RADI−007 only group indicating induction of metabolic machinery. CAT, GR and SOD resumption of activity was observed on 5th day in RADI−007+2Gy as compared to negative control. However, non-significant decrease in GPx and GST radiation control revealed that low radiation dose sensitivities vary among different tissues (Table 2). Table 3 depicts levels of non-enzymatic antioxidants in neural and hepatic tissues of Sprague Dawley rats on 5th post-irradiation day amongst various groups studied. In RADI−007+2Gy, enhanced MDA level has been restored to control values (1.6 times lower than 2Gy group) by 5th day. The significant decrease (P<) in levels of intrinsic glutathione and ascorbate were also resumed by administration of RADI−007 in the specified test period. In hepatic tissue, induction of lipid peroxidation was slightly higher than neural tissue (1.7 times) which has been mitigated significantly ($P<0.05$) by administration of RADI−007. RADI−007 prophylaxis has also resumed physiological levels of in glutathione and ascorbate as compared to positive control.

Table I Observable Impact of CS administration (100-1500mg/Kg bwt.)

Dose*	Lethargy	Fecal count	Anxiety
100	+	+	+
200	+	++	++
300	++	++	++
400	++	++	+++
500	+++	+++	+++
750	+++	+++	+++
1000	+++	+++	++++
1250	++++	++++	++++
1500	+++++	+++++	+++++

#dose i.p. administered in mg/kg.b.wt.;+ : minimal; ++: moderate level; +++slightly higher; ++++; higher; +++++" significantly higher; with respect to control(100mg/kg b.wt.)

Table II Effect of CS on levels of Antioxidant Enzymes in neural and hepatic tissues

Enzymes#	Control	2Gy	CS	CS +2Gy
Neural				
CAT	6.714±.045	3.45±0.613	3.89±0.98	7.34±3.56**
GR	1.2045±0.011	0.629±0.0031	0.998±0.0089	1.3032±0.0109**
GPx	9.24±0.18	6.29±.29	8.27±1.16	9.72±0.964*
GST	14.65±1.22	9.68±0.97	12.91±0.88	13.79±0.98*
SOD	40.69±9.69	32.48±4.32	38.92±3.46	41.34±2.39*
Hepatic				
CAT	52.34±0.88	46.714±.045	55.79±0.88	63.45±0.613**
GR	0.161±0.071	0.154±0.016	0.169±0.05	0.198±0.045*
GPx	0.0549±0.0064	0.0536±0.027	0.0586±0.0026	0.0635±0.0038*
GST	7.81±0.47	7.00±0.29	9.89±0.90	11.91±0.71**
SOD	39.93±1.052	18.85±3.3	34.32±4.76	42.99±5.41**

#Catalase (CAT); Glutathione Reductase (GR); Glutathione Peroxidase (GPx); Glutathione Transferase (GST); Superoxide Dismutase (SOD) Activity estimated in nmoles/min/mg of protein in respective tissue homogenates of Sprague Dawley Rats administered with CS (300mg/kg b.wt.) on 0day 1 hr prior to 2Gy exposure, sacrificed on 5th post-irradiation (n= 6 per group).* $P<0.05$ & ** $P< 0.01$ indicates Significant changes in activity with respect to radiation control (2 Gy)

