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THE POTENTIAL RADIOPROTECTIVE ROLE OF SELENIUM ON GAMMA IRRADIATED RATS' SALIVARY GLANDS ⁎

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Key words: Selenium, Gamma radiation, Salivary glands, Antioxidants, Irradiated rats.

الدور الوقائى الكامن للسيلينيوم على الغدد اللعابية للجرذان المعرضة لأشعة جاما

سلوى فريد أحمد و عواطف إبراهيم دراز و داليا الروبى
و إيمان محمد فتحى المغربى

خلاصة

تهدف هذه الدراسة إلى تقييم مدى كفاءة السيلينيوم فى حماية الغدد اللعابية من أشعة جاما باعتبار ه مادة طبيعية مضادة للأكسدة، وذلك باستخدام طرق نسيجية و نسجية كيميائية و مناعية نسجية كيميائية. تم استخدام ٤٨ جرذ ذكر أبيض تم تقسيمها إلى ٦ مجموعات بكل مجموعة ٨ جرذ وقد تم تعريض المجموعة الأولى والثانية لأشعة جاما بجرعة ٤ و ٦ جراى لمرة واحدة فقط، على التوالى، أما المجموعة الثالثة تم حقن الجرذان داخل الغشاء البريتونى بجرعة يومية من الصوديوم سيلينيت (٥ اميكروجرام لكل كيلو جرام وزن) لمدة ثلاثة أسابيع، بينما المجموعة الرابعة و الخامسة تم حقن الجرذان داخل الغشاء البريتونى بجرعة يومية من الصوديوم سيلينيتلمدة أسبو ع قبل التعرض لجرعة واحدة بمقدار ٤ و ٦ جراى من أشعة جاما، على التوالى، لمدة أسبوعين بعد ذلك، أما المجموعة الضابطة لم يتم تعريض الجرذان فيها للإشعاع أو السيلينيوم. تم ذبح الجرذان بعد يوم وأسبوعين وأربعة وثمانية أسابيع من التعرض للأشعة وتم استخراج الغدتين تحت الفكية وتحت اللسانيةثم صباغة شرائح البرافين بالهيماتكسلين و الأيوسين و بصبغة الألشيان الأزرق المزدوجة مع الباس وصبغة الفولجين كذلك تم الكشف عن وجود دليل التكاثر

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الخلوى النووى (PCNA) باستخدام صبغة مناعية نسجية كيميائية. و قد تم تعيين النسبة المئوية للمساحة المصبوغة بكل من الفولجين و PCNA باستخدام الكمبيوتر لتحليل الصورة.

أوضح الفحص النسيجى وجود فجوات داخل سيتوبلازم خلايا الإفراز فى الغدة التحت فكية كذلك لوحظ نقص فى محتوى القنوات الملتوية المحببة من الحبيبات المفرزة وكانت هذه التغيرات أكثر وضوحاً فى المجموعة المشعة بجرعة ٦جراى حيث كانت خلايا الإفراز ضامرة أو متحللة تماماً. وقد أظهرت صبغة الألشيان الأزرق / باس المزدوجة و صبغة الفولجين نقصاً فى كلا من الغدد اللعابية بعد يوم وأسبوعين من التشعيع وظهرت هذه التغيرات بشكل بسيط فى المجموعات المعالجة بالسيلينيوم قبل التشعيع. وأوضحت الصبغة المناعية النسجية الكيميائية لدليل التكاثر الخلوى النووى وجود تناقص مبدئى فى عدد الخلايا التى تتكاثر فى كل قطاعات الغدد اللعابية بعد يوم من التشعيع وقد تلى ذلك وجود زيادة فى تكاثر الخلايا بعد ١٤ يوم من التشعيع. و قد لوحظ تغيير مماثل فى المجموعات المشعة المعالجة بالسيلينيوم و إن كان معدل التكاثر أقل من المجموعات المشعة.

و بذلك أوضحت الدراسة دور السيلينيوم فى الوقاية من الآثار الضارة للإشعاع على الغدد اللعابية، حيث ساعد فى الحفاظ على النمط النسجى الطبيعى و على المعدل الطبيعى للبروتينات و الكربوهيدرات و الأحماض النووية داخل الخلايا.

ABSTRACT

This study was conducted to evaluate the radioprotective efficacy of selenium, a naturally occurring antioxidant nutrient, against gamma radiation damage in rats' salivary glands by histological, histochemical and immunohistochemical assessments. A total of 48 male Albino rats were equally divided into 6 groups: Groups I and II were subjected to single gamma irradiation doses of 4Gy and 6Gy, respectively. Group III was injected with an intraperitoneal daily dose of sodium selenite (15 μg/kg) for three weeks. Groups IV and V were injected intraperitoneally with Se for one week before exposure to gamma rays at doses of 4 Gy and 6 Gy, respectively, and for two weeks after. The control group received neither radiation nor selenium. Animals were sacrificed 1 day, 2 weeks, 4 weeks and 8 weeks after radiation exposure. Paraffin sections of the submandibular and sublingual glands were stained by haematoxylin and eosin, alcian /PAS stain and Feulgen reaction, in addition to proliferating cell nuclear antigen (PCNA) immunostaining. The Feulgen and PCNA reactivity was estimated by an image analyzer computer system.

Histological examination of the irradiated groups revealed intracytoplasmic vacuoles in the acinar cells of both glands. A reduction in the number and content of secretory granules was noted in the submandibular granular convoluted tubules (GCT). In the 6 Gy group, some acini appeared atrophied or

completely degenerated. A decreased alcian/PAS and Feulgen histochemical staining was noted in both glands one day and 2 weeks after irradiation. A milder degree of these changes was noted in the selenium treated groups. Two months post irradiation, the glandular tissue appeared normal in both the irradiated and the selenium treated irradiated groups. PCNA immunostaining revealed an initial decline in the number of proliferating cells in all glandular compartments 1 day post-irradiation in both glands followed by a subsequent increase after 14 days. The same pattern was generally detected in the selenium treated irradiated groups.

The results revealed that the selenium treatment prevented some of the deleterious effects of radiation and helped maintaining the normal histological appearance of the salivary glands and their contents of carbohydrates and nucleic acids.

INTRODUCTION

Radiotherapy is commonly employed in the treatment of malignant neoplasms of the head and neck and is associated with numerous side effects. The effects produced in the body by ionizing radiation are classified as acute and late. Acute changes occur within hours or days of treatment depending on how it is scheduled - whereas late changes occur after months or years. Acute effects are believed to be due to depletion of stem cells in an organ or tissue. Late changes are thought largely to be related to endothelial injury that leads to exposure of basement membrane which activates the formation of platelet fibrin thrombi (Moulder et al., 1998).

Radiation protection is based on physical principles which aim at lowering the radiation exposure dose and subsequently the risk of radiation injury. This method includes proper shielding and monitoring devices. Other means are chemical radioprotectors which aim at control of the initial lesion induced in the biological system at the molecular level before proceeding to detectable physiological or micro-anatomical changes. This method is based on the use of certain chemicals that prevents the development of radiation injury through modulation of immune system of exposed organisms, prevention of absorption of radionuclides (Cs-137) and free radical scavenging system activation (Cherupally et al., 2001).

Radioprotective agents could be identified as chemical compounds capable of ameliorating the biological influence of ionizing radiation when administered before radiation exposure. The efficiency of these radioprotectors is greatly dependent on their chemical properties, period of treatment and post-irradiation time elapsing after radioprotectors application. An ideal radioprotector agent should be active, rapidly absorbed and easily distributed in the body tissues. It must be without any side effects or at least with minimal toxicity which gives insurance that no cumulative consequences will be expected from their repeated usage (Slyshenkov et al., 1999).

