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Purple grape juice as a protector against acute x-irradiation induced alterations on mobility, anxiety, and feeding behaviour in mice

Félix A. A. Soares¹, Cristiane L. Dalla Corte¹, Edson R. Andrade², Raquel Marina¹, Paquita González⁴ and Juan P. Barrio⁴


Abstract
The aim of this work was to test the hypothesis that a moderate intake of organic purple grape juice shows a positive radiomodifier effect over early behavioural damage following acute X-irradiation in mice. Anxiety-, locomotion-, and feeding-related responses to 6 Gy total body X-irradiation (TBI) were studied via open field, Rotarod, and feeding/drinking recording. Thirty-two male mice weighing 25-30 g were grouped according grape juice (J) or water (W) ad libitum drinking and either non-irradiated (N) or irradiated (R). 24 h post-TBI the access frequency to the center and corners of the open field was decreased, and the total stay in the corners increased, in RW vs. NW mice. Anxiety-related parameters decreased in RJ vs. RW mice. Rotarod latency times increased 72 h post-TBI in RJ vs RW mice. No overall changes in food and drink intake were observed along the experimental period. On the irradiation day, bout number was increased and bout duration was decreased in RW mice. The changes were reversed by purple grape juice intake. Grape juice intake before and after TBI can overcome several radiation-induced changes in behaviour within 24-72 hours after sub-lethal X-irradiation. This beneficial effect on short-term anxiety and mobility-related activities could probably be included in the list of flavonoid bio-effects. The present findings could be relevant in designing preventive interventions aimed to enhance body defense mechanisms against short-term irradiation damage.

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Key words: Anxiety. Behaviour. Grape juice. Ionizing radiation. Mice.

Correspondence: J. P. Barrio.
Departamento de Ciencias Biomédicas.
Universidad de León.
24071 León. Spain
E-mail: jpbarl@unileon.es
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Abbreviations

HPLC: High pressure liquid chromatography.
LD50/30: Radiation dose that is expected to kill half of the exposed population within 30 days.
MDA: Malondialdehyde.
NJ: Non-irradiated mice drinking purple grape juice.
NW: Non-irradiated mice drinking water.
RJ: X-irradiated mice drinking purple grape juice.
RW: X-irradiated mice drinking water.
TBI: Total body irradiation.

Introduction

Radiation injury of the central nervous system, consisting of the brain and spinal cord, may have important clinical consequences. Studies have shown a number of quick and often transient radiation-induced changes in both rodent and human behaviour, with median effective doses for early performance decrement between 3 and 7 Gy. Pathophysiological consequences of ionizing radiation strike might result in cell death or malfunction due to interaction with free highly reactive agents such as hydroxyl radical (OH•) and other oxygen and nitrogen reactive species, which can damage membrane lipids, proteins and nucleic acids. Dysfunction of signal transduction pathways and decrease in hippocampal neurogenesis have been pointed out as directly related to cognitive deficits, and stem–cell therapy is currently being tested as a new approach to overcome cognitive decline associated to cranial radiotherapy. On the other hand, low-dose (less than 1 Gy) ionizing radiation exposure has been shown to activate brain-based pro-inflammatory responses underlying early locomotor activity decrease in mice.

Epidemiological investigations have proved that an adequate intake of fruit and vegetables is helpful in maintaining health and preventing the development of chronic degenerative diseases due the presence of polyphenol-based substances. Among these commonly consumed foods, grapes and their processed products – including wine and juices – are a rich source of phenolic compounds with significant biological activities. Animal model studies clearly suggest the ability of grape polyphenols, including the stilbene resveratrol, to improve neuronal function. Animal model studies clearly suggest the ability of grape polyphenols, including the stilbene resveratrol, to improve neuronal function.