IV. DISCUSSION

Exposure of ionizing radiation is known to cause a significant neurobehavioral impact, including loss of appetite and water consumption, causing nausea, vomiting with reported incidences of anorexia and adipsia. It might be attributed to radiation-induced gastrointestinal syndrome due to attenuation of ion transport in colon; release of serotonin (5HT) from the gastric enterochromaffin cells and manifested changes in 5HT-mediated pathways [44] leading to conditioned taste aversion in cancer patients undergoing radiotherapy. This has indicated the probable usage of this test in evaluation of radiation countermeasure agent. Green tea is obtained from the leaves of Camellia sinensis (family Theaceae), an evergreen shrub, that grows in several countries across the globe. In Asia, especially China and India, green tea has been used as a beverage for more than 4000 years. Green tea owes its antioxidant activity mainly due to the presence of polyphenols like flavonoids, flavanols, flavandiols and phenolic acids, which scavenge destructive ROS/RNS generated in the body. Polyphenols make up 30-40% of the green tea dry weight. (-) Epicatechin (EC), (-) epicatechin gallate (ECG), (-) epigallocatechin (EGC) and (-) epigallocatechin gallate (EGCG) are the key catechins present in green tea while small amounts of (-)-epigallocatechin-3(3'methyl)-gallate (EGCMG), (+)-catechin (C) and (+)-gallocatechin-3-gallate (GTG) have also been reported. These catechins and their gallate derivatives belong mainly to the flavan-3-ol class and are known to exhibit number of health benefits including cardiovascular and neuroprotectant effect [45]. EGCG is known to prevent radiation-induced increase of lipid peroxidation in liver and has been suggested as an orally effective radioprotector with veminisculery little toxicity [46][45][46][46]. The analysis of spectrum of extinction of taste aversion in conditioned animals over a period of 5-days revealed that the interactive effects of days and treatment are significant. The results clearly indicates that extinction of CTA by Green Tea Extract is time-dependent and its efficacy is based on alterations induced in physiological milieu. The dosage of 300 mg/kg body weight of CS was found to be most effective in extinguishing radiation-induced taste aversion and completely restored the normal taste preference by the fifth post-irradiation day against 2 Gy (negative control) fig-2. The preliminary screening of toxicological manifestation revealed non-significant changes upto 1500mg/kg bwt., indicating its safe usage due to broad therapeutic index (Table 1). However, the neuroprotective efficacy is limited to extinction of CTA, though acquisition of aversion was not observed. This could be attributed to irreversible damage that occurred in neural tissue. Green tea is well known for its safe consumption and associated befeficial health effects. The safety record of several thousand years of usage by human and health promoting properties associated with Green Tea Extract make it useful as a potential Radiation Countermeasure (RCM) agent for first responders operating in radiological and nuclear warfare zones, cancer patients undergoing radiotherapy and a prophylaxis dose regime against planned exposure during space travels. Thus, to understand the utility of Green Tea in manifesting neurobehavioral radiation protection, various antioxidant enzymes and endogenous antioxidants levels of both neural and hepatic tissues were evaluated.

A comparative analysis revealed that the CAT, GR, GPx, and SOD activity resumed to its normal level within 5 days in both tissues, however, hepatic GST exhibited an increase, while neural GST exhibited a decrease in activity (Table 2&3). This could be attributed to activation of Phase I and Phase II toxicological enzymes in response to administration of drug, liver being major site of drug metabolism, which is not in case of neural tissue. Further, the dose regime dosage imparted significant ($P<0.05$) membrane protection in both neural and hepatic (2-fold Protection in each case) tissue homogenates by suppression of levels of MDA. Such similar ability of maintaining membrane integrity has been reported earlier [54] using different plants attributing towards its protective behavior. In case of resuming normal the baseline thresholds of Glutathione (GSH) and Ascorbic Acid, the effect of CS is significant, however induction of these endogenous antioxidant was not observed, such balanced action could be related to significant induction of antioxidant enzymes for which these biomolecules act as substrates. The overall analysis revealed that Green Tea Extract (CS) induced antioxidant enzymes and supplements endogenous antioxidant levels both in neural and hepatic tissue implying its protective efficacy against radiation exposure, which could be attributed to the presence of compounds like EGCG; flavonal-3-ol class and polyphenols.

The role of the brain in mediation of taste disorder is supported by the taste alterations described in patients with neurological and psychiatric disorders. It indicates taste dysfunction might be inter-linked as a consequent combined impact of radiation on hepatic and neural tissue. Moreover, 5-HT also affects food intake both in humans and animals. The neurobehavioral effect of CS extract on radiation-induced CTA can be attributed to the above-mentioned properties. In addition, the 5-HT antagonistic action of Z. officinale negates the serotonin released in the intestine, restoring gastrointestinal integrity and blocking the visceral pathway , thereby counteracting the 5-HT mediated suppression of fluid intake and development of aversion for saccharin. CS extract, through its neuromodulatory effect, may be acting at the brain level thereby protecting it from radiation-induced activation of sensory receptors and mitigating the disturbances in neural activity. Its action could also be mediated via suppression of nitric oxide production or by virtue of its anti-cyclooxygenase activity, it may be acting at the humoral level, suppressing inflammatory prostaglandins [48].Green tea has been used against accumulation of eicosanoids and formation of oxygen free radicals that have been implicated in the pathogenesis of ischemia/reperfusion brain injury. In addition, it is also used in reduction of neuronal cell death [47]. Green tea extract administration is effective in enhancing learning and memory in aged rats, and hence, may be useful in reversing age-related deficits[48]. The ability of green tea extract in modulating taste preferences and ingestion behavior are following radiation have been studied using CTA neurobehavioral model, and the results are in coherence with our previous studies using another herbal extract Zingiber officinalis [49]. In conclusion our studies indicate the C. sinensis can be safely used as a radiation countermeasure agents during nuclear and radiological emergencies (NREs) to develop herbal formulation prophylactic for performance increment and during operation in contaminated scenarios.

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