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The role of Selenium in Mitigating The adverse Effect of Cyclophosphamide on The rat Submandibular Salivary Glands

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THE goal of this study is to see if selenium can counteract the effects of cyclophosphamide on albino rats' submandibular salivary gland tissues. 24 male rats were separated into four groups, each with 6 rats aged 12 -16 weeks weighing 300-400 grams: the first was given physiological saline orally, the second was given selenium (200 mg/kg) orally for 14 days, and the third group was given cyclophosphamide orally once (150 mg /kg). On day 8, group 5th group(IV) got selenium (200 Mg/kg) and cyclophosphamide (150 mg/kg) intraperitoneally (i.p.). On day 15, all rats were sedated and slaughtered, and the salivary glands of the submandibular region were collected. In comparison to the other groups, the third group lost weight significantly. The histopathological study revealed mucosal acini necrosis with edema surrounding the striated ducts, as well as mucosal acini cell atrophy and vacuoles in the serous acini. A decrease in the number of granulocytic convoluted tubules, an increase in fibrous tissue surrounding the interlobular ducts, and serous acinar apoptosis characteristics were also seen. In comparison to the other groups with moderate to severe foci, immunohistochemical results in this group demonstrated modest to the negative expression of (anti-apoptotic Bcl-2 protein) in stromal cells among the acinar cells of the salivary glands. We concluded that cyclophosphamide caused structural alterations in the submandibular salivary glands of rats and that selenium alleviated these side effects, as seen by histological investigation and immunohistochemistry analysis of Bcl-2 expression.

Keywords: Selenium, Cyclophosphamide, Submandibular salivary gland, Rat

Introduction

Surgery was the unique way to treat cases of solid tumors in the initial periods of the last century, which caused a high rate of high mortality [1]. Later on, and during the last four decades, there was a progressive enhancement of the rates of cancer patients' survival via anticancer agents including cyclophosphamide (CY) [2].

In fact, oral mucositis remains the frequent adverse effect of anticancer agents [3]; .patient complaints of several symptoms include difficulty swallowing, difficulty speaking, taste disorders, infection, and pain in the oral cavity besides dental caries and even sepsis which leads to different sequels [4].

The oral tissue integrity needs an optimal structure and function of salivary glands as saliva which contributes in different functions as antibacterial, anti-inflammatory, antifungal beside the role to lubricate the oral cavity and preservation of teeth [5]. The decrease in saliva amount (Xerostomia) which is associated with the use of anticancer agents may be manifested as oral mucositis [6] and exaggerate their toxic action [6]. For this reason ,the reports on the protection against the toxicity of anticancer agents are

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numerous. In addition, the chief mechanism of the anticancer drugs (including cyclophosphamide) is the oxidative stress [7].

In fact, chemotherapy may adversely impact the multiple organs which may be associated with the oral cavity [2, 3]. Cyclophosphamide is an alkylating agent belonging to the nitrogen mustard class that is mainly applied in cancer chemotherapy and autoimmune diseases and in bone marrow transplant while it has been associated with various organ toxicities [8], and authors reported its action on the submandibular salivary gland which is considered as the second largest salivary gland, which produces about two thirds of saliva secretion at rest, as it affects the glycogen as a substrate using in metabolism [9].

Physiologically, the biochemical reactions in cells metabolism produce reactive oxygen species. Several antioxidants are found normally to act as scavengers of reactive oxygen species [10]. Any imbalance between the oxidant and antioxidant systems may cause oxidative stressing and subsequent destruction of cellular components including organelles and deoxyribonucleic acid and proteins followed by cellular death [11].

Biologically, among the fundamental trace element is Selenium (that is found in food as meat products, cereals, fruits, vegetables, and milk). It obtains a remarkable interest as it is vital human's micronutrient as it contributes as in immune responses and endocrine functions beside different cellular and physiological mechanisms [12, 13] with antioxidant characters [14]. Several degenerative disorders are related with Selenium deficiency and it has been extensively used in the treatment of various pathophysiological states as in cervical, hepatic and renal malignancies [15].

This study aims to define the role of selenium in attenuating the impact of cyclophosphamide on submandibular salivary glands in rats using histological techniques and via the immune-histochemical expression of B cell lymphoma 2 .

Material and Methods

Chemicals

The cyclophosphamide (CY) Endoxan® 500 mg vial (Baxter Oncology GmbH, Baxter International Inc. India), and the (Se) selenium 200 Mcg tablet (AMS® American Medic & Science) it was freshly prepared each day before the treatment. The Endoxan® and selenium were obtained from the local pharmaceutical market of Iraq, Mosul.

Animals

This study involved Twenty-four adult male albino rats, about 300-400 grams body weight and aged about 12-16-weeks old, which were utilized. All rats were obtained from Animal House, (College of Veterinary Medicine, University of Mosul, Mosul, Iraq). They were housed in plastic cages with wire mesh covers under the standard environment condition at 21±2◦C, 12 h light/12 h dark cycle, and were allowed *ad libitum* to a standard diet and drinking water.

Ethical approval

This work was conducted according to the guidelines followed by the University of Mosul/ college of Dentistry, the pharmacology laboratory at the Department of Dental Science and the Ethics Committee University of Mosul /college of Dentistry (UoM.Dent/A.L.10/22) granted the ethical approval.

Experimental design

All rats were randomly divided into four groups , six rats in each group (n =6), as described below:

I: Control group: includes rats which were received normal slain orally using oral needle gavage for fourteen days.

II: Selenium **(Se)** group: include rats which were received (0.2 mg /kg b.w. /day) of selenium orally that diluted in normal saline for fourteen days via oral needle gavage.

III: Cyclophosphamide **(CY)** group: includes rats which were received (150 mg/kg of b.w.) of cyclophosphamide (diluted in normal saline) injected intraperitoneally (i.p) on the 8th day.