Materials and methods

Grape juice

Ecologically-produced (organic) purple grape juice was obtained from Econatura Produtos Ecológicos e Naturais LTDA in the city of Garibaldi, in the main grape-growing region of the state of Rio Grande do Sul, Brazil. Grapes were cultivated in 2007 and grape juice was prepared from the same crop. The concentration (mg/L) of phenolic compounds in grape juice was previously determined by high pressure liquid chromatography (HPLC) as follows: Resveratrol 3.95 ± 0.01, Quercetin 8.95 ± 0.09, Rutin 3.75 ± 0.03, Gallic acid 81.07 ± 2.03, Caffeic acid 30.28 ± 2.00.

Animals

This study used thirty-two male Swiss ICR/CD1 mice weighing 25-30 g (Harlan, Barcelona, Spain), housed in groups of 4 in polycarbonate cages (425×265×150 mm) with stainless steel lids. Environmental conditions were controlled (12-hour photophase from 8:00 am, 800 lux average photophase illumination, 40% relative humidity, 20 ± 2 °C). Animal handling was conducted at the animal facility of University of León, approved according to Spanish law.

Experimental procedure

According drinking solution treatment (diluted grape juice – J, or water – W) and irradiation (non-irradiated – N, or irradiated – R), animals were divided in four groups (NW, NJ, RW, and RJ). After one week adaptation, mice were allowed to drink grape juice...
(diluted 1:3 with water, referred to as ‘grape juice’ unless otherwise stated) or water, depending on their assigned group, along seven days of treatment. The rationale behind using diluted grape juice in NJ and RJ animals was to provide enough water intake, since previous trials showed decreased voluntary intake of whole purple grape juice\(^\text{11}\). After seven days of grape juice/water drinking, mice underwent a single dose of X-irradiation and behavioural parameters were monitored by 3 more days (under its respective treatment). The experimental protocol was approved by the University of León Ethical Committee, and adhered to the European Community Guiding Principles for the Care and Use of Animals.

**Irradiation procedure**

In order to avoid undesirable behavioural side-effects of different anesthetizing agents\(^\text{14,19}\) we used non-anesthetized animals in the present study. Four mice were placed on a circular Plexiglas cage 20 cm in diameter immediately before the irradiation process. To avoid jumping out, the container was covered with perforated transparent film. Animals were irradiated at a time and exposed to a single dose of 6 Gy TBI from an X-ray machine (200 kV) MAXISHOT 200 (YXLOON, Copenhagen, Denmark), at a radiation dose rate of 0.40 Gy/min, with a source-skin distance of 50 cm. The radiation dose was chosen as sublethal following the reported estimated LD50,30 value of 9.0 Gy for mice, irrespective of strand, sex or age\(^\text{20}\). The actual irradiation process started about 12:00 pm. Non-anesthetized animals in the present study. Four square adjacent open field spaces were set up with white floor and walls, each 60 cm width and 40 cm height, illuminated from above with about 150 Lux cold light (4100 K) fluorescent bulbs. Animals were individually placed at the center of each open field space and a video camera (512×512 pixels) was set up 2 m high to record the trials. All four open field spaces were tracked simultaneously using Noldus Ethovision 3.0 (Noldus Information Technology, Wageningen, The Netherlands). Animal activity (5 min) was recorded at 12:00 am the day prior to TBI as well as each of the three days following TBI. Four square corner zones 15 cm wide were defined on each open field, leaving a center square zone 30 cm wide, and four wall zones. The zones were later grouped into center, corners, and walls for analytic purposes. The behavioural parameters studied were Frequency of entering each zone (the number of times the mouse entered a given zone of the open field arena), Total duration of the stay (total time spent by the animal inside corners, walls or the center of the open field arena, in seconds), Latency to the first occurrence in each zone (time elapsed until the mouse first entered any zone, in seconds), and Distance moved (overall distance travelled by the mouse in each zone during the 5-min open field test, in cm).

**Rotarod test**

Motor coordination was evaluated using the Rotarod apparatus (TSE Systems GmbH, Bad Homburg, Germany), consisting of a rotating rod 30 cm long and 3 cm in diameter divided into five compartments by discs 24 cm in diameter. The rod rotation speed was set at 10 rpm. Animals were given a training session before the first trial in order to acclimate them to the Rotarod apparatus. Latency to first fall from the rod and number of falls were noted. The cut-off time was 120 s.

