The Ameliorative Role of Grape Seed Oil on Irradiated Rat Fungiform Papillae

Samah S Mehanni1 Eman M El- Maghraby2*, Alyaa R Hassan3, Dalia A H El-Baz1

1Oral Biology Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt.
2Health Radiation Research Department, National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt.
3Supplementary science department, Faculty of Oral and Dental Medicine, Future University, Cairo, Egypt

emey_maghraby@hotmail.com

Abstract: This study was held to clarify changes in the epithelium of the fungiform papillae after irradiation at the microscopic level as well as the effect of grape seed oil (GSO) on these changes and the changes in the innervation of these papillae. Thirty five male Swiss Albino rats were included in this study. The animals were divided into, Grape seed irradiated group (GSI gp): GSO was administrated orally in accompanied with radiation. Irradiated group (IR gp): The same protocol was applied to this group except that the animals received distilled water instead. Control group(C gp): only distilled water was administrated orally. The right tongue halves were prepared for Ultrastructural studies. The left halves were used for detection of the innervations using S100. Degeneration of the cell nuclei and organelles in stratum basale and spinosum of the fungiform papillae in IR gp was obvious. In the GSI gp the nuclei had prominent nucleoli and granulated chromatin. The regeneration of the epithelium in the IR gp was delayed compared with GSI gp. Regeneration of the cytoskeleton was advanced in the GSI gp. Toward the end of the experiment, increase in the innervations was more detectable in GSI gp. For these reasons, grape seed oil is recommended to be consumed as a dietary supplement and could be useful in synergizing the hazardous of radiation on the tongue papillae.

1. Introduction

Taste deficits as a consequence of head and neck radiation were recently referred to as post-irradiation gustatory dysfunction. It is a common problem among head and neck radiation therapy (RT) patients [1]. It is generally accepted that taste disorders caused by radiochemotherapy result from epithelial changes [2]. Direct damage of the mucosa occurs through inhibition of DNA replication and mucosal cell proliferation [3]. Radiation is known also to generate reactive oxygen species such as superoxide radicals, hydrogen peroxides and hydroxyl radicals. These products have been found to injure cells, leading to mucositis [4]. Mucositis is a significant and painful side effect that can affect the ability to eat, swallow, speech leading to secondary infection and weight loss as well can limit cancer treatment [5,6]. Mucositis is an acute injury to the mucosal lining and histologically characterized by ulceration and pseudomembranous formation caused by cancer therapies [7,8]. Radical scavenging by antioxidants and hence a reduction of radiation effects at their onset have been suggested to prevent the side effects of RT [9]. Little is known about the changes in the epithelium of the fungiform papillae after radiation at the microscopic level as well as the effect of grape seed oil, as an antioxidant [10-12], on these changes. Thus this study was held to clarify these changes ultrastructurally and the changes that occurred in the innervation of these papillae.

2. Methods

Animals

Thirty five male Swiss Albino rats (100-120 gm) purchased from the Animal House of the Faculty of Medicine, Cairo University were included in the study. The experimental protocol used was approved by the department of Animal care, Cairo University that adhered to the European Communities Council guiding principles for the care and use of Laboratory Animals. The animals were housed in a controlled environment at 25°C with 12 hrs light /dark cycle and were acclimated for three-five days before use. All animals were allowed free access to food and tap water.

Grape seed oil administration:

Grape seed oil (GSO) was administrated orally at a dose of 100mg/kg body weight [13] obtained from Sigma, Chemical Co. (Nasr City, Cairo, Egypt).

Radiation exposure:
Irradiation of animals was performed at National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt using 137 Cesium Gamma Cell 40 giving a dose rate of 0.795 Gray/ min at the time of experiment.