IV: cyclophosphamide with selenium **(CY+Se)** group: includes rats which were received a (0.2 mg /kg b.w. /day) of selenium orally for fourteen days, as well as, a single dose of (150mg/kg of b.w.) of cyclophosphamide diluted in normal saline were given on the day 8th of an experiment.

All rats in the four groups were weighted in the first and the final day of the experimental period using the digital balance, the difference between the mean body weight (initial and final) body weight was calculated .

At the end of the experiment, all rats in the four groups were anesthetized with an injection of ketamine and xylazine. Subsequently, submandibular salivary glands (SMG) were

excised for histology and immunohistochemistry. The specimens of submandibular glands for all groups were taken and immersed in neutral buffer formalin of 10% for 24h for fixation and the tissue blocks that collected were serially sectioned by using the microtome and placed on a glass slide to be stained with hematoxylin & eosin (H&E) stain, and examined by light microscope.

The immunohistochemical examination via the immune-histochemical expression of Bcl2 which is an essential influential gene during the apoptosis process and controls the normal development through the balance between mitotic activity and apoptosis [16].

The immunostaining Bcl-2 in this study was carried out by using a FLEX Monoclonal Mouse Anti-Human BCL2 Oncoprotein, Clone 124, Ready-to-Use (Link), Code IR614, for use with Dako EnVision FLEX detection system is intended for use in immunohistochemistry together with Autostainer Link instruments. The staining procedure sections of the instructions included with detection system were followed. Positive and negative controls were run simultaneously with biopsy specimen, And was used in this study the scoring system of Immunohistochemical expression of BCL-2 protein in the rat salivary gland tissue for negative cytoplasmic patterns expression =0, mild cytoplasmic patterns expression =1, moderate cytoplasmic patterns expression =2, and strong cytoplasmic patterns expression =≥ 3 (Table 1) [17].

Statistical analysis

Sigmaplot software (Sigmaplot version 14.0; Systal software Inc.) was utilized for statistical analysis. by One-Way ANOVA and the Duncan post hoc test. Mean ± standard error was used for result expression. P value was statistically significant at less than 0.05.

Results

Rats' body weight

The mean body weights (initial and final) showed significant differences between the **CY** group (letter **B**) with the other remaining three groups with letter A which means a significant weight loss, that means than there was a significant weight loss in the (CY) group with other groups , Moreover, there were no significant differences between the CY , Se and CY+Se groups , in spite of the weight gain in the CY and Se groups and the weight loss in the CY+Se group (**Table 1, Histogram 1).**

Histogram 1. The difference between the mean body weight (initial and final) of the rats

B- Histopathological results:

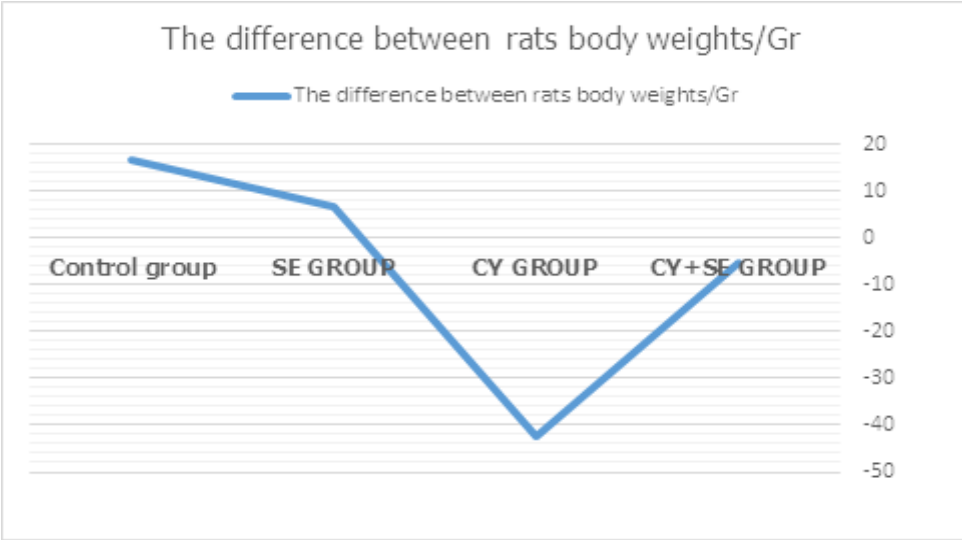
Light microscopic examination, with H&E stain, of rats' submandibular salivary glands sections of group **(C)** showed normal architecture represented by intact mucous acini, granular convoluted tubules, striated ducts and interlobular ducts (Fig. 1). Submandibular salivary glands sections of **(Se)** group also showed normal architecture represented by intact mucous acini, granular convoluted tubules, striated ducts and interlobular ducts **(Fig. 2).** The submandibular salivary glands sections of **(CY)** group revealed several histological changes: These changes include features of necrosis of mucous acini ,striated duct and granular convoluted tubules epithelial cells with edema surrounding striated ducts, also atrophy of mucous acini cells was seen, with vacuolations in serous acini , In addition, there were a decreased number of granular convoluted tubules, an increase in the fibrous tissue surrounding interlobular ducts and ultimately features of apoptosis of serous acini also has been seen (Fig. 3). Whereas the submandibular salivary glands sections of **(CY+Se)** group showed semi-normal appearance of serous acini, mucous acini, and striated duct, a mild degree of degeneration

TABLE 1. The difference between the mean body weight (initial and final) of the rats

	Control group	(Se) group	(CY) group	(CY+Se) group
The difference between rats body weights	16.6 ± 11.9^{A}	6.6 ± 12.4^{A}	-42.4 ± 10.6^{B}	-5.4 ± 15.7^{A}

Data expressed as Mean ± Stander error

The difference letters mean there are significant differences between groups at $p<0.05$



Histogram 1. The difference between the mean body weight (initial and final) of the rats

