Glycyrrhiza Glabra Root Extract Alleviates Cyclophosphamide Induced Mucositis of the Tongue in Adult Male Albino Rats

Original Article

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ABSTRACT

Introduction: Cyclophosphamide (CPA) is an anti-cancer medication, utilized in chemotherapy. Recently, inflammation of oral mucosa (mucositis) is thought to be one of the most serious side effects of anticancer therapy.

Aim of the Work: To investigate the role of glycyrrhiza glabra (Licorice) root extract on rat tongue mucositis induced by CPA.

Materials and Methods: Forty adult male albino rats were classified into four groups, (each included 10 rats): Group I (Control group), Group II (Licorice group): each rat received licorice root extract by an oral daily dose of 200 mg/kg/day for 10 days, Group III (CPA group): each rat received CPA in a single intraperitoneal dose of 300 mg/kg on the second day of experiment and Group IV (Licorice CPA group): each rat received licorice root extract by an oral daily dose of 200 mg/kg/day for ten days, on the second day of experiment, the rats received CPA in a single intraperitoneal dose of 300 mg/kg. At the end of the experiment, the rats were anesthetized, and the tongue was excised for histological, immunohistochemical and ultrastructural analysis. Morphometric and statistical analysis were also done.

Results: Licorice root extract reduced the severity of CPA induced oral mucositis by increasing the epithelial thickness, normalization of congested blood vessels in the lamina propria of tongue mucosa, significant amelioration of immunoreactivity to anti-Ki-67, anti-E-cadherin and anti-P53.

Conclusion: CPA chemotherapy has a detrimental effect on the oral mucosa resulting in marked morphometric and microscopic changes. Glycyrrhiza glabra extract can protect the oral mucosa from CPA-induced toxicity and reduce the associated injury.

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Key Words: Cyclophosphamide, glycyrrhiza glabra root extract, mucositis, rat.

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INTRODUCTION

Cyclophosphamide (CPA) is a chemotherapeutic drug used to treat cancers such as multiple myeloma, sarcoma, and breast cancer. CPA is a nitrogen mustard with anti-neoplastic properties due to alkylation[1]. CPA is activated by hepatic cytochrome P450 enzymes resulting in the formation of its two metabolites: phosphoramide mustard and acrolein. These metabolites impede the tissue antioxidant defense system, resulting in several reactive oxygen species (ROS) that attack protein amino acids leading to structural and functional cellular changes[2]. In addition, CPA treatment targets cellular DNA resulting in DNA fragmentation and cell death[3]. Furthermore, CPA treatment disrupts humoral and cell-mediated immunity by acting on B and T lymphocytes, respectively[4].

Mucositis is the inflammation of the mucous membrane that covers the alimentary tract. Once it affects the mucous membrane of the oral and oropharyngeal regions, it is known as oral mucositis[5]. Mucositis is a common side effect of cancer treatment, especially CPA, which has numerous systemic consequences[6,7].

Glycyrrhiza glabra (Licorice) is a traditional medicinal herb, grown widely throughout the world, and known for its bioactive properties, which include antioxidant, antifungal, anti-ulcer, anti-