Among the chemical radioprotectors which modulate the immune system and activate the free radical scavenging system is selenium (Se) which is an essential micronutrient trace element for animals and humans because of its role in the

antioxidant enzyme glutathione peroxidase. This enzyme protects cell membranes from damage caused by lipids peroxidation (Tinggi, 2003 and Rasmussen et al., 2006).

Selenium occurs in staple foods such as corn, wheat and soybean as selenomethionine. Selenium supplements may contain sodium selenite and sodium selenate; two inorganic forms of selenium. Selenium is also available in high selenium yeasts, which may contain as much as 1 to 2 micrograms of selenium per gram (Schrauzer, 2001 and 2003). In man, low dietary Se intakes are associated with health disorders including oxidative stress-related conditions, reduced fertility and immune functions and an increased risk of cancers (Broadley et al., 2006). Recently, Fujieda et al. (2007) found that selenium (Se) deficiency reduces glutathione peroxidase (GPx) activity, resulting in increased oxidative stress.

Epidemiological studies, preclinical investigations and clinical intervention trials support the role of selenium compounds as potent cancer chemopreventive agents. The dose and the form of selenium are critical factors in cancer prevention and induction of apoptosis and inhibition of cell proliferation are considered important cellular events that can account for the cancer preventive effects of selenium. Prior to induction of apoptosis, selenium compounds alter the expression and/or activities of a number of cell cycle regulatory proteins, signalling molecules, proteases, mitochondrial associated factors, transcriptional factors, tumour suppressor genes and glutathione levels (Sinha and El-Bayoumy, 2004).

Combs et al. (2001) suggested that selenium affects cancer risk in two ways. As an anti-oxidant, selenium can protect the body from the damaging effects of free radicals and may also prevent or decrease tumour growth. Certain breakdown products of selenium are believed to prevent tumour growth by enhancing immune cell activity and suppressing development of blood vessels within the tumour**.**

Selenium has been proven to help chemotherapy treatment by enhancing the efficacy of the treatment, reducing the toxicity of chemotherapeutic drugs and preventing the body's resistance to the drugs (Caffery, 1998 and Vadgama et al., 2000). However, the radioprotector effect of selenium on salivary glands has not been thoroughly investigated, therefore, the aim of the present study was to evaluate the radioprotective efficacy of selenium, a naturally occurring antioxidant nutrient, as a free radical scavenger against gamma radiation damage in rats' salivary glands by histological, histochemical and immunohistochemical assessments.

MATERIALS AND METHODS

I- Experimental animals:

A total of 48 male Albino rats of comparable weight between 100 - 150 grams were used in the present study. The animals were fed on a standard rodent diet.

Food and water were made available *ad libitum* at all times throughout the experimental period. The rats were divided into 6 groups, each composed of 8 rats.

Control group: rats served as a control and received neither radiation nor selenium.

Group I: rats were exposed to a single whole body gamma irradiation at dose of 4 Gray (Gy).

Group II: rats were exposed to a single whole body gamma irradiation at dose of 6 Gray (Gy).

Group III: rats were injected intraperitoneally by a daily dose of sodium selenite (15 µg/kg) for three weeks.

Group IV: rats were injected intraperitoneally by a daily dose of sodium selenite (15 µg/kg) for one week before exposure to gamma rays at dose of 4 Gy and two weeks after.

Group V: rats were injected intraperitoneally by a daily dose of sodium selenite (15 µg/kg) for one week before exposure to gamma rays at dose of 6 Gy and two weeks after.

II- Irradiation technique:

The whole body irradiation of animals was performed at National Centre of Radiation Research and Technology, Cairo, Egypt, using Gamma cell 40. It is cesium-137 source provides a dose rate of 0.48 Gy / min. Animals of group I and IV were exposed to a dose of 4 Gy while those of group II and V were exposed to a dose of 6 Gy.

III- Selenium treatment:

Selenium in the form of sodium selenite (Na_2SeO_3) was purchased from Technogen Company, Dokki, Egypt. Sodium selenite was dissolved in saline and injected intraperitoneally at a dose of 15 µg/kg body weight.

At 1 day, 2 weeks, 4 weeks and 8 weeks after radiation exposure, 2 rats from each group were sacrificed by decapitation. The skin was removed then the submandibular and sublingual salivary glands were carefully dissected. Submandibular and sublingual salivary glands were immediately fixed in 10 % formalin. Fixed specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylol then embedded in paraffin. Five microns thick paraffin sections were cut. The prepared sections were stained with Harris's haematoxylin and eosin for histological evaluation of any structural changes in the salivary glands (Bancroft and Gamble, 2002), combined alcian blue (pH 2.5) with periodic acid schiff (PAS) for detection of neutral and acidic mucopolysaccharides (Bancroft and Gamble, 2002) and Feulgen reaction for detection of DNA content (Bancroft and Gamble, 2002). Four to five microns thick sections were cut on tissue adhesive coated slides. The prepared sections were stained by commercially available proliferating cell nuclear antigen (PCNA) primary antibody.

IV-Immunohistochemical technique:

Slides were incubated in a solution of 3% hydrogen peroxide in methanol (180 ml methyl alcohol + 20 ml H_2O_2) for 20 minutes then washed in phosphate buffer saline (PBS). To unmask the antigens affected by formalin fixation, slides were

placed in a glass container filled with sufficient target retrieval solution prepared by adding 20 cc of target retrieval solution (DAKO, Denmark) to 180 cc of distilled water in water bath. Slides were heated in a microwave oven at 100°C for three successive trades, 5 minutes each. Slides were then placed in PBS for 5 minutes. The anti-PCNA primary antibody (PC 10, Cat. # U 7032, DAKO, Denmark) was used in a dilution of 1:50 in PBS. The slides were left in a covered humid chamber over night at 4°C. One to two drops of the biotinylated secondary anti-immunoglobulin (Cat. No. 404050) (k0673) were added on each section. The slides were kept horizontal in a humid chamber at room temperature (37°C) for 10 minutes. Slides were then rinsed in PBS for 5 minutes and one to two drops of streptavidin reagents were added to each section for 10 minutes. After PBS rinse, the antigen was localized by the addition of di-amino benzene (DAB) substrate chromogen solution for 5-10 minutes. Slides were counterstained with Mayer's hematoxylin for 1 minute. The cover slips were mounted using purified Canada balsam (DPX, Sigma).

V- Evaluation of staining:

Feulgen reaction and immunoreactivity for PCNA were measured by an image analyzer computer system using the software Leica Qwin 500. Measuring was performed in the form of an area and perimeter of the nuclei expressing the Feulgen reaction and as area percent for PCNA immunoreactivity in a standard measuring frame per 10 fields using a magnification x400 by light microscopy transferred to the monitor's screen. For evaluation of the area percent of PCNA immunoreactivity, areas containing positively immunostained tissues were chosen for evaluation regardless the intensity of staining. These areas were masked by a blue binary colour that could be measured by the computer system.

VI- Statistical evaluation:

The measured values were expressed as mean values ± SD (standard deviation). The statistical importance of the difference in these values between different groups was estimated using simple student test (t-test). A P-value less than 0.05 was considered significant.

RESULTS

I- Haematoxylin and eosin:

Control group:

The terminal secretory portions of the submandibular gland were predominantly of the serous type and triangular acinar cells surrounded the rounded narrow lumen. The intralobular duct system consisted of intercalated and striated ducts.

The granular convoluted tubules (GCT) were lined by a single layer of columnar cells, containing eosinophilic granules and round basal nuclei. The secretory portions of the sublingual glands were predominantly of the mucous type and were

composed of high cuboidal cells with an eosinophilic spongy cytoplasm and an angular basal nucleus (fig. 1).