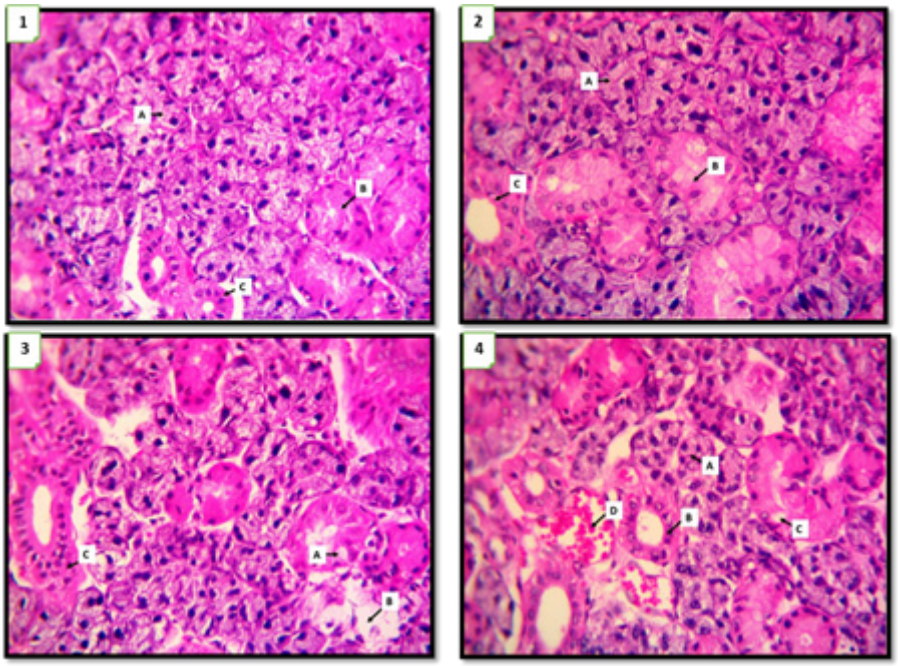


Fig. 1. A photomicrograph of a rat's submandibular salivary gland section of (C) group with normal architecture representing by intact serous acini(arrow),and mucous acini (A), granular convoluted tubules (B) and striated ducts (C). H&E stain, 400X.

Fig. 2. Aphotomicrograph of rat's submandibular salivary gland of (Se) group shows normal architecture representing by intact serous acini(arrow), and mucous acini (A), granular convoluted tubules (B) and striated ducts (C). H&E stain, 400X.

Fig. 3. A photomicrograph of a rat's submandibular salivary gland of (CY) group with degeneration of granular convoluted tubules epithelial cells (A), necrosis and atrophy of mucous acini cells (B) and hyperplasia of striated ducts epithelial cells (C).Features of apoptosis of serous acini(arrows)is noticed H&E stain, 400X.

Fig. 4. A photomicrograph of a rat's submandibular salivary gland of (CY + Se) shows intact serous acini (arrow),and mucous acini (A) and striated duct (B), mild degeneration of granular convoluted tubules epithelial cells (C) and congestion of blood vessels (D). H&E stain, 400X.

of granular convoluted tubules epithelial cells with mild congestion of blood vessels (Fig. 4), also presented with an improvement of cyto architecture in comparison with those of **(CY)** group.

C-The Immunohistochemical (Bcl-2) Results:

Immunohistochemical stain of BCL-2 protein of the lymph node as (control positive) reveals strong cytoplasmic patterns expression (brown color in the lymphocytes) (score-4) (Fig. 5) and Immunohistochemical stain of BCL-2 protein of salivary glands as (control negative) (without antibody) reveals negative expression (without brown color) in the stromal cells between acini (Fig. 6) .

Immunohistochemically stained of Bcl-2 protein of rats' submandibular salivary gland sections of (C) group reveals a moderate to strong cytoplasmic patterns expression (positive brown color immune reaction for Bcl-2 in the stromal cells between acinar cells of the salivary glands (Figures 7,8), and have a scores range from 2 to 3 as showed in (Table 2). The (Se) group reveals moderate to strong cytoplasmic patterns expression (positive brown color immune reaction for BCL-2 in the stromal cells between acinar cells of the salivary glands (Figures 9,10) and have a scores range from 2 to 4 as showed in (Table 2). While the (CY) group reveals negative to mild cytoplasmic patterns expression (negative to weak immune reaction for bcl2) in the stromal cells between acini of the salivary glands (Figures 11,12), and have a scores range from 0 to 1 as showed in (Table 2). whereas (CY+Se) group reveals moderate to strong cytoplasmic patterns expression (positive brown color immune reaction for bcl-2 in the stromal cells between acinar cells of the salivary glands (Figures 13,14), and have ascores range from 2 to 3 as showed in (Table 2). That mean there are no ssignificant differences between the (C) group, (Se) group and the (CY+Se) group, with P- Value greater than 0.05, but there is a significant differences between these three groups with the (CY) group, with P-Value = 0.002 (less than 0.05) as showed in (Table 2, Histogram 2)

The difference letters mean there are significant differences between groups at $p \leq 0.05$

Histogram 2 : Immunohistochemistry Scores of Bcl-2

Discussion

Regarding anticancer drugs, including cyclophosphamide, each one of them has its specialized adverse effects which may interfere with a patient compliance and life [18]. Generally, the targeting of DNA and oxidative stress are the most common causes of the adverse effects of chemotherapy [19]. Cyclophosphamide acts by damaging DNA thereby inhibiting cell division and exerting its cytotoxic action [20]. It is metabolized inside the body by the Cytochromes *P450* system in the liver to produce phosphoramide mustard, acrolein, and other cytotoxic metabolites, Acrolein, which is considered a toxic compound