Experimental design:

The animals were divided into three groups, first two groups consisted of 15 animals while the third group consisted of five animals only: Grape seed irradiated group: GSO was administered for seven consecutive days by a curved gastric intubation using a plastic syringe with special stainless steel needle. This group received radiation as a single dose of (6.4Gy –Cesium 137) whole body irradiation, and GSO was maintained for 14 additional days. Irradiated group: The same protocol was applied to this group except that the animals received distilled water instead of GSO in a similar manner along with procedure. Control group: only distilled water was administrated orally by gastric intubation. Following their exposure to radiation, the animals were placed individually in the cages. The rats of the Grape seed group and Irradiated group were decapitated under mild anesthesia (50 mg/kg, i.p. ketamine) three, seven and fourteen days post radiation. The rats of the control group were decapitated under mild anesthesia after one week of housing.

Transmission electron microscopic studies:

The right tongue halves were dissected and fixed in the modified Karnovsky solution, containing 2.5% glutaraldehyde, 2% paraformaldehyde in a 0.1 M (pH 7.3) sodium cacodilate buffer. This group received radiation as a single dose of (6.4Gy –Cesium 137) whole body irradiation, and GSO was maintained for 14 additional days. Irradiated group: The same protocol was applied to this group except that the animals received distilled water instead of GSO in a similar manner along with procedure. Control group: only distilled water was administrated orally by gastric intubation. Following their exposure to radiation, the animals were placed individually in the cages. The rats of the Grape seed group and Irradiated group were decapitated under mild anesthesia (50 mg/kg, i.p. ketamine) three, seven and fourteen days post radiation. The rats of the control group were decapitated under mild anesthesia after one week of housing.

Immuno-histochemical studies:

For detection of the nerves, the left tongue halves were fixed in 4% glutaraldehyde fixer. S100: anti-human clone (MAC 387) catalog number ZO-311 Rabbit monoclonal, (DAKO Corporation) was used in dilution of 1:50.

Measurements of area % of S100 immunopositivity were done at ten different fields within the fungiform papillae by image analyzer computer system using the software Leica Qwin 500 and the mean area % was calculated for each specimen.

Statistical Analysis:

Data were expressed as mean ± standard deviation (X±SD). Comparison of S100 expression between the groups was made using the post-hoc test in one way analysis of variance (ANOVA). Representative charts were generated from the data.

3. Results

Electron microscopic results

At the third day post irradiation, the radiation hazards were clear and obvious in both the Stratum basale and spinosum of the fungiform papillae. The nuclei of the basal cells in the irradiated group were degenerated as well as most of the cell organelles. Sporadic multinucleated cells were detected (Fig.1). On the other hand, the grape seed irradiated group did not reveal these multinucleated cells but the cell organelles were still ill defined except of few survived free ribosomes. The nuclei had prominent nucleoli and granulated chromatin. The cytoplasm appeared vacuolated. Remnants of desmosomes and tonofibrils could be detected (Fig. 2).

Both groups displayed kerato-hyaline granules and few homogenous Odland’s bodies that lack the normal lamellated appearance in the stratum granulosum (Fig.3).

The keratin layer showed irregular surface and appeared homogenous with ill defined cell borders, desmosomes and cell organelles especially in the irradiated group (Fig.4).

At the seventh day post irradiation, degeneration of the different cell layers was clear in the irradiated group as shown in Fig. 5. There were loss of cell boundaries and junctions. Vacuolization increased in great manner. Thinning of the epithelium and sloughing of the keratin layer were noticeable. The basal cells of the fungiform papillae of the grape seed irradiated group, in contrast, disclosed regenerated rough endoplasmic reticulum intermingled with cytoplasmic vacuoles. The cells retained their boundaries but lost their junctions (Fig. 6).

The regeneration of the epithelium in the irradiated group was delayed till the fourteenth day post irradiation. Regeneration of the cytoskeleton was advanced in the grape seed irradiated group (Fig. 7).

Statistical results:
Significant obvious decrease in the innervations was indicated in the irradiated group at the third day post radiation compared with both grape seed irradiated and control groups. A marked increase in these innervations was detected at the seventh and fourteenth days post radiation but still far away from that of the control group. On the other hand, when comparing grape seed irradiated to the control group, the increase in the innervations was more detectable such that seemed to be normal after the fourteenth day post irradiation (Figs. 8-10, Table 1).