Effect of radiation:

In group I, one day post 4 Gy irradiation**,** the submandibular glands revealed few intracytoplasmic vacuoles within the acinar cells. Some degenerated acinar cells were also found. The GCT showed a decrease in their content of the secretory granules. Both intralobular and interlobular ducts were normal. The connective tissue stroma contained dilated blood vessels**.** These alterations were still observed after 2 weeks in addition to progressive acinar vacuolization (fig. 2). In the one month observation period, the intracytoplasmic vacuoles became smaller with restoration of the normal acinar architecture. Re-granulation of GCT was also noticed. After two months, the submandibular glands showed complete recovery.

One day post 4 Gy irradiation, the mucous acinar cells of sublingual glands were appeared almost normal. After two weeks, the sublingual glands revealed few intracytoplasmic vacuoles in the mucous cells. Some acini showed alteration of their normal architecture. The duct system appeared normal. The connective tissue stroma contained many dilated blood vessels. Normal histological appearance was noted in the subsequent observation periods (fig. 3).

In group II, one day post 6 Gy irradiation, the submandibular glands revealed acinar intracytoplasmic vacuolization. Some acini were atrophied while others became completely degenerated. Marked reduction in number of GCT was noticed, which showed loss of the secretory granules. The connective stroma contained many dilated blood vessels. These alterations were more obvious after 2 weeks. Restoration of the glandular architecture started after month. Complete recovery was observed in the last observation period (fig. 4).

One day post 6 Gy irradiation, the sublingual glands revealed intracytoplasmic vacuolization of the acinar cells and many dilated blood vessels. After two weeks, the vacuoles became numerous and larger size compared to the previous observation period. Few degenerated acinar cells were also detected. The duct system was normal. In the subsequent dates, the sublingual glands revealed almost normal acinar structure and intact duct system.

Effect of selenium treatment:

Group III treated by selenium only revealed normal glandular appearance with slight increase in the number of the blood vessels noted starting from the 2nd week.

In group IV, one day post selenium treatment and radiation dose of 4 Gy, mild intracytoplasmic vacuolization was detected in the acinar cells of the submandibular glands. The GCT showed slight decrease in the content of the secretory granules. The duct system was normal. After two weeks, the gland appeared almost normal, exhibiting very mild vacuolization of acinar cells with re-granulation of the GCT. Complete restoration of the normal histological appearance was observed in the subsequent observation periods (fig. 5).

Very mild acinar vacuolization was developed in the sublingual glands 2 weeks post 6 Gy irradiation. Recovery of normal glandular architecture was observed after 1 and 2 months.

In group V, one day post selenium treatment and radiation dose of 6 Gy, the submandibular glands revealed acinar cells vacuolization, alteration of acinar architecture and de-granulation of the GCT. The duct system appeared normal. Two weeks post selenium treatment and radiation dose of 6 Gy, the above-mentioned changes were still present but with a milder degree. Restoration of the normal histological appearance was observed in the subsequent observation periods (fig. 6).

Similarly, one day post selenium treatment and radiation dose of 6 Gy, the sublingual glands revealed occasional vacuoles in the acinar cells in addition to slight alteration of acinar architecture. These changes were ameliorated after 2 weeks and completely recovered thereafter.

II- Alcian / periodic acid schiff:

Control group:

Examining paraffin sections stained by alcian blue (pH 2.5) combined with periodic acid shiff (PAS) technique for demonstration of acidic and neutral mucosubstances, respectively, showed that the serous acini of the submandibular gland presented a moderately positive reaction for periodic acid shiff and a mild positive reaction for alcian blue (pH 2.5). This indicated that they contain a considerable amount of neutral mucosubstances. The GCT were packed with intensely PAS +ve granules. The cytoplasm of the intercalated, striated and excretory duct lining cells showed a moderate reaction for neutral mucosubstances. In the sublingual glands, the mucous acini revealed a moderate to intense reaction for acidic mucosubstances. The cytoplasm of the intercalated, striated and excretory duct lining cells showed a mild reaction for both neutral and acidic mucosubstances (fig. 7).

Effect of radiation:

In group (I), one day and two weeks post 4 Gy irradiation, the reaction of the acini of the submandibular gland to both neutral and acidic mucosubstances was decreased. The GCT contained decreased amount of intensely PAS +ve granules. In the sublingual glands, one day post 4 Gy irradiation, most of the acini revealed moderate reaction for acidic mucosubstances, while after two weeks, some acini presented a mild reaction to both neutral and acidic mucosubstances, whereas others showed a moderate reaction for acidic mucosubstances only. One month and two months post 4 Gy irradiation, both glands revealed a staining reactivity similar to the control.

In group (II), one day and two weeks post 6 Gy irradiation, the staining reactivity of the acini of the submandibular gland to both neutral and acidic mucosubstances was further decreased as compared to the previous group. The GCT revealed few moderately PAS +ve granules. One day post 6 Gy irradiation, most acini of the

sublingual glands presented a mild reaction to acidic mucosubstances, while only few acini showed a moderate reaction, whereas after two weeks, most acini presented a mild reaction for neutral and acidic mucosubstances. The duct system revealed mild reaction for both neutral and acidic mucosubstances.

One month and two months post 6 Gy irradiation, the submandibular gland revealed a staining reactivity similar to the control, while the acini of the sublingual gland revealed intense reaction for acidic mucosubstances. Mild reaction for neutral and acidic mucosubstances was observed in the sublingual duct system.

Effect of selenium treatment:

In Group (III), the serous acini of the submandibular gland presented a moderately positive reaction for periodic acid shiff with condensation in the basement membrane. The GCT were packed with intensely PAS +ve granules. The intercalated, striated and excretory duct lining cells showed a moderate reaction for neutral mucosubstances. The above mentioned changes were observed in all experimental periods. The sublingual gland of group III revealed a staining reactivity similar to the control throughout the experiment.

In group (IV), one day and two weeks post selenium treatment and radiation dose of 4 Gy, the staining reaction of the acini and ducts of the submandibular gland was mild to moderate for both neutral and acidic mucosubstances. The GCT revealed intensely PAS +ve granules. The mucous acini of the sublingual glands revealed an intense reaction for acidic mucosubstances. The cells of the duct system presented a mild reaction for neutral mucosubstances with mild reaction for acidic mucosubstances at the luminal border.

One month and two months post selenium treatment and radiation dose of 4 Gy, the reaction of the submandibular acini for both mucosubstances was mild to moderate. The GCT were packed with intensely PAS +ve granules. Moderate reaction for acidic mucosubstances was detected in the sublingual mucous acini. The cytoplasm of the intercalated, striated and excretory duct lining cells showed a moderate reaction for both neutral and acidic mucosubstances.

In group (V), one day and two weeks post selenium treatment and radiation dose of 6 Gy, the staining reaction of the acini and ducts of the submandibular gland was moderate for the neutral mucosubstances and mild for the acidic type. The GCT contained a decreased amount of intensely PAS +ve granules. One month and two months post selenium treatment and radiation dose of 6 Gy, the reaction for both neutral and acidic mucosubstances was similar to the control. The sublingual gland demonstrated an intense reaction for acidic mucosubstances in the mucous acini and moderate reaction for neutral and acidic mucosubstances in the duct system cells all over the investigation period (fig. 8).

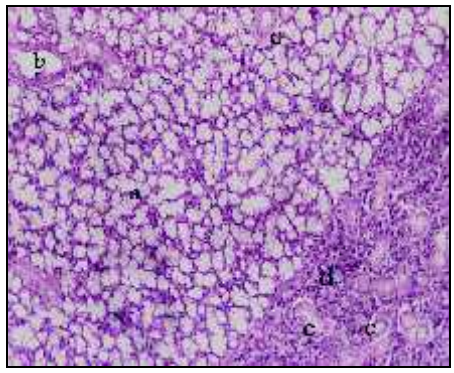

Fig. (1): Photomicrograph of sublingual and submandibular glands of the control group revealing mucous acini of sublingual gland (a), excretory duct of sublingual gland (b), striated duct of sublingual and submandibular glands (c), serous acini of submandibular gland (d) and GCT of submandibular gland (e), (H & E x 200).