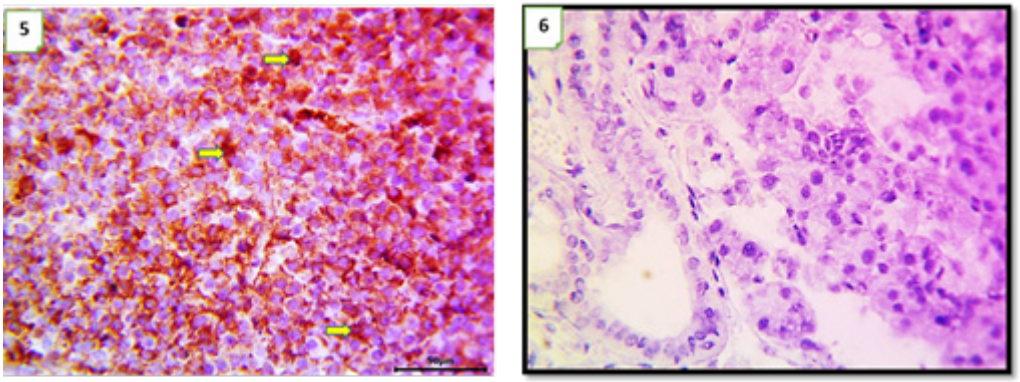


Fig. 5. Immunohistochemical stain of BCL-2 protein reveals strong cytoplasmic patterns expression (brown color in the lymphocytes) of the lymph node as the control positive (score-4); hematoxylin; Scale bar = 50µm.

Fig. 6. Immunohistochemical stain of BCL-2 protein (without antibody) reveals negative expression in the stromal cells between acini (without brown color) of the salivary glands as control negative (score-0); hematoxylin; Scale bar = 50µm.

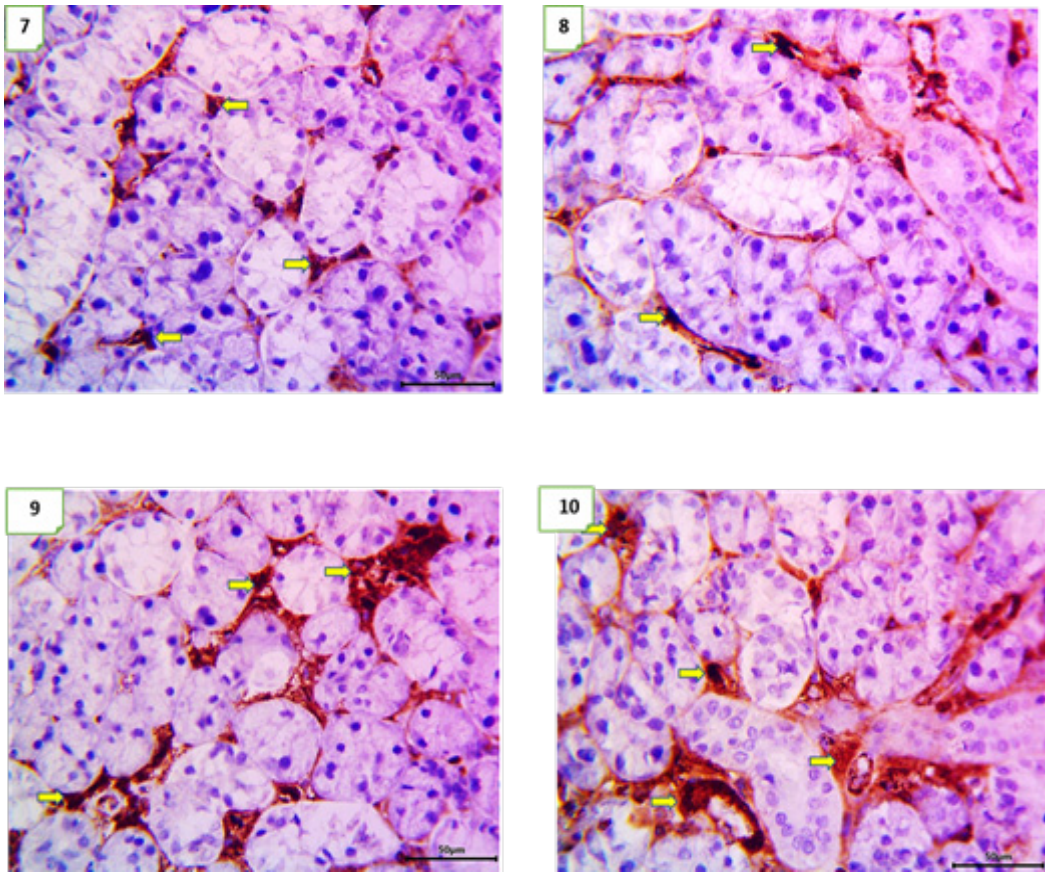


Fig. 7. Immunohistochemical stain of BCL-2 protein reveals strong cytoplasmic patterns expression in the stromal cells between acini (brown color) of the salivary glands of the (C) group (score-3); hematoxylin; Scale bar = 50µm.

Fig. 8. Immunohistochemical stain of BCL2 protein reveals moderate cytoplasmic patterns expression in the stromal cells between acini (brown color) of the salivary glands of the (C) group (score-2); hematoxylin; Scale bar = 50µm.

Fig. 9. Immunohistochemical stain of BCL-2 protein reveals strong cytoplasmic patterns expression in the stromal cells between acini (brown color) of the salivary glands of the (Se) group (score-3); hematoxylin; Scale bar = 50µm.

Fig. 10. Immunohistochemical stain of BCL2 protein reveals strong cytoplasmic patterns expression in the stromal cells between acini (brown color) of the salivary glands of the (Se) group (score-4); hematoxylin; Scale bar = 50µm.