**Table 1:** Difference in the mean area percent between different groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (n=5)</th>
<th>Irradiated (n=5)</th>
<th>Grape seeds n=(5)</th>
<th>F-ratio</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x ± SD</td>
<td>x ± SD</td>
<td>x ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area percent at 3 days</td>
<td>12.8 ± 0.93 a</td>
<td>45.52 ± 2.99 a,b</td>
<td>451.68</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Area percent at 7 days</td>
<td>56.18 ± 3.15</td>
<td>26.6 ± 2.53 a</td>
<td>51.97 ± 1.49 a,b</td>
<td>207.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Area percent at 14 days</td>
<td>44.64 ± 1.61 a</td>
<td>54.83 ± 3.33 a</td>
<td></td>
<td>25.3</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

a: Significant difference between groups versus control group.
b: Significant difference between groups versus irradiation group.

**Fig.1:** An electron micrograph of the stratum basal of the irradiated group at the third day post irradiation shows multinucleated cell (C). X1000

**Fig.2:** An electron micrograph of the stratum basal of the grape seed irradiated group at the third day post irradiation shows remnants of desmosomes (arrows) and the nuclei containing granulated chromatin as well as prominent nucleoli (N) and. X3000

**Fig.3:** An electron micrograph of the stratum granulosum of the irradiated group at the third day post irradiation shows nucleus (N), kerato-hyaline granules (arrowheads) and Odland’s bodies (arrows). X2000
Fig. 4: An electron micrograph of the keratin layer of the grape seed irradiated group at the third day post irradiation appears homogenous with an irregular surface. X3000

Fig. 5: An electron micrograph of the fungiform papillae of the irradiated group at the seventh day post irradiation shows vacuoles of different sizes all over the cell and aborted nucleus (N). X1000

Fig. 6: An electron micrograph of the stratum basal of the grape seed irradiated group at the seventh day post irradiation shows rough endoplasmic reticulum (RER) and cytoplasmic vacuoles (V). X1000

Fig. 7: An electron micrograph of the epithelium of the grape seed irradiated group at the fourteenth day post irradiation shows regenerated cytoskeleton and vacuolated cytoplasm. X2000

Fig. 8: A photomicrograph of the tongue lamina propria of irradiated group at the seventh day after irradiation shows weak S100 immunoreactivity in the nerve fibers. S100x400
the oral mucosa lining the mouth, which can reveal systemic conditions. Modern anticancer therapy represents a significant challenge to the integrity of the oral mucosa. Chemotherapeutic agents and radiation therapy limit the proliferative ability of the epithelium so that it becomes thin or ulcerated. This is manifest first in the more rapidly proliferating tissues, such as gastrointestinal and oral lining mucosa [21]. There may also be indirect effects, such as damage to the salivary glands that will reduce salivary production and impair barrier efficiency and a reduction in immunocompetence as a result of myeloablative therapy. This will increase the risk of local infection from oral organisms [22-24]. The ongoing study revealed degenerative nuclei as well as most of the cell organelles in the basal cell layer of the fungiform papillae of IR group. Multinucleated cells also could be detected. This histo-pathological finding was detected by Obinata et al. [18] and Just et al. [19]. Yamazaki et al. [20] also revealed that the basal cells of the mice circumvallate papillae had almost disappeared when exposed to a single 15 Gy dose of X-ray irradiation. This finding could be a hazard of the radiation that causes DNA damage in basal epithelial cells and generates reactive oxygen species. Further damage occurs to cells and blood vessels in the submucosa [25]. Meanwhile, the grape seed irradiated group revealed persistent nuclei and covering of taste pores by epithelial cells are likely reasons for taste loss during radio-chemotherapy [19]. Yamazaki et al. [20] revealed that there was not clear change in the spindle-shaped taste cells on day 4 after irradiation. The number of taste cells had decreased on day 8, and then remained unchanged until day 20, after which they increased and recovered to their original number by day 24. For these debates about the effect of radiation on fungiform or circumvallate papillae and taste buds that influence taste sensation directly or indirectly, the present study concerned with the epithelial changes that occurred in the fungiform papillae, neglecting their numbers, and the innervations of these papillae regardless the presence or absence of the taste buds.