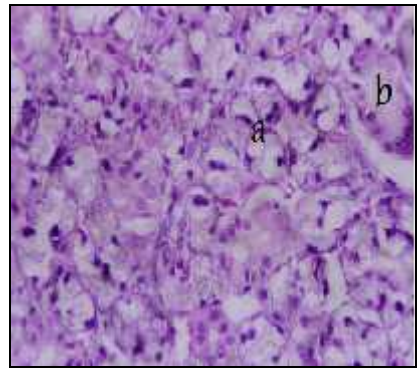

Fig. (2): Photomicrograph of submandibular gland two weeks post 4 Gy irradiation revealing acinar vacuolization and loss of architecture of acini (a), in addition to decrease in GCT granules (b), (H & E x 400).

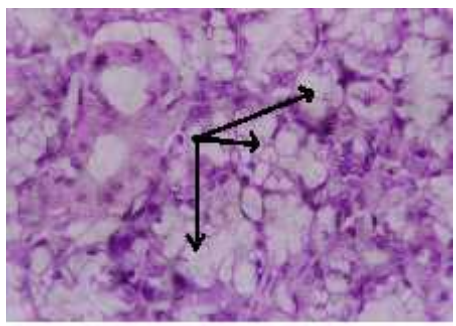

Fig. (3): Photomicrograph of sublingual gland two weeks post 4 Gy irradiation revealing vacuolization of the mucous acini and loss of normal acinar architecture (arrows), (H & E x 400).

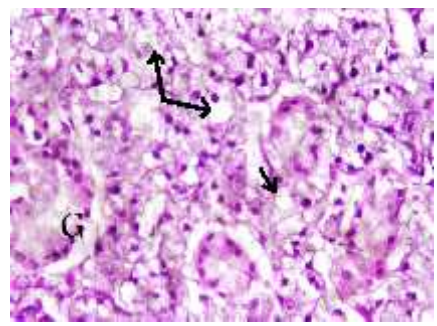

Fig. (4): Photomicrograph of submandibular gland two weeks post 6 Gy irradiation revealing acinar vacuolization and loss of normal acinar architecture (arrows). Note the degranulation of GCT (G), (H & E x 400).

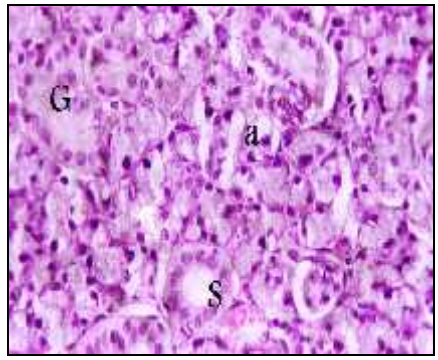

Fig. (5): Photomicrograph of submandibular gland two weeks post selenium and 4 Gy irradiation revealing very mild acinar vacuolization (a), mild degranulation of GCT (G) and normal appearance of duct system (S), (H & E x 400).

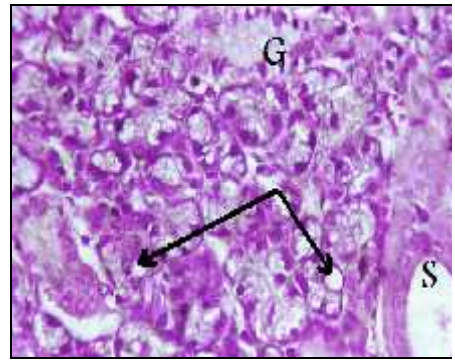

Fig. (6): Photomicrograph of submandibular gland one day post selenium and 6 Gy irradiation revealing acinar vacuolization (arrows), mild degranulation of GCT (G) and normal appearance of striated duct (S), (H & E x 400).

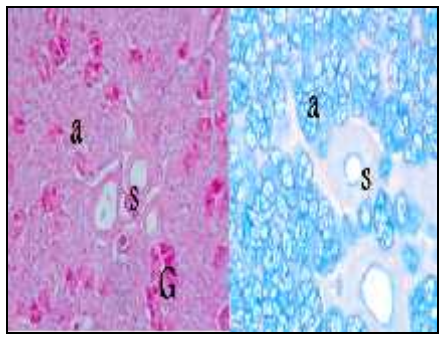

Fig. (7): Photomicrograph of control submandibular gland (left) revealing moderate reaction for neutral mucosubstances and mild reaction for acidic mucosubstances in the acini (a), intensely PAS +ve granules in the GCT (G) and moderate reaction for neutral mucosubstances in striated and excretory ducts (S), (alcian / PAS x 100). The control sublingual gland (right) reveals moderate to intense reaction for acidic mucosubstances in the mucous acini (a) and mild reaction for both neutral and acidic mucosubstances in striated ducts (S), (alcian / PAS x 200).

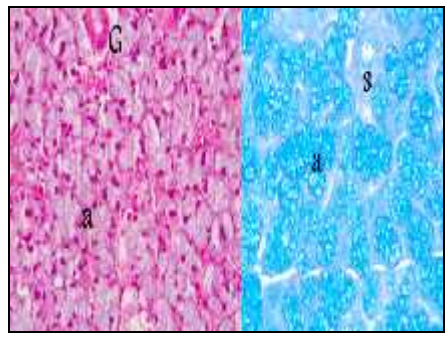

Fig. (8): Photomicrograph of submandibular gland two weeks post selenium treatment and 6 Gy irradiation (left) revealing moderate and mild reaction for neutral and acidic mucosubstances, respectively, in the acini (a) and decreased amount of intensely PAS +ve granules in the (G), (alcian / PAS x 200). The sublingual gland one month post selenium treatment and 6 Gy irradiation (right) revealed intense reaction for acidic mucosubstances in the mucous acini (a) and moderate reaction for neutral and acidic mucosubstances in striated ducts (S), (alcian / PAS x 200).

III- Feulgen reaction:

Control group:

Examining paraffin sections stained by the Feulgen reaction for DNA, The nuclei of the cells of acini and duct system of both glands were well organized and of uniform normal size. They demonstrated a moderate to intense staining reaction.

Effect of radiation:

In group (I), one day post 4Gy irradiation, the nuclei of the acini of the submandibular gland were small, pale in colour and haphazardly arranged.

On the other hand, the nuclei of duct system and GCT had a more or less organized arrangement and moderate staining reaction. Two weeks post 4 Gy irradiation, the staining reactivity of the nuclei was decreased and the size was slightly improved. The irregular arrangement of the acinar nuclei was still observed. In the sublingual glands, one day and two weeks post 4 Gy irradiation, the nuclei of the acini and duct system were small and moderately stained. The nuclei of the acini were irregularly arranged, however, nuclei of the duct system were organized. In the subsequent observation periods, the normal arrangement of the nuclei was restored. In both glands, the nuclei had a normal size and colour similar to the control group (fig. 9).

In group (II), one day and two weeks post 6 Gy irradiation, the nuclei of the submandibular acini and GCT were haphazardly arranged. The nuclei of duct system exhibited a lesser degree of disorganization. The nuclei of the whole gland appeared with smaller size and paler colour as compared to normal. Similarly, the nuclei of the sublingual glands were pale and irregularly distributed (fig. 10). In the subsequent observation periods, the normal staining reaction, size and arrangement of the nuclei was in the submandibular gland while in the sublingual glands, the nuclear arrangement was not fully restored.

Effect of selenium treatment:

The nuclei of the acini and GCT of the group treated with selenium only (group III) were variable in staining reaction. The nuclei of striated and excretory ducts were organized and moderately stained. In all experimental periods, the nuclei of the sublingual gland revealed a normal organization and size. The acinar nuclei were dark while those of duct system were moderately stained.