TABLE 2. Immunohistochemical intensity scores of cl-2 expression in samples from the salivary gland of rats' of all groups

Groups	Control group	(Se) group	(CY) group	(CY+Se) group	P- Value (N= 5)
Scores of Bcl-2	2.5 ± 0.28^{A}	2.7 ± 0.47^{A}	0.5 ± 0.28^{B}	2.5 ± 0.28^{A}	0.002

N. Total specimens = 5

Data expressed as Mean ± Stander error

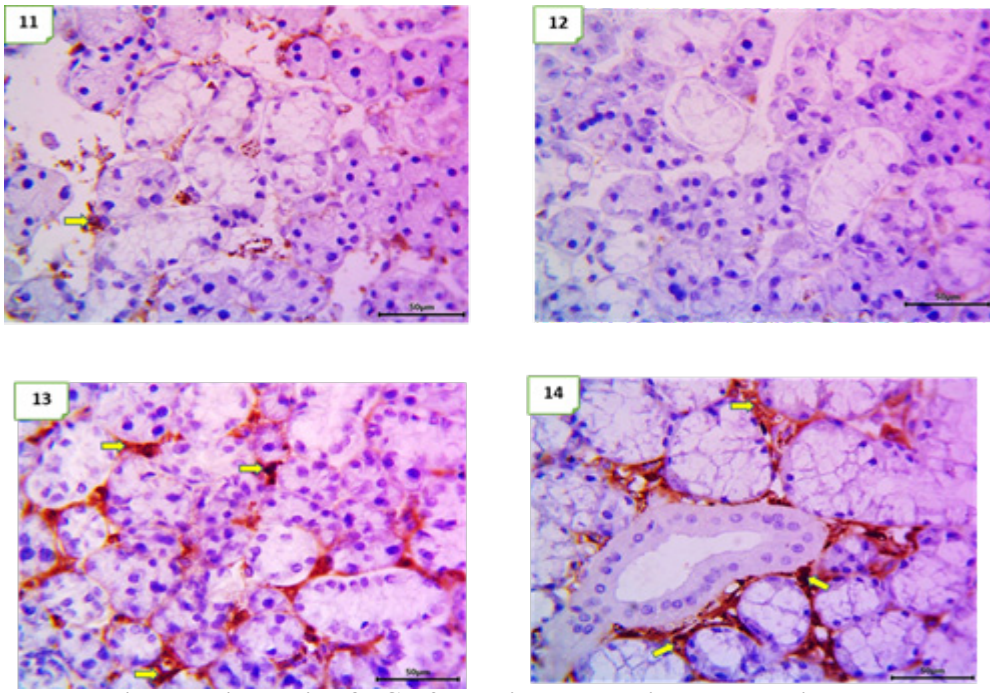
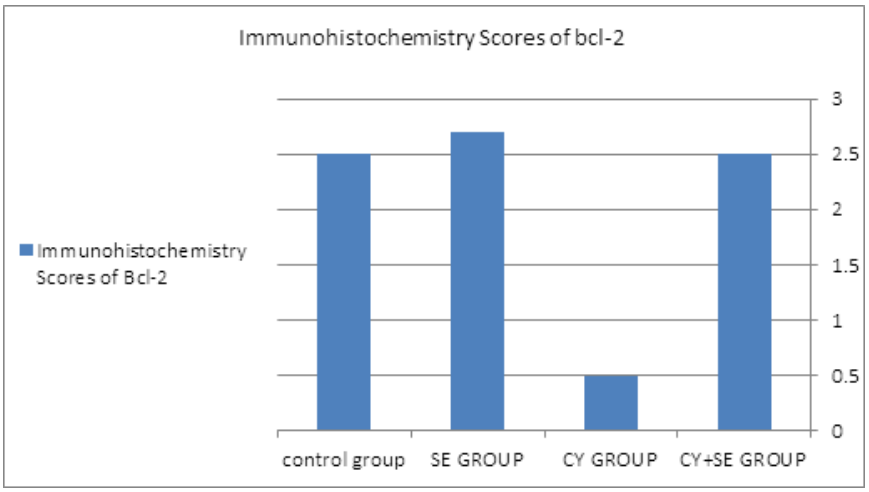


Fig. 11. Immunohistochemical stain of BCL-2 protein reveals mild cytoplasmic patterns expression in the stromal cells between acini (brown color) of the salivary glands of the (CY) group (score-1); hematoxylin; Scale bar = 50µm.

Fig. 12. Immunohistochemical stain of BCL-2 protein reveals negative expression in the stromal cells between acini (without brown color) of the salivary glands of the (CY) group (score-0); hematoxylin; Scale bar = 50µm.

Fig. 13. Immunohistochemical stain of BCL-2 protein reveals strong cytoplasmic patterns expression in the stromal cells between acini (brown color) of the salivary glands of the (CY+Se) group (score-3); hematoxylin; Scale bar = 50µm.

Fig. 14. Immunohistochemical stain of BCL-2 protein reveals strong cytoplasmic patterns expression in the stromal cells between acini (brown color) of the salivary glands of the (CY+Se) group (score-3); hematoxylin; Scale bar = 50µm.



Histogram 2 . Immunohistochemistry Scores of Bcl-2

that increases the rate of apoptosis by causing oxidative stress [21].

Several studies revealed that the co-administration of anti-oxidants including the Selenium beside the chemotherapy is used to curb these toxicities, by reducing the free radicals and ultimately preventing oxidative stress [20]. However, there are contradictory reports on the use of antioxidants besides chemotherapy [22]. This study aims to demonstrate if selenium has an ameliorating effect on rats' submandibular salivary glands toxicity induced by cyclophosphamide.

The chemical agent –selenium as an agent of multivitamins nature and antioxidant agent, it regulates the physiological redox balance and it is incorporated into selenoproteins [23]. It is utilized to decrease the damage of salivary glands after exposure to radiation, and xerostomia by a protective process against oxidative stress damage [24].

The current work demonstrates that there is a remarkable body weight loss in the CY group in comparison with those of other groups. This finding could be explained by many mechanisms that can be the cause of body weight loss after chemotherapy. One of these mechanisms reported by one study suggested that causes of the weight loss are the adverse effects of the chemotherapy such as nausea and vomiting or appetite loss that led to reduction the oral food intake. In addition to another report that records the body weight loss to some degree is due to anorexia [25]. (CY+Se) group shows no remarkable weight loss that demonstrates that there is an ameliorating effect of selenium on weight loss that induced by Cyclophosphamide, results agree with another study in which selenium alleviated the chemotherapeutic adverse effect by modulating effect in circadian clocks, improving the fatigue, nausea, and impaired physical function. As well as, the renal and liver functions also can be improved [26].