**Lipid peroxidation (TBARS assay)**

TBARS levels (μmol of MDA/mg of protein) were measured at 532 nm\(^\text{25}\). Protein contents were determined following standard procedures\(^\text{11}\).
Lipid peroxidation data were analyzed using two-way ANOVA. Since behavioural data were recorded from the same individuals over a number of days, the statistical analysis procedure used was repeated-measures ANOVA. The experimental design used in this work involved testing two fixed factors Treatment (water and grape juice) and Radiation (X-irradiation and sham-irradiation) and one within-subjects factor (Day) for each behavioural variable studied. An additional third fixed factor Zone was also included in open field analysis. In feeding behaviour analysis, two within-subjects (repeated measures) factors were considered: Timing (before and after irradiation), and Day (1 to 3 within Timing). The Newman-Keuls test was used for post-hoc group comparisons using Statistica v8.0 (Statsoft Inc., Tulsa OK, USA). Results were considered statistically significant at p < 0.05.

Results

Organ weights and lipid peroxidation status

Both body weight and brain weight were unaffected at the end of the experiment irrespective of grape juice or water treatment and radiation (for groups NW, NJ, RW, and RJ, respectively). Body weight (g) was 34.5 ± 0.7, 34.2 ± 0.9, 34.0 ± 1.1, and 33.0 ± 1.1; brain weight (g) was 0.45 ± 0.1, 0.48 ± 0.1, 0.47 ± 0.1, and 0.49 ± 0.2. A significant interaction was found between Radiation and Treatment factors in brain lipid peroxidation [F(1, 28) = 4.865, p = 0.035], expressed as an increase in RW mice (NW 3.92 ± 0.71; RW 6.27 ± 0.61; NJ 4.29 ± 0.68; RJ 4.03 ± 0.49 umol MDA/mg prot). This effect was prevented in RJ mice.

Open field

The data on behavioural parameters Frequency of access, Total duration, Latency to the first occurrence, and Distance moved obtained for the different zones defined in the Ethovision software (center, corners, and walls) on the day previous to TBI and the three days following TBI for X-irradiated and sham-irradiated animals are presented in table I. The analysis of Frequency of access yielded significant effects for factors Treatment [F(1, 74) = 4.036, p = 0.048], Radiation [F(1, 74) = 4.507, p = 0.037], and Day [F(3, 222) = 2.765, p = 0.042], as well as interactions Day*Treatment [F(3, 222) = 7.753, p = 0.000], and Day*Radiation [F(3, 222) = 3.184, p = 0.024]. The analysis of Total duration in each zone provided significant effects for factor Zone [F(2, 72) = 2242.81, p = 0.000] and interactions Day*Zone [F(6, 216) = 2.426, p = 0.027] and Day*Treatment*Zone [F(6, 216) = 3.569, p = 0.002]. The analysis of Latency to first occurrence revealed significant effects for factor Zone [F(2, 70) = 50.267, p = 0.000] and interactions Day*Treatment [F(3, 150) = 7.093, p = 0.000], Day*Radiation [F(3, 150) = 5.159, p = 0.002], and Day*Radiation*Zone [F(6, 150) = 2.807, p = 0.013]. The analysis of the Distance moved resulted in significant effects for factor Zone [F(2, 70) = 74.141, p = 0.000] and interaction Day*Treatment [F(3, 150) = 3.480, p = 0.017]. Post hoc analysis revealed significant group differences that can be summarized as follows. Frequency of access was decreased in corners and walls as a result of TBI in WR mice, and it decreased in the walls in JR mice. The duration of the stay in the corners was increased in WR mice but it was decreased in the walls, indicative of a redistribution of time with a preference by the corners. The distance moved along the walls was also significantly decreased by X-irradiation in WR mice.