In group (IV), one day post selenium treatment and radiation dose of 4 Gy, the organization of the nuclei of the submandibular acinar cells was slightly altered. However, the nuclei appeared of relatively normal staining reaction and size. Nuclei of the sublingual acini presented altered arrangement and staining reaction with decreased size. These changes were still present in both glands after two weeks but to a milder degree. The nuclei of the cells of duct system were almost normal in both glands throughout the experiment. One and two months post selenium treatment and 4 Gy irradiation, restoration of the normal nuclear organization, size and staining reactivity were progressively observed in both glands (fig. 9).

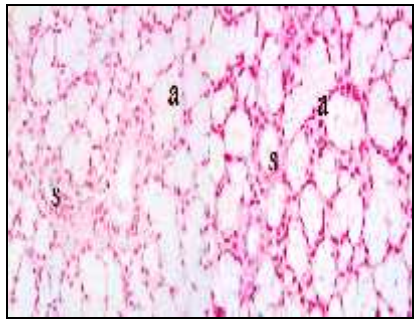

Fig. (9): Photomicrograph of sublingual gland two weeks post 4 Gy irradiation (left) revealed moderately stained acinar nuclei (a), moderately stained, regularly arranged nuclei of striated ducts (S). The sublingual gland two weeks post selenium treatment and 4 Gy irradiation (right) revealed well organized, slightly enlarged, darkly stained nuclei of acini (a) and normal size, distribution and staining of nuclei of striated ducts (S), (Feulgen x 200).

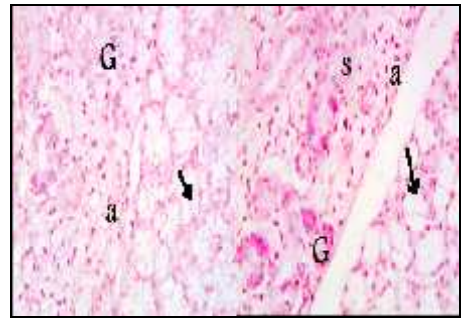

Fig. (10): Photomicrograph of sub-mandibular and sublingual glands two weeks post 6 Gy irradiation (left) revealed small, lightly stained and irregularly distributed nuclei of the acini (a) and pale but organized nuclei of GCT (G). Two weeks post selenium treatment and 6 Gy irradiation (right) variable staining reactions were noted in the submandibular acinar (a) and GCT nuclei (G). A moderate staining reaction is noted in nuclei of the striated ducts (S) while sublingual acinar nuclei appeared organized but moderately stained (arrow), (Feulgen x 200).

Table (1): Mean nuclear area revealing Feulgen reaction in the submandibular glands of the different studied groups (mean ± SD).

O.P.	Control		4 Gy		6 Gy		Selenium		Se & 4 Gy		Se & 6 Gy	
	M	L	M	L	M	L	M	L	M	L	M	L
1 Day	487.5 ± 706.5	503.7 ± 735.9	305.9 ± 373.9	267.9 ± 346.1	417.1 ± 140.7	397.3 ± 69.9	554.8 ± 247	555.5 ± 224.3	440.4 ± 103.6	386.7 ± 77.7	483.3 ± 293.7	488.4 ± 163.1
14 Days	490.4 ± 711.3	507.6 ± 737.4	414.3 ± 458.4	415.0 ± 647.4	657.8 ± 553.7	456.8 ± 178.1	556.1 ± 379.9	564.5 ± 224.0	559.0 ± 386.5	622.9 ± 377.9	592.4 ± 439.1	658.9 ± 311.5
60 Days	490.1 ± 710.5	500.7 ± 736.1	504.0 ± 752.9	541.7 ± 550.4	531.7 ± 283.4	584.8 ± 296.3	537.3 ± 236.4	558.1 ± 188.9	495.8 ± 214.4	444.2 ± 192.7	506.8 ± 238.6	563.6 ± 294.9

O.P.: Observation period, M: submandibular, L: sublingual.

Table (2): Significance of the difference in mean nuclear area in the submandibular and sublingual glands of the different studied groups (t-test).

Groups	1 Day		14 Days		60 Days	
	P (M)	P (L)	P (M)	P (L)	P (M)	P (L)
Control ≠ 4 Gy	4.14347^{-8} E	7.5194^{-11}E	0.056246	0.049602*	0.709737	0.451443
Control ≠ 6 Gy	0.438603	1.3188^{-7}E	0.000201*	0.005128*	0.288954	0.181832
Control ≠ (Se)	0.328491	0.280796	0.249313	0.211525	0.390558	0.264817
4 Gy ≠ 6 Gy	0.021352*	0.517794	1.60715^{-7}E	0.473891	0.52675	0.365728
4 Gy ≠ Se & 4 Gy	0.000007*	0.332415	0.0023*	0.000008*	0.851997	0.030417E
6 Gy ≠ Se & 6 Gy	0.107076	0.337226	0.075518	1.73707^{-10}E	0.21218	0.517824
Se & 4 Gy ≠ Se & 6 Gy	0.092863	0.082579	0.265277	0.293637	0.533315	0.000018 E
Se ≠ Se & 4 Gy	0.000001*	0.034927*	0.937531	0.041219*	0.059393	1.06588^{-8}*
Se ≠ Se & 6 Gy	0.096492	0.002132*	0.368195	0.000289*	0.18263	0.818359

** : Denotes a statistically significant difference.*

E : Denotes a high statistically significant difference.

P(M) = P value in the submandibular gland, P(L)= P value in the sublingual gland.

In group (V), one day post selenium treatment and radiation dose of 6 Gy, the nuclei of the acinar cells of both glands had an almost normal size but alterations in the staining reaction and organization were occasionally found. The nuclei of the duct system were almost normal. After two weeks, the acinar and GCT nuclei were variable in staining reaction while the nuclei of the striated ducts were moderately stained (fig. 10). One month and two months post selenium treatment and radiation dose of 6 Gy, complete restoration of the normal appearance of the nuclei was observed in all cell types in both glands. The mean nuclear area expressing the Feulgen reaction in the different studied groups of both glands and the statistical significance of the difference are presented in tables (1) and (2).

IV- PCNA immunostaining:

Control group:

Examining sections immunohistochemically stained for demonstration of the proliferating cell nuclear antigen (PCNA) showed that the control submandibular gland revealed few proliferating acinar and GCT cells. Proliferation was also detected in the intercalated and striated ducts at low level. The control sublingual gland expressed a small percentage of proliferating cells within the different gland compartments (fig. 11).

Effect of radiation dose of 4 Gy:

In group (I), one day after 4 Gy irradiation, the mean area percent of PCNA was decreased (mean area percent = 1.98 ± 0.64) as compared to control. The acinar cells were the most affected cells by this reduction. This was followed by a subsequent increase in PCNA expression at two weeks post irradiation (mean area percent = 7.15 ± 3.6). The acinar cells showed the highest proliferation (fig. 11). Afterwards, the PCNA expression was decreased until one month after irradiation where it became similar to the control. Almost the same level of expression was maintained up to two months after irradiation.

In group (II), one day post 6 Gy irradiation, the proliferation was minimum among the different cell types of submandibular gland (mean area percent of PCNA positivity = 0.26 ± 0.12) and in the intercalated ducts of the sublingual glands. Two weeks after, the proliferation reached a maximum level (mean area percent of PCNA positivity = 11.76 ± 5.04) which was higher than the 4 Gy irradiation group. The proliferation was higher in the acinar cells followed by the intercalated duct cells (fig. 12). The proliferation returned to the normal level at one month post irradiation. Two months after irradiation, the proliferation index was similar to the control.

Effect of selenium treatment:

In group (III), the proliferation index of selenium treated submandibular glands was higher as compared to the control group while the percentage of proliferating cells in the sublingual gland was comparable to that of the control group throughout the investigation period.