On the other hand, light microscopic examination of sections of rats exposed to a systemic dose of cyclophosphamide (CY) group exhibited different structural features in the submandibular salivary gland in comparison with those of (C) group including the degeneration of granular convoluted tubules epithelial cells, necrosis and atrophy of mucous acini cells with edema surrounding it, also vaccuolations and features of apoptosis of serous acini was noticed [27].

The features of vacuolations may be due to swelling of mitochondria and revealed that the accumulation of lipid droplets that lted from cellular dysfunction (unutilized fatty acids). These fat droplets might merge with each other to form a large vacuole, beside the disturbance in cellular metabolism and entering sodium ions that lead to the cellular damage [28]. The other causes of cytoplasmic vacuolations might be due to degeneration of other cell organelles such as the Golgi apparatus which appear as empty spaces [29].

In fact, cyclophosphamide leads to several changes due to its oxidative stress effect that induced cellular damage in the biosystem by lipid peroxidation [21]. Several Authors demonstrated that DNA damage and genetic alterations with apoptosis(programmed cell death) via oxidative action [30].

One of the tissues that are used to clarify the fundamental pharmacological problems induced by the chemotherapy toxicities is the salivary glands as they responded adversely affected by the action of anticancer (cyclophosphamide) which is cause a decrease in the saliva production[31].

The current study revealed that the use of selenium with cyclophosphamide may attenuate the adverse effect of this anticancer agent on the submandibular salivary gland as shown by the histological analysis. Selenium has an ameliorating effect on the action of several chemotherapeutic drugs including Cyclophosphamide by restoring the levels of antioxidant enzymes system, also at the same time improving its antitumor efficacy, the co-administration of selenium with Cisplatin causes a decrease in parameters of host toxicity including (nephrotoxicity, myeloid suppression, and weight loss). In addition, it was reported that selenium has a protective effect against cisplatin-induced nephrotoxicity in mice [32]. selenium has a regenerative effect on the salivary glands of Aging rats Furthermore the antioxidants such as selenium protect the growing follicles in normal ovarian tissue from the toxic effects Induced by chemotherapy the selenium attenuates the cyclophosphamide- pulmonary toxicity induced by cyclophosphamide via improvement of oxidant parameters. The promising role of Selenium was found in different aspects like aging and regenerative field regarding the salivary glands [17], In addition, antioxidant supplementation during chemotherapy also promises higher

therapeutic efficiency and increased survival times in patients [33].

Regarding the immunohistochemical analysis of Bcl-2 protein expression in submandibular salivary glands, this work revealed that sections of rats in (C) group that were exposed to normal saline or in (Se) group exhibited strong cytoplasmic patterns expression in the stromal cells between acini of the salivary glands, while those of rats of (CY) group showed mild or negative cytoplasmic patterns expression in the stromal cells between acini. These findings were in accordance with another study showed the anti-apoptotic effect of selenium by increasing the BCL-2 protein expression [34].

On the other hand, rats that were received the cyclophosphamide and Selenium (CY+Se) group were manifested by moderate to strong cytoplasmic patterns expression in the stromal cells between acini, and there is no significant differences between the (C) group and the (Se) group but there is a significant difference with the (CY) group. The difference in the Bcl2 expression indicated that selenium has anti-apoptotic action. This is a finding in accordance with those researcher mentioned previously, cyclophosphamide has a toxic effect on submandibular salivary tissues. This fact is agreed with the current study results (where the negative immunoreaction of BCL-2) in (CY) group demonstrated stimulation of apoptotic pathway in salivary gland tissue, this could be lead to the fact that cyclophosphamide administration has been related to the increased reactive oxygen species which in turns results in more oxidative stresses and finally promote the apoptosis [35].

In conclusion, this study revealed the presence of structural changes in rats› submandibular salivary glands after exposure to cyclophosphamide. These changes may be attenuated by selenium as shown by the microscopic examination and confirmed by immunohistochemical analysis of BCL-2 protein expression.

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Conflict of interest

There is no conflict of interest with self-funding statement.

Funding statement

Self-funding

References

1. Alieva, M., van Rheenen, J., and Broekman, M. L. Potential impact of invasive surgical procedures on primary tumor growth and metastasis. *Clinical & Experimental Metastasis*, **35**(4), 319-331(2018). https://doi.org/10.1007/s10585-018-9896-8

2. DeVita, V.T. and Chu, E. A history of cancer chemotherapy. *Cancer Research*, **68**(21), 8643-8653(2008). doi.org/10.1158/0008-5472.CAN-07-6611

3. Willershausen, I., Schmidtmann, I., Azaripour, A., Kledtke, J., Willershausen, B. and Hasenburg, A. Association between breast cancer chemotherapy, oral health and chronic dental infections: a pilot study. *Odontology*, **107**(3), 401-408 (2019).

4. Thomsen, M. and Vitetta, L. Adjunctive treatments for the prevention of chemotherapy- and radiotherapy-induced mucositis. *Integrative Cancer Therapies*, **17**(4), 1027-1047 (2018). https://doi.org/10.1177/1534735418794885

5. Roblegg, E., Coughran, A. and Sirjani, D. Saliva: An all-rounder of our body. *European Journal of Pharmaceutics and Biopharmaceutics*, **142**, 133-141(2019).

6. Yousef, D. M., Abd El-Fatah, S. S., Al-Semeh, M. D. and Amira, E. Oxidative Stress Changes Induced by Methotrexate on Parotid Gland Structure of Adult Male Albino Rat: Can Vitamin C Ameliorate These Changes?. *The Medical Journal of Cairo University*, **87**(June), 2555-2565(2019).. DOI: 10.21608/mjcu.2019.54869.