Table I

Open field parameters in non-irradiated and irradiated animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rad.</th>
<th>Zone</th>
<th>Frequency of access</th>
<th>Total duration (s)</th>
<th>Latency (s)</th>
<th>Distance moved (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>N</td>
<td>center</td>
<td>8.3 ± 3.5</td>
<td>8.5 ± 14.6</td>
<td>140.0 ± 23.0</td>
<td>189.8 ± 179.4</td>
</tr>
<tr>
<td>W</td>
<td>N</td>
<td>corners</td>
<td>44.6 ± 3.5</td>
<td>261.0 ± 8.9</td>
<td>8.2 ± 14.1</td>
<td>1344.2 ± 109.9</td>
</tr>
<tr>
<td>W</td>
<td>N</td>
<td>walls</td>
<td>45.5 ± 3.5</td>
<td>78.2 ± 8.9</td>
<td>27.8 ± 14.1</td>
<td>1060.5 ± 109.9</td>
</tr>
<tr>
<td>W</td>
<td>R</td>
<td>center</td>
<td>4.3 ± 3.5</td>
<td>9.7 ± 12.7</td>
<td>168.3 ± 19.9</td>
<td>149.7 ± 155.4</td>
</tr>
<tr>
<td>W</td>
<td>R</td>
<td>corners</td>
<td>31.2 ± 3.5*</td>
<td>298.4 ± 8.9*</td>
<td>1.6 ± 14.1</td>
<td>1142.0 ± 109.9</td>
</tr>
<tr>
<td>W</td>
<td>R</td>
<td>walls</td>
<td>30.6 ± 3.5*</td>
<td>51.1 ± 8.9*</td>
<td>43.3 ± 14.1</td>
<td>759.4 ± 109.9*</td>
</tr>
<tr>
<td>J</td>
<td>N</td>
<td>center</td>
<td>5.3 ± 3.5</td>
<td>9.1 ± 11.3</td>
<td>113.1 ± 17.8</td>
<td>154.2 ± 139.0</td>
</tr>
<tr>
<td>J</td>
<td>N</td>
<td>corners</td>
<td>36.5 ± 3.5</td>
<td>281.9 ± 8.9</td>
<td>3.5 ± 14.1</td>
<td>1236.9 ± 109.9</td>
</tr>
<tr>
<td>J</td>
<td>N</td>
<td>walls</td>
<td>41.1 ± 3.5</td>
<td>69.8 ± 8.9</td>
<td>22.8 ± 14.1</td>
<td>1027.0 ± 109.9</td>
</tr>
<tr>
<td>J</td>
<td>R</td>
<td>center</td>
<td>4.2 ± 3.5</td>
<td>9.0 ± 25.3</td>
<td>179.3 ± 39.9</td>
<td>176.3 ± 310.8</td>
</tr>
<tr>
<td>J</td>
<td>R</td>
<td>corners</td>
<td>34.3 ± 3.5</td>
<td>297.5 ± 8.9</td>
<td>0.4 ± 14.1</td>
<td>1202.1 ± 109.9</td>
</tr>
<tr>
<td>J</td>
<td>R</td>
<td>walls</td>
<td>28.8 ± 3.5*</td>
<td>51.3 ± 11.3</td>
<td>40.0 ± 17.8</td>
<td>763.8 ± 139.0</td>
</tr>
</tbody>
</table>

N: non-irradiated. R: irradiated. W: water. J: grape juice. Unweighted repeated-measures ANOVA means ± SEM of daily 5 minutes tests conducted over 4 days. n = 8 per group. *p < 0.05 from the respective N group.
In order to understand the contribution of the different days on the above mentioned differences in irradiated animals, as well as to ascertain the effects of grape juice intake, figure 1 shows the observed changes in open field parameters, arranged to compare the animal scores on the 24 h previous to TBI (the first exposure to the open field arena) with those of the 24, 48, and 72 h following TBI.