The oral cavity has sometimes been described as a mirror that reflects the health of the individual. Changes indicative of disease are seen as alterations in the oral mucosa lining the mouth, which can reveal systemic conditions. Modern anticancer therapy represents a significant challenge to the integrity of the oral mucosa. Chemotherapeutic agents and radiation therapy limit the proliferative ability of the epithelium so that it becomes thin or ulcerated. This is manifest first in the more rapidly proliferating tissues, such as gastrointestinal and oral lining mucosa [21]. There may also be indirect effects, such as damage to the salivary glands that will reduce salivary production and impair barrier efficiency and a reduction in immunocompetence as a result of myeloablative therapy. This will increase the risk of local infection from oral organisms [22-24]. The ongoing study revealed degenerative nuclei as well as most of the cell organelles in the basal cell layer of the fungiform papillae of IR group. Multinucleated cells also could be detected. This histo-pathological finding was detected by Obinata et al. [18] and Just et al. [19]. Yamazaki et al. [20] also revealed that the basal cells of the mice circumvallate papillae had almost disappeared when exposed to a single 15 Gy dose of X-ray irradiation. This finding could be a hazard of the radiation that causes DNA damage in basal epithelial cells and generates reactive oxygen species. Further damage occurs to cells and blood vessels in the submucosa [25]. Meanwhile, the grape seed irradiated group revealed persistent nuclei and covering of taste pores by epithelial cells are likely reasons for taste loss during radio-chemotherapy [19]. Yamazaki et al. [20] revealed that there was not clear change in the spindle-shaped taste cells on day 4 after irradiation. The number of taste cells had decreased on day 8, and then remained unchanged until day 20, after which they increased and recovered to their original number by day 24. For these debates about the effect of radiation on fungiform or circumvallate papillae and taste buds that influence taste sensation directly or indirectly, the present study concerned with the epithelial changes that occurred in the fungiform papillae, neglecting their numbers, and the innervations of these papillae regardless the presence or absence of the taste buds.

The oral cavity has sometimes been described as a mirror that reflects the health of the individual. Changes indicative of disease are seen as alterations in the oral mucosa lining the mouth, which can reveal systemic conditions. Modern anticancer therapy represents a significant challenge to the integrity of the oral mucosa. Chemotherapeutic agents and radiation therapy limit the proliferative ability of the epithelium so that it becomes thin or ulcerated. This is manifest first in the more rapidly proliferating tissues, such as gastrointestinal and oral lining mucosa [21]. There may also be indirect effects, such as damage to the salivary glands that will reduce salivary production and impair barrier efficiency and a reduction in immunocompetence as a result of myeloablative therapy. This will increase the risk of local infection from oral organisms [22-24]. The ongoing study revealed degenerative nuclei as well as most of the cell organelles in the basal cell layer of the fungiform papillae of IR group. Multinucleated cells also could be detected. This histo-pathological finding was detected by Obinata et al. [18] and Just et al. [19]. Yamazaki et al. [20] also revealed that the basal cells of the mice circumvallate papillae had almost disappeared when exposed to a single 15 Gy dose of X-ray irradiation. This finding could be a hazard of the radiation that causes DNA damage in basal epithelial cells and generates reactive oxygen species. Further damage occurs to cells and blood vessels in the submucosa [25]. Meanwhile, the grape seed irradiated group revealed persistent nuclei and covering of taste pores by epithelial cells are likely reasons for taste loss during radio-chemotherapy [19]. Yamazaki et al. [20] revealed that there was not clear change in the spindle-shaped taste cells on day 4 after irradiation. The number of taste cells had decreased on day 8, and then remained unchanged until day 20, after which they increased and recovered to their original number by day 24. For these debates about the effect of radiation on fungiform or circumvallate papillae and taste buds that influence taste sensation directly or indirectly, the present study concerned with the epithelial changes that occurred in the fungiform papillae, neglecting their numbers, and the innervations of these papillae regardless the presence or absence of the taste buds.