7. Soltanbeigi, A, Dastyar, N., Khajoei Nejad, F. and Shokri, S. Medicinal Herbs Affecting Cancers in Iran and the World. *Plant Biotechnol Persa*., **4** (1): 1-3(2022).

8. Hasan, H. F., Abdel-Hamid, G. R. and Ebrahim, S. I. Antioxidant and Anti-Inflammatory Effects of Diallyl Disulfide on Hepatotoxicity Induced by Cyclophosphamide in Rats. *Natural Product Communications*, **15**(10), 1934578X20969083, (2020). doi.org/10.1177/1934578X20969083

9. Temel, Y., Kucukler, S., Yıldırım, S., Caglayan, C. and Kandemir, F. M. Protective effect of chrysin on cyclophosphamide-induced hepatotoxicity and nephrotoxicity via the inhibition of oxidative stress, inflammation, and apoptosis. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **393**(3), 325-337(2020).

10. Bachmeier, E., López, M. M., Linares, J. A., Brunotto, M. N. and Mazzeo, M. A. 5-Fluorouracil and Cyclophosphamide modify functional activity in submandibular gland of rats. *Journal of Oral Research*, **8**(5), 363-369 (2019). DOI: 10.17126/joralres.2019.056

11. Gulcin, İ. Antioxidants and antioxidant methods: An updated overview. *Archives of Toxicology*, **94**(3), 651-715 (2020). doi.org/10.1007/s10266-019-00411-z

12. Kurutas, E. B. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition Journal*, **15**(1), 1-22 (2015). DOI 10.1186/s12937-016-0186-5

13. Zhu, K., Jiang, L., Chu, Y. and Zhang, Y. Protective effect of selenium against cisplatin-induced nasopharyngeal cancer in male albino rats. Oncology Letters, 12, 5068-5074 (2016). https://doi.org/10.3892/ol.2016.5346

14. Yousif, D. M., Abd EL-Fatah, S. S., Al-Semeh, M. D., and Amira, E. Oxidative Stress Changes Induced by Methotrexate on Parotid Gland Structure of Adult Male Albino Rat: Can Vitamin C Ameliorate These Changes?. The Medical Journal of Cairo University, 87(June), 2555-2565, (2019). DOI: 10.21608/mjcu.2019.54869

15. Nematbakhsh, M. and Nasri, H. The effects of vitamin E and selenium on cisplatin-induced nephrotoxicity in cancer patients treated with cisplatin-based chemotherapy: A randomized, placebo-controlled study. *Journal of Research in Medical Sciences*, **18**(7), 625(2013).

16. Puspitasari, I. M., Abdulah, R., Yamazaki, C., Kameo, S., Nakano, T. and Koyama, H. Updates on clinical studies of selenium supplementation in radiotherapy. Radiation Oncology, **9**(1),1-9 (2014).

17. Abd El-Moneim, O. M., Abd El-Rahim, A. H. and Hafiz, N. A. Evaluation of selenium nanoparticles and doxorubicin effect against hepatocellular carcinoma rat model cytogenetic toxicity and DNA damage. *Toxicology Reports*, **5**,771–776(2018). https://doi.org/10.1016/j.toxrep.2018.07.003

18. Elsaied, H.A. Histological and Immunohistochemical Study of Selenium Regenerative Effect on Submandibular and Sublingual Glands of Aging Rats. *Egyptian Dental Journal*, **65**, 3413-3426(2019).

19. Fazeli-Nasab, B. and Bidarnamani, F. Medicinal Plants and Herbal Compounds: Cancer Prevention and Treatment. Plant *Biotechnol Persa*., **4** (1), 25-36(2022).

20. Punnoose, E., Peale, F. V., Szafer-Glusman, E., Lei, G., Bourgon, R., Do, A. D. and Vitolo, U. Bcl2 expression in first-line diffuse large B-cell lymphoma identifies a patient population with poor prognosis. *Clinical Lymphoma Myeloma and Leukemia*, **21**(4), 267-278(2021). https://doi.org/10.1016/j.clml.2020.11.004

21. Singh, K., Bhori, M., Kasu, Y. A., Bhat, G. and Marar, T. Antioxidants as precision weapons in war against cancer chemotherapy induced toxicity–Exploring the armoury of obscurity. *Saudi Pharmaceutical Journal*, **26**(2), 177-190(2018). https://doi.org/10.1016/j.jsps.2017.12.013

22. Yahyazadeh, A. Effect of curcumin on rat sublingual gland exposed to cyclophosphamide. *Cukurova Medical Journal*, **46**(3), 897-903(2021). DOI: 10.17826/cumj.895357

23. Lehmann, F. and Wennerberg, J. Evolution of nitrogen-based alkylating anticancer agents. *Processes*, **9**(2), 377 (2021).

24. Afkhami-Ardakani, M., Hasanzadeh, S., Shahrooz, R., Delirezh, N. and Malekinejad, H. *Spirulina platensis* (*Arthrospira platensis*) attenuates Cyclophosphamide-induced reproductive toxicity in male Wistar rats: Evidence for sperm apoptosis and p53/Bcl-2 expression. *Journal of Food Biochemistry*, **45**(8), e13854 (2021).

25. Yousif, D. M., ABD EL-Fatah, S. S., Al-Semeh, M. D. and Amira, E. Oxidative Stress Changes Induced by Methotrexate on Parotid Gland Structure of Adult Male Albino Rat: Can Vitamin C Ameliorate These Changes?. *The Medical Journal of Cairo University*, **87**(June), 2555-2565 (2019). DOI: 10.21608/mjcu.2019.54869

26. Chakraborty, P., Sk, U. H. and Bhattacharya, S. Chemoprotection and enhancement of cancer chemotherapeutic efficacy of cyclophosphamide in mice bearing Ehrlich ascites carcinoma by diphenylmethyl selenocyanate. *Cancer Chemotherapy and Pharmacology*, **64**(5), 971-980 (2009).