Fig. 1.—Frequency of access (A), Total duration of the stay (B), Latency to first entry (C), and Distance moved (D) in the center (ce), corners (co), and walls (wa) of the open field arena by mice drinking water (W) or grape juice (J) 24 h before and 24, 48, and 72 h after total body irradiation. Mean values for 8 animals ± SEM. *p < 0.05 relative to water-drinking mice values. #p<0.05 relative to -24 h values.
Results from figure 1A show a TBI-induced significant decrease in the frequency of access to all zones in the open field arena in water-drinking mice at 24h post-irradiation, but the values were not significantly different afterwards. The frequency of each animal accessing all zones increased 24h post-irradiation in RJ compared with RW mice. It can also be observed that the frequency of entry into corners at 48 h and walls at 72 h was significantly decreased in RJ animals. There were no changes induced by X-irradiation in the duration of the stay in each zone at 48 and 72 h after TBI, but the time spent at the corners was higher 24h post-TBI in RW mice (fig. 1B). The duration of the stay in the center and walls was also significantly higher in RJ compared to RW mice on the first day post-TBI. There was a big increase in the latency to first enter the center in water-drinking mice at 24 h post-TBI, without differences at 48 and 72 h. The latency to access the center zone was significantly lowered at 24 hours post-irradiation in RJ compared to RW mice (fig. 1C). The distance moved by RW mice along the walls was significantly decreased 24 h after TBI. The total distance moved was significantly increased in center and walls 24 hours after TBI in RJ compared to RW mice (fig. 1D). This effect was not noticeable on the 2nd and 3rd days after irradiation.

The increase of both time spent in the center zone of the open field and the ratio center/total locomotion, as well as the decrease in the latency to entering the center zone, are considered as indications of anxiolysis24. With the aim of further exploring anxiety, data from non-radiated (-24 h) animals and radiated mice at 24 h post-irradiation were analyzed by repeated measures ANOVA considering Treatment as main factor and two levels of within-factor Day. Table II summarizes data on anxiety-related open field parameters (Total duration in the center zone, Latency to first entering the center, and ratio Center/Total distance moved) obtained the day prior and the day immediately next to TBI. No significant main effects or interactions were found involving Treatment or Day when analyzing parameters Total duration in the center and Latency to first entry in the center. The analysis of the Center/Total distance ratio revealed significant interaction Treatment*Day $[F(1, 30) = 5.2104, p = 0.0297]$. However, post hoc tests revealed decreased total duration in the center, increased latency and decreased center/total distance ratio in WR mice at 24 h post-TBI, and reversed effects in JR mice, which suggest a significant decrease in anxiety markers early after TBI as a result of the animals drinking grape juice.

### Rotarod

The analysis of the time (latency) mice were able to stand against 10 rpm rotation in the Rotarod apparatus yielded no significant effects for factors Treatment [$F(1, 28) = 0.895, p = 0.352$] and Radiation [$F(1, 28) = 1.403, p = 0.246$]. However, there was a significant effect of the repeated-measures factor Day [$F(3, 84) = 4.617, p = 0.004$], indicative of different mobility responses depending of the day after irradiation. A high inter-individual variability precluded finding significant differences in latency at -24, 24 and 48 h post-irradiation, but post-hoc group analysis showed that JR animals were able to maintain balance significantly longer ($p = 0.026$) than WR animals at 72 h post-irradiation (fig. 2). Grape juice intake thus prevented the decrease in motor coordination on the 3rd day after TBI.

### Feeding behaviour

The data for feeding behaviour parameters are presented in figure 3. Preliminary repeated-measures analysis using three levels each for within-subjects factor Timing revealed no significant main effects or interactions for fixed factors Radiation and Treatment. However, when including a second within-subjects factor Day, significant differences emerged from the data set. Analyzing food intake, significant effects were found for interactions Timing*Day in Number of bouts [$F(2, 8) = 11.309, p = 0.004$], Timing*Day*Group in Number of bouts [$F(6, 8) = 6.447, p = 0.009$], Timing*Day*Group in Intake duration [$F(6, 8) = 5.788, p = 0.013$], factor Timing in Bout duration [$F(1, 4) = 18.003, p = 0.013$], interaction Timing*Day*Group in Bout duration [$F(6, 8) = 4.583, p = 0.026$]. For fluid intake analysis, significant effects were found for factor Day in Number of bouts [$F(2, 8) = 13.635, p$...
interaction Day*Group in Number of bouts $[F(6, 8) = 12.620, p = 0.001]$, factor Timing in Intake size $[F(1, 4) = 74.004, p = 0.001]$, factor Timing in Bout size $[F(1, 4) = 35.115, p = 0.004]$, interaction Day*Group in Bout size $[F(6, 8) = 8.954, p = 0.003]$, and interaction Timing*Day*Group in Bout size $[F(6, 8) = 7.927, p = 0.005]$. Post hoc group analysis revealed a decrease in Number of bouts and Intake duration, as well as an increase in Bout size, for both food and fluid intake during the day X-irradiation took place. These changes only appeared in RW mice.