In group (IV), one day post selenium treatment and radiation dose of 4 Gy, the mean area percent of PCNA expression was slightly decreased (3.02 ± 0.53) as compared to control group. Afterwards, the proliferation was increased to reach the maximum level after two weeks (PCNA mean area percent = 5.09 ± 1.49) and returned to the control level at one month post-irradiation.

In group (V), the proliferative pattern in the submandibular gland of this group was similar to the previous one. However, the decrease in PCNA expression at day one post-irradiation was greater (mean area percent positivity = 2.66 ± 0.72) (fig. 13) and the subsequent increase after two weeks was less (mean area percent positivity = 4.39 ± 1.81). One month and two months post irradiation, the proliferative index was similar to the control and the same pattern was observed in the sublingual gland (fig. 13).

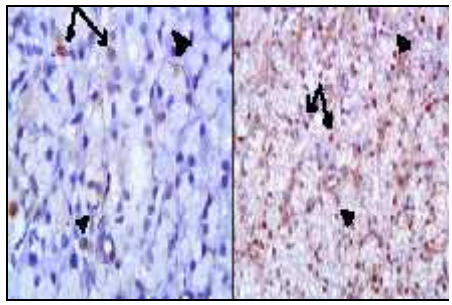

Fig. (11): Photomicrograph of control submandibular gland (left) revealed a few PCNA positive acinar (arrowheads) and ductal cells (arrows), (PCNA x 400).

The submandibular gland two weeks post 4 Gy irradiation (right) revealed many PCNA positive cells in the acini (arrowheads) and ducts (arrows), (PCNA x 200)

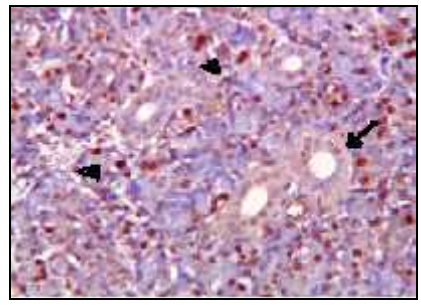

Fig. (12): Photomicrograph of submandibular gland two weeks post 6 Gy irradiation revealed numerous PCNA positive cells in the acini (arrowheads) and ducts (arrows), (PCNA x 200).

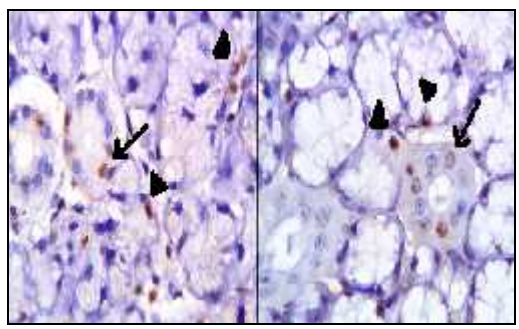

Fig. (13): Photomicrograph of submandibular gland two weeks post selenium treatment and 6 Gy irradiation (left) revealed numerous PCNA positive acinar cells (arrowheads) and few positive ductal cells (arrows), (PCNA x 400).

The sublingual gland one month post selenium treatment and 6 Gy irradiation (right) revealed few PCNA positive acinar (arrowheads) and ductal cells (arrows), (PCNA x 400).

Table (3): Significance of the difference in PCNA expression in the submandibular glands of the different studied groups (student's t-test).

Groups	1 Day		14 Days	
	P	**Significance**	**P**	**Significance**
Control ≠ 4 Gy	0.000871	*	0.032464	*
Control ≠ 6 Gy	6.11265^{-7}	E	0.000296	*
Control ≠ Se	0.117348		0.111811	
4 Gy ≠ 6 Gy	1.44136^{-7}	E	0.030418	*
4 Gy ≠ Se & 4 Gy	0.000935	*	0.110498	
6 Gy ≠ Se & 6 Gy	4.41984^{-9}	E	0.000387	*
Se & 4 Gy ≠ Se & 6 Gy	0.217151		0.358911	
Se ≠ Se & 4 Gy	0.001772	*	0.45807	
Se ≠ Se & 6 Gy	0.000724	*	0.159276	

** : Denotes a statistically significant difference.*

E : Denotes a high statistically significant difference.

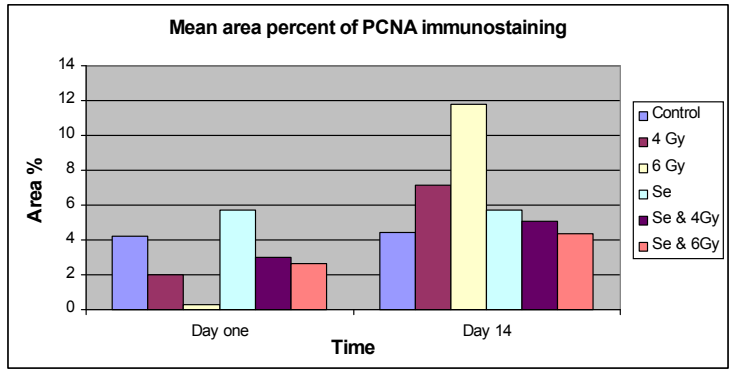

Fig. (14): A histogram representing the mean area percent of PCNA immunostaining in the submandibular glands of the different studied groups.

In the one day observation period in the submandibular glands, the mean area percent of PCNA expression was greatest in the Se group (5.706 ± 2.26) followed by the control group (4.244 ±1.68) then the Se and 4Gy (3.021 ± 0.53) while the lowest expression was noted in the 6 Gy group (0.26 ± 0.12). After 14 days, the greatest mean area percent of PCNA expression was noted in the 6 Gy group (11.758 ± 5.04) followed by the 4 Gy group (7.154 ± 3.6) and the Se group (5.738, ±2.28) while the lowest expression was noted in the Se and 6 Gy group (4.388 ±1.81), (fig. 14). The statistical significance of the difference between the measured values is presented in table (3).

DISCUSSION

Salivary gland is one of the organs affected by radiotherapy because it is often not possible to exclude salivary glands from the treatment field (Price et al., 1995). The secretory cells of the salivary glands are the most radiosensitive, especially the serous secretors (Coppes et al., 2002).

Naturally occurring antioxidants may provide an extended window of protection against low dose and low dose rate irradiation, including a therapeutic potential when administered after irradiation. However, they are less protective but also less toxic than synthetic radioprotectors (Weiss and Landauer, 2003).

The Institute of Medicine, Food and Nutrition Board (2000) considers the mineral selenium to be a dietary antioxidant, defined as a substance in foods that significantly decrease the adverse effects of reactive species (such as reactive oxygen and nitrogen species) on normal physiological functions in humans.

The present study was designed to assess the histological, histochemical and immunohistochemical effects of radiation and/or selenium on submandibular and sublingual glands of the rats.

The intracytoplasmic vacuoles detected in the present study in both the irradiated submandibular and sublingual glands were similar to those reported by Sagowski et al.(2003, 2004, 2005) and Boraks et al. (2008) in the parotid gland, Vissink et al (1991), Bralic et al. (2005), Urek et al. (2005), Lee et al. (2006) and Muhvic et al. (2006) in the submandibular gland.

Atrophy of serous acini and granular convoluted tubules observed in the submandibular and sublingual glands were also reported by Ahlner et al. (1993) and O'Connell et al. (1999) in the submandibular gland of the rabbit and rat, respectively, Nagler (1998) and Radfar and Sirois (2003) in both the submandibular and parotid glands and Onodera et al. (2006) in the parotid gland. Late atrophy was the direct result of acute loss of serous acini and reflects a lack of regeneration of acinar cells receiving acute injury (Stephens et al., 1986b).

Guchelaar et al. (1997) showed that the early response resulting in atrophy of the secretory cells without inflammation might be due to radiation-induced apoptosis. Other studies have proposed that progressive loss of acini in the parotid and submandibular glands can be correlated to the activation of mast cells and release of their secretory products (Henriksson et al., 1994).