27. Ohkawa, K., Tsukada, Y., Dohzono, H., Koike, K. and Terashima, Y. The effects of co-administration of selenium and cis-platin (CDDP) on CDDP-induced toxicity and antitumour activity. *British Journal of Cancer*, **58**(1), 38-41(1988).

28. Chakraborty, P., Roy, S. S., Sk, U. H. and Bhattacharya, S. Amelioration of cisplatin-induced nephrotoxicity in mice by oral administration of diphenylmethyl selenocyanate. *Free Radical Research*, **45**(2), 177-187(2011).

29. Elsaied, H. A. Histological and Immunohistochemical Study of Selenium Regenerative Effect on Submandibular and Sublingual Glands of Aging Rats. *Egyptian Dental Journal*, **65**, 3413-3426(2019).

30. Gürgen, S. G., Erdoğan, D., Elmas, Ç., Kaplanoğlu, G. T. and Özer, Ç. Chemoprotective effect of ascorbic acid, α-tocopherol, and selenium on cyclophosphamide-induced toxicity in the rat ovarium. *Nutrition*, **29**(5), 777-784(2013).

31. Ghosh, P., Bhattacharjee, A., Basu, A., Singha Roy, S. and Bhattacharya, S. Attenuation of cyclophosphamide-induced pulmonary toxicity in Swiss albino mice by naphthalimide-based organoselenium compound 2-(5-selenocyanatopentyl)-benzo [de] isoquinoline 1, 3-dione. *Pharmaceutical Biology*, **53**(4), 524-532(2015).

32. Kuršvietienė, L., Mongirdienė, A., Bernatonienė, J., Šulinskienė, J. and Stanevičienė, I. Selenium anticancer properties and impact on cellular redox status. *Antioxidants*, **9**(1), 80, (2020).

33. Al-Refai, A. S., Khaleel, A. K. and Ali, S. The effect of green tea extract on submandibular salivary gland of methotrexate treated albino rats: immunohistochemical study. *Journal of Cytology & Histology*, **5**(2), 212 pages 1-6 2014)). DOI: 10.4172/2157-7099.1000212

34. Mahmoud E. and Mahmoud M: Effect of pomegranate peel extract on submandibular salivary glands of streptozotocin induced diabetes in rats: histological, immunohisto-chemical and ultrastructural study. *Adva. Biol. Biotech. J.,* **13**, 1-15(2017).

35. Horvat, R., Weninger, W. and Ulrich, W.: Expression of bcl-2 in Salivary Glands and Salivary Gland Adenomas: a contribution to the reserve cell theory. *Pathol. Res. Prac. J.*, **191**(1), 35-41 (1995). doi: 10.1016/S0344-0338(11)80920-9.

36. 35. Vardi, N., Parlakpinar, H., Ates, B., Cetin, A. and Otlu, A: Antiapoptotic and antioxidant effects of beta-carotene against methotrexate-induced testicular injury. *Fertil. Steril. J.*, **92**, 2028–2033(2009).

دور السيلينيوم في التخفيف من التأثير الضار للسيكلوفوسفاميد على الغدد اللعابية تحت الفكية في الجرذان

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تهدف هذه الدراسة إلى تحديد التأثير المحسن للسيلينيوم ضد تأثير سيكلوفوسفاميد على أنسجة الغدد اللعابية تحت الفكية السفلية للجرذان البيضاء. قسمت ٢٤ من ذكور الجرذان إلى ٤ مجاميع ٦ جرذان لكل مجموعة: جرعت المجموعة الاولى محلول ملحي فسلجي عن طريق الفم ، المجموعة الثانية جرعت بالسيلينيوم (٢٠٠ ميكروغرام لكل كيلوغرام) فمويا لمدة ١٤ يوما ، المجموعة الثالثة حقنت مرة واحدة بالسيكلوفوسفاميد (١٥٠ مجم لكل كجم) داخل الصفاق (i.p) في اليوم الثامن بينما تلقت المجموعة الرابعة السيلينيوم (٢٠٠ ميكروجرام \ كجم) و سيكلوفوسفاميد (١٥٠ ملغم\ كغم). في اليوم ١٥ ، تم تخدير جميع الفئران والتضحية بها ، وتم جمع الغدد اللعابية تحت الفك السفلي, أظهرت المجموعة الثالثة انخفاضًا ملحوظًا في الوزن مقارنة بالمجاميع الأخرى. أظهر الفحص النسجي المرضي في هذه المجموعة وجود نخر أسيني مخاطي مع وذمة تحيط بالقنوات المخططة ، كما لوحظ ضمور في خلايا أسيني المخاطية ، مع وجود فجوات في أسيني المصلي. بالإضافة إلى ذلك ، كان هناك انخفاض في عدد الأنابيب الملتوية الحبيبية ، كما لوحظت زيادة في الأنسجة الليفية المحيطة بالقنوات بين الفصوص، لوحظت ملامح موت الخلايا المبرمج للأسيني المصلي. أظهرت النتائج الهيستوكيميائية المناعية في هذه المجموعة تعبيرًا خفيفًا إلى سلبي (بروتين Bcl-2 المضاد للاستماتة) في الخلايا اللحمية بين خلايا أسينار في الغدد اللعابية ، مقارنة بالمجاميع الأخرى التي أظهرت تغبيرا معتدل إلى شديد. خلصت الدراسة وجود تغييرات هيكلية في الغدد اللعابية تحت الفك السفلي للجرذان بعد التعرض للسيكلوفوسفاميد وقد خفف السيلينيوم هذه التأثيرات الضارة كما يتضح من الفحص النسجي المرضي وما يؤكده التحليل الكيميائي المناعي لتعبير Bcl2.

الكلمات المفتاحية: السيلينيوم ، سيكلوفوسفاميد، الغدد اللعابية تحت الفكية، الجرذان.