The above results can be interpreted as follows. There was no overall effect of Treatment and Radiation on food and fluid intake parameters considering data obtained before and after irradiation. RW mice yielded a smaller number of both food and fluid intake bouts in the X-irradiation day, but the amount of food/fluid ingested per bout (Bout size) was higher, so that the daily food/fluid intake remained similar to the other days, either pre- or post-irradiation. It can also be shown from figure 3 that fluid daily intake duration was lower in the X-irradiation day for RW mice but, since the number of bouts was also decreased, Bout duration was not significantly altered by X-irradiation. The same trend appears for food Intake duration, but this parameter strongly increases in the day following X-irradiation, keeping food bout duration higher during the post-irradiation period. These changes in feeding behaviour parameters induced by TBI were reversed by grape juice supplementation, so that number of bouts, bout size and food/fluid intake remained within baseline values in RJ animals.

Discussion

Acute exposure to ionizing radiation has since long been demonstrated to elicit important changes in behaviour, both military and clinically relevant. There is a widely acknowledged potential for ionizing radiation to induce significant cognitive effects in adults and children undergoing radiotherapy, and most studies have been focused on long-term cognitive deficits, especially hippocampal damage-related effects. Work on short-term behavioural effects of ionizing radiation, either cranially or total body-administered, has also been conducted, especially on learning and memory, but also on motor performance (see Obenaus for a review). Since previous studies by our team have shown a positive radiomodifying effect of grape juice on hematological parameters and liver oxidative damage in rats, the hypothesis tested in the present work has been that antioxidant-rich grape juice intake can prevent acute sublethal TBI-induced effects in mouse behaviour. Measurements were made on anxiety, locomotion, sensorimotor coordination, and feeding behaviour-related parameters using open field and Rotarod observations and feeding/drinking sensor readings. The results of the present study are suggestive that daily intake of grape juice for 7 days before and 3 days after TBI in mice is able to modify the irradiation-induced anxiety status, as well as the decrease in motor coordination at 72 h post-TBI. Grape juice intake also impairs the increase in brain lipid peroxidation three days after sublethal X-irradiation. This decrease in lipid peroxidation in the brain (as a consequence of the antioxidant effect of grape juice) has also been observed in the cerebral cortex of rats supplemented with ‘green juice’ (prepared with orange, apple, lettuce, cabbage, and cucumber) and orange juice.

Anxiety can be evaluated in the open field test by measuring the ratio of the distance traveled in the center to the total distance moved. Classical behavioural parameters in the open field test can not only reflect anxiety but also locomotor and exploratory behavior, so that to get a quick look at immediate overall behavioural effects induced by radiation, the open field study was completed.
Purple grape juice as a protector against acute X-irradiation induced alterations on mobility...

Fig. 3.—Changes in feeding behaviour parameters: Number of bouts, Intake size, and Intake duration for food (right) and fluid intake along six consecutive days in non-irradiated (N) and radiated animals (R) drinking water (W) or grape juice (J). The actual irradiation day was day 0. Mean values for 8 animals ±SEM. In each group, means with different letters are significantly different (Newman-Keuls, p < 0.005).
with the Rotarod test, RW mice showed decreased motor performance relative to RJ, but this motor deficiency showed up at 72 h, while open field behavioral changes were more explicit at 24 h post-irradiation.