The content of secretory granules of the serous cells of both submandibular and sublingual glands and granular convoluted tubules of submandibular glands were reduced. Similar results were reported in GCT of the submandibular gland by Vissink et al. (1991), in the rabbit submandibular gland by Ahlner et al. (1993), in the serous cells of parotid and submandibular gland by Peter et al. (1994) and in the parotid gland by Onodera et al. (2006). Regranulation and restoration of normal structure was observed after one month and was consistent with that reported by Vissink et al. (1991) and Ahlner et al. (1994).

In the present study, the serous acinar cells were more sensitive to irradiation damage than mucous acinar cells and ductal cells. These results are consistent with those observed by Stephen et al. (1986a , 1991) and Nagler (2003) in the monkeys and by Vissink et al. (1990), Gemryd et al. (1992) and Sagowski et al. (2003) in the rats. Stephen et al. (1986b) and Peter et al. (1994) demonstrated degeneration and necrosis of both serous and mucous cells, which may be due to higher dose of radiation and the mechanism is not clear but it has been suggested that serous secretion granules have proteolytic and metallic transmission enzymes, while the mucous secretion granules mainly contain glycoproteins. The transmission materials are known to promote the formation of free radicals and potentiating the damage to the serous granule membranes. As a result, the proteolytic enzymes may infiltrate and damage the cytoplasm causing autolysis and cellular death (Abok et al., 1984 and Coppes et al., 2000).

In the current study, sodium selenite diminished the deleterious effects of radiation at the different experimental periods studied but did not completely prevent all the radiation-induced pathological alterations. The radioprotective effect of sodium selenite treatment was manifested by decrease in the number and size of vacuoles, less development of GCT degranulation and absence of degenerated acinar cells. These results were consistent with that of Yanardag et al. (2001) who reported that sodium selenate minimized or even prevent the radiation induced degenerative changes of the liver of the rat. Similarly, Sagowski et al. (2005) observed a delayed development of the vacuolization, oedema and degranulation as well as reduction of the amount of necrotic acinar cells in selenium treated irradiated rat's submandidular and parotid glands.

Pontual et al. (2007) suggested that sodium selenite probably helped to maintain the integrity of the secretion granules, preventing leakage of their contents into the cellular cytoplasm, subsequent rupture of the organelles and cell destruction.

In the present study, the alcian/PAS staining technique was used to demonstrate the neutral and acidic mucopolysaccharides contents of the examined salivary glands. Reduction in the mucopolysaccharide content of the 4 Gy or 6 Gy irradiated submandibular and sublingual glands was consistent with Eid et al. (1989) and Lin et al. (2001). Similar results were achieved in the small intestine, stomach, lungs, and liver of guinea pigs and small intestine of rats (Sheremet et al., 2001) and in the liver of rats (El-Ghazaly et al., 2004 and Soliman et al., 2007). Lombaert et al.

(2006) revealed a similar reduction in the salivary gland tissues of irradiated mice as assisted by PAS staining method.

The present investigation denoted the effect of selenium in preserving the mucopolysaccharides content of the irradiated salivary glands. This finding emphasizes the role of this natural antioxidant in maintaining the secretory function of the salivary gland components as a part of its radioprotective action.

Using the Feulgen reaction, the results of the present study revealed that DNA content of submandibular and sublingual salivary gland cells were reduced after irradiation. Yang and Qin (2005) also reported that DNA content was decreased evidently after radiation.

In the study under investigation, sodium selenite was found to maintain normal size, shape, staining reaction and organization of salivary gland nuclei. The concentration of DNA was higher in the Se treated groups as compared to the irradiated group and was similar to the control.

The initial decline in the number of the proliferating cells in all gland compartments at day 1 post-irradiation using PCNA immunostaining in the present study was also reported in the parotid gland by Peter et al. (1994) and Hakim et al. (2002) and in the submandibular gland by O'Connell et al. (1998) and Bralic et al. (2005). Takahashi et al. (2000) attributed the decreased expression of PCNA in the nuclei of acinar cells one day post-irradiation to the widely dispersed intranuclear alterations. According to Bralic et al. (2005), the cell arrest observed in all cell types at day 1 post-irradiation was resulted from delay of the S-phase of the cell cycle induced by irradiation. After the initial decline, there was a subsequent increase in the proliferation rate at 14 days after radiation in all parenchymal cell types. This finding is consistent with those reported by Ballagh et al. (1994) and Peter et al. (1994) in parotid and submandibular glands and by Bralic et al. (2005) and Muhvic et al. (2006) in submandibular gland. Two months after irradiation, the proliferation index of all gland compartments were similar to the control, which in agreement with Bralic et al. (2005) and Muhvic et al. (2006).

The results of this study suggest that proliferation takes place in all glandular compartments but at different levels. Denny et al. (1993) indicated that repopulation of irradiated glands can be explained by self proliferation of cells. They added that approximately 70% of the cell population was maintained by self proliferation as opposed to 30% by differentiation from progenitor cells.

In the present study, sodium selenite treatment was found to promote cell proliferation and ameliorated the initial decline in proliferation in irradiated salivary glands. Zeng (2002) stated that low levels of Se up-regulated the expression of numerous cell cycle-related genes, including c-Myc, cyclin C, proliferating cell nuclear antigen, cyclin-dependent kinase (CDK1), CDK2, CDK4 and cyclin B. This led to the promotion of cell cycle progression, particularly G2/M transition and/or the reduction of apoptosis, primarily in G1 cells.

In summary, the results of the present study demonstrated that selenium has some promise in preventing the histopathological alterations induced by gamma

irradiation in the salivary glands. Selenium ameliorated the radiation-induced alterations in the mucopolysaccharides and DNA content of the salivary glands, attenuated the radiation-induced cell cycle inhibition and assisted the regenerative process in the irradiated glands.

CONCLUSION

Selenium can be used as a natural radioprotective agent during radiotherapy schedules to decrease radiation side effects. Further investigations are required to elaborate the molecular mechanisms responsible for the radioprotective effect of selenium and to evaluate the benefit of using this natural micronutrient as an adjunct to radiotherapy in clinical practice.

REFERENCES

Abok, K., Brunk, U., Jung, B. and Ericsson, J., Virchows Arch. B Cell Pathol. Incl. Mol. Pathol., 45, 443-460 (1984).

Ahlner, B.H., Hagelqvist, E., Lind, M.G. and Ruden, B.I., Acta Otolaryngol., 113(2), 210-219 (1993).

Ahlner, B.H., Hagelqvist, E. and Lind, M.G., Ann. Otol. Rhinol. Laryngol., 103(2), 125-134 (1994).

Ballagh, R.H., Kudryk, K.G., Lampe, H.B., Moriarty, B., Mackay, A., Burford, A.P. and Dardick, I., Oral Surg. Oral Med. Oral Pathol., 77(1), 27-35 (1994).

Bancroft, J.D. and Gamble, M., Churchill Livingstone, Edinburgh, London, NewYork, Oxford, Philadelphia, St Louis, Sydney, Toronto, 5th Ed., p. 125-242 (2002).

Boraks, G., Tampelini, F.S., Pereira, K.F. and Chopard, R.B., Braz. Dent. J., 19(1), 1590-1592 (2008).

Bralic, M., Muhvic, U.M., Stemberga, V., Golemac, M., Jurkovic, S., Borcic, J., Braut, A. and Tomac, J., Acta Med. Okayama, 59(4), 153-159 (2005).

Broadley, M.R., White, P.J., Bryson, R.J., Meacham, M.C., Bowen, H.C., Johnson, S. E., Hawkesford, M.J., McGrath, S.P., Zhao, F.J., Breward, N., Harriman, M. and Tucker, M., Proc. Nutr. Soc., 65(2), 169-181 (2006).

Caffery, P.B., Biological Trace Element Research, 65(3), 187-195 (1998).