The oral cavity has sometimes been described as a mirror that reflects the health of the individual. Changes indicative of disease are seen as alterations in the oral mucosa lining the mouth, which can reveal systemic conditions. Modern anticancer therapy represents a significant challenge to the integrity of the oral mucosa. Chemotherapeutic agents and radiation therapy limit the proliferative ability of the epithelium so that it becomes thin or ulcerated. This is manifest first in the more rapidly proliferating tissues, such as gastrointestinal and oral lining mucosa [21]. There may also be indirect effects, such as damage to the salivary glands that will reduce salivary production and impair barrier efficiency and a reduction in immunocompetence as a result of myeloablative therapy. This will increase the risk of local infection from oral organisms [22-24]. The ongoing study revealed degenerative nuclei as well as most of the cell organelles in the basal cell layer of the fungiform papillae of IR group. Multinucleated cells also could be detected. This histo-pathological finding was detected by Obinata et al. [18] and Just et al. [19]. Yamazaki et al. [20] also revealed that the basal cells of the mice circumvallate papillae had almost disappeared when exposed to a single 15 Gy dose of X-ray irradiation. This finding could be a hazard of the radiation that causes DNA damage in basal epithelial cells and generates reactive oxygen species. Further damage occurs to cells and blood vessels in the submucosa [25]. Meanwhile, the grape seed irradiated group revealed persistent nuclei and covering of taste pores by epithelial cells are likely reasons for taste loss during radio-chemotherapy [19]. Yamazaki et al. [20] revealed that there was not clear change in the spindle-shaped taste cells on day 4 after irradiation. The number of taste cells had decreased on day 8, and then remained unchanged until day 20, after which they increased and recovered to their original number by day 24. For these debates about the effect of radiation on fungiform or circumvallate papillae and taste buds that influence taste sensation directly or indirectly, the present study concerned with the epithelial changes that occurred in the fungiform papillae, neglecting their numbers, and the innervations of these papillae regardless the presence or absence of the taste buds.
noticed from day three in the grape seed irradiated group. Donnetti et al. 2009 [31] have performed immunohistology and immunocytochemistry of desmosomal cadherin expression (desmoglein 1/desmoglein 3) and demonstrated that the distribution of desmoglein 1 was not affected, whereas the expression of desmoglein 3 decreased in the suprabasal layers of irradiated samples. They suggested that this has consequences for the mechanical integrity of the mucosa and promotes the development of radiation mucositis. Persistent desmosomal junctions that were noticed in the grape seed irradiated group of the herein study suggested improvement effect of grape seed oil on these desmosomal cadherin.

Besides the foregoing mentioned epithelial changes that were perceived in this study and their possible effect on taste sensation, radiation-induced taste dysfunction could be also associated with damage to the nerve fibers. One of the most fascinating finding of the present investigation was significant reduction in the innervations of the fungiform papillae three days after irradiation. This reduction was greatly improved by feeding grape seed oil till reach to the normal level of innervations after fourteen days post irradiation. Such as was detected in this study. Thus the changes that were detected in this research could be of dual causes; the direct action of the radiation on the papillae and indirect effect on their innervation. Sadows et al. 2006 [34] on the other hand, stated that radiation-induced taste dysfunction can be associated with damage to either the intrageminal nerve cells or taste cells, or both. Because nerve fibers are non-dividing and do not proliferate, damage to these cells is unlikely to recover, and the related radiation-induced taste loss would not recover. However, damage to taste cells, which replenish approximately every 10 days, would likely recover at least partially, unless the proliferative capacity of the receptor cells is disrupted. In this study the nerve fibers regenerate especially in the grape seed irradiated group and thus taste sensation expected to be improved in this group.

Conclusion

For these reasons, preservation of the oral mucosa integrity and their innervations, grape seed oil is recommended to be consumed as a dietary supplement and could be useful in synergizing the hazardous of radiation on the tongue papillae.

Corresponding author

Eman M El-Maghraby

http://www.americanscience.org

5. References