Compared to non-irradiated animals, 24 h after TBI the frequency of access to the center, corners, and walls on the open field was decreased by X-irradiation in RW mice (fig. 1A). Frequency of access, total duration, and distance travelled in center and walls were increased at 24 h as a result of grape juice treatment in RJ animals relative to RW animals, returning to values similar to non-irradiated mice (figs. 1A, 1B, 1D). Similarly, the total duration of the stay in the corners (fig. 1B) increased at 24 h in RW animals with respect to NW mice, to be decreased in RJ mice.

Open field behaviour is subjected to habituation. It is worth to mention that RJ mice showed a progressive decrease in frequency of access and movement duration at the walls, while RW animals maintained frequency values similar to those of the first open-field exposure day (24 hours before TBI). This is in agreement with the known fact that habituation to the open field does not develop in animals with prefrontal or hippocampal lesions.

There was a clear decrease in locomotor activity in the walls of the open field in RW animals at 24 h post-irradiation, which was reverted in RJ animals (fig. 1D). Similar decreases in 24 h spontaneous motor activity have been previously reported to be related to increased cerebellar oxidative stress. Considerable evidence has been reported of the deleterious effect of sublethal total body irradiation on the cerebellum, involving oxidative stress, inflammation and calcium neurotoxicity mechanisms. Moreover, increased cerebellar lipid peroxidation in mice genetically impaired to synthesizing ascorbic acid lead to decreased sensorimotor function as tested by the Rotarod apparatus and other tests. In relation to this we found a significant increase in oxidative stress in RW mice brain, which was decreased in grape juice-drinking animals with a parallel improvement in motor coordination at 72 h post-TBI (fig. 2).

Many literature references report a decrease in total food and water intake after ionizing radiation in mice. Most of these studies only cite these changes, without quantification. A recent study on the Ash for meal patterning, there are few studies addressing spontaneous feeding behaviour of mice. After considering reported alterations in the behaviour of individually housed mice and the effects of strong magnetic fields on feeding and drinking of mice kept in group cages, in this work we decided to study feeding behaviour on groups of four animals, that is, using the whole cage as single measuring subject. To overcome the strong decrease in purple grape juice voluntary intake, mice were forced to drink diluted grape juice solution as sole source of water for seven days prior TBI, after the initial one-week acclimation upon its arrival to the animal house. The alternative use of oral gavage pure grape juice administration was discarded because of the animal handling involved. No open-field or rotarod-tested behavioural effects were found on the day immediately before X-irradiation, but an unexplained decrease in number of bouts was found for NJ mice on the second day after TBI, with no effect on total food/fluid intake (fig. 3). However, the immediate outcome of X-irradiation on food/fluid intake parameters was a decrease in the number of bouts and the duration of food/water intake in RW mice. These effects, together with the increase in bout size in the TBI day and the lack of alterations in overall food/fluid intake size, could be attributed to motor changes rather than gastrointestinal alterations in the immediate days following X-irradiation. This is in agreement with previous work reporting no alteration in intestinal morphology at 3 days post-irradiation (10 Gy) in mice. Data in table I indicate that irradiated mice decrease the duration of the stay in the open-field wall zones and the distance walked along the walls, while they increase the time spent at the corners. This is suggestive of a reduced mobility 24 h after TBI, which could help explain the changes in bout size and bout duration on the basis of an overall depression of motile activities. Because of the gnawing activity to feed from food pellets in a feeder arrangement hanging from the top of the lid, albeit at the same distance from the floor than the tip of the fluid container, food intake should conceivably be harder than fluid intake for an animal less prone to moving after being irradiated, and food bout duration (fig. 3) is consistent with this idea.

We can conclude that voluntary intake of purple grape juice along several days before TBI is able to overcome several aspects of radiation-induced changes in feeding behaviour, also improving anxiety and locomotion within 24 to 72 hours after sub-lethal X-irradiation. To our knowledge, this is the first time radiaton-induced behavioural changes are found to be modified by antioxidant-rich grape juice intake in mice immediately after total body irradiation. The present findings could be relevant in designing preventive interventions aimed to enhance body defense mechanisms against short-term irradiation damage.

Acknowledgments

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Declaration of interests

The authors hereby declare not to be in situation of known real, potential or apparent conflict of interest that could affect their objectivity and independence in this work.
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