Cherupally, K.K., Dillip, K. and Taisei, N., J. Radiat. Res., 42, 21-37 (2001).

Combs, G.F., Clark, L.C. and Turnbull, B. W., Biofactors, 14, 153-159 (2001).

Coppes, R.P., Roffel, A.F., Zeilstra, L.J., Vissink, A. and Konings, A.W., Radiat. Res., 135, 339-346 (2000).

Coppes, R.P., Vissink, A. and Konings, A.W., Radiother. Oncol., 63, 321-328 (2002).

Denny, P.C., Chai, Y., Klauser, D.K. and Denny, P.A., Anat. Rec., 235, 475-485 (1993).

Eid, F.A., Farid, N.M., Osman, A., El-Aaser, B.A. and Osman, N.H., Egyptian J. Radiation Sciences and Applications, 7(2), 153-168 (1989).

El-Ghazaly, M.A., Ramadan, L.A., Ashry, O.M. and Kafafy, Y.A., Egyptian J. Radiation Sciences and Applications, 17(1), 25-45 (2004).

Fujieda, M., Naruse, K., Hamauzu, T., Miyazaki, E., Hayashi, Y., Enomoto, R., Lee, E., Ohta, K., Yamaguchi, Y., Wakiguchi, H. and Enza, H., Pediatr. Nephrol., 22(2), 192-201 (2007).

Gemryd, P., Norberg, L.E. and Lundquist, P.G., J. Otorhinolaryngol. Relat. Spec., 54(1), 33-37 (1992).

Guchelaar, H.J., Vermes, A. and Meerwaldt, J.H., Support Care Cancer, 5(4), 281-288 (1997).

Hakim, S.G., Kosmehl, H., Lauer, I., Nadrowitz, R., Wedel, T. and Sieg, P., Anticancer Res., 22(6), 4121-4128 (2002).

Henriksson, R., Frojd, O., Gustafsson, H., Johansson, S., Yi-Qing, C., Franzen, L. and Bjermer, L., Br. J. Cancer, 69(2), 320-326 (1994).

Institute of Medicine, Food and Nutrition Board, Washington DC, National Academy Press, p.384 (2000).

Lee, H., Lee, Y., Kwon, H., Bae, S., Kim, S., Min, J., Cho, C. and Lee, Y., American J. Pathology, 169, 1601-1611 (2006).

Lin, A.L., Johnson, D.A.Y., Wong, W.G., Ebersole, J.L. and Yeh, C.K., Archives of Oral Biology, 46(11), 1085-1089 (2001).

Lombaert, I.M.A., Pieter, K.W., Tineke, K., Harm, H.K., Gerald, D. and Robert, P.C., Clinical Cancer Research, 12, 1804-1812 (2006).

Moulder, J.E., Robbins, M.E., Cohen, E.P., Hopewell, J.W. and Ward, W.F., Cancer Treatment Review, 93, 129-151 (1998).

Muhvic,U.M., Bralic, M., Curic, S., Pezelj, R.S., Borcic, J. and Tomac, J. , Physiol. Res., 55, 89-95 (2006).

Nagler, R.M., Anticancer Res., 18(1), 315-320 (1998).

Nagler, R.M., In Vivo, 17(4), 369-375 (2003).

O'Connell, A.C., Lillibridge, C.D., Zheng, C., Baum, B.J., O'Connell, B.C. and Ambudkar, I.S., J. Cell Physiol., 177(2), 264-273 (1998).

O'Connell, A.C., Redman, R.S., Evans, R.L. and Ambudkar, I.S., Radiat. Res., 151(2), 150-158 (1999).

Onodera, T., Nasu, M., Iwata, H. and Yosue, T., Oral Radiology, 22(1), 1-6 (2006).

Peter, B., Van Waarde, M.A., Vissink, A., Gravenmade, E.J. and Konings, A.W., Radiat. Res., 140(2), 257-265 (1994).

Pontual, M.A., Tuji, F.M., Barros, S.P., Bóscolo, F.N., Novaes, P.D. and Almeida, S.M. , J. Appl. Oral Sci., 15(3), 162-168 (2007).

Price, R.E., Ang, K.K., Stephens, L.C. and Peters, L.J. , Radiother. Oncol., 36(2), 156-157 (1995).

Radfar, L. and Sirois, D.A., Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod., 96(3), 267-274 (2003).

Rasmussen, L.B., Mejborn, H., Andersen, N.L., Dragsted, L.O., Krogholm, K.S., Larsen, E.H., Ovesen, L.F. and Tjonneland, A.M., Ugeskr Laeger., 168(39), 3311-3313 (2006).

Sagowski, C., Wenzel, S., Tesche, S., Jenicke, L. and Jaehne, M., Eur. Arch. Otorhinolaryngol., 260(9), 513-517 (2003).

Sagowski, C., Wenzel, S., Riehle, I., Tesche, S., Jenicke, L., Zywietz, F., Roeser, K. and Metternich, F.U., HNO, 52(1), 25-32 (2004).

Sagowski, C., Wenzel, S., Jenicke, L., Metternich, F.U. and Jaehne, M., Eur. Arch. Otorhinolaryngol., 262, 459-464 (2005).

Schrauzer, G.N., J. Am. College Nutr., (20), 1-4 (2001).

Schrauzer, G.N., Adv. Food Nutr. Res., (47), 73-112 (2003).

Sheremet, Z.I. , Manteifel, V.M. and Rayshenbakh, M.O., Meditsinskaya Radiologiya, 4(12), 25-36 (2001).

Sinha, R. and El-Bayoumy, K., Curr. Cancer Drug Targets, 4(1), 13-28 (2004).

Slyshenkov, V.S., Omelyanchik, A.G., Petushak, N.E. and Wojtezak., L., Acta Biochem. Pol., 46(2), 239-248 (1999).

Soliman, O., Hafez, M.N. and Abd El Maguid, A., Egyptian J. Radiation Sciences and Applications, 20(1), 195-214 (2007).

Stephens, L.C., Ang, K.K., Schultheiss, T.E., King, G.K., Brock, W.A. and Pseters, L. J., Radiother. Oncol., 7(2), 165-174 (1986a).

Stephens, L.C., King, G.K., Peters, L.J., Ang, K.K., Schultheiss, T.E. and Jardine, J.H., Am. J. Pathol., 124(3), 469-478 (1986b).

Stephens, L.C., Schultheiss, T.E., Price, R.E., Ang, K.K. and Peters, L.J., Cancer, 67(6), 1539-1543 (1991).

Takahashi, S., Nakamura, S., Suzuki, R., Islam, N., Domon, T. and Yamamoto, T., Tissue Cell, 32, 457-463 (2000).

Tinggi, U., Toxicol. Lett., 137(1-2), 103-110 (2003).

Urek, M.M., Bralic, M., Tomac, J., Borcic, J., Uhac, I., Glazar, I., Antonic, R. and Ferreri, S., Arch. Med. Res., 36(4), 339-343 (2005).

Vadgama, J.V., Wu, Y., Shen, D., Hsia, S. and Block, J., Anticancer Research, 20, 1391-1414 (2000).

Vissink, A., Gravenmade, E.J., Ligeon, E.E. and Konings, A.W., Radiat. Res., 124(3), 259-265 (1990).

Vissink, A., Kalicharan, D., Gravenmade, E.J., Jongebloed, W.L., Ligeon, E.E., Nieuwenhuis, P. and Konings, A.W., J. Oral Pathol. Med., 20(9), 449-456 (1991).

Weiss, J.F. and Landauer, M.R., Toxicology, 189(1-2), 1-20 (2003).

Yanardag, R., Bolkent, S. and Kizir, A., Biol. Trace Elem. Res., 83(3), 263-273 (2001).

Yang, J. and Qin, L., Chinese J. Radiological Medicine and Protection, 25(6), 533-536 (2005).

Zeng, H., J. Nutr., 132, 674-679 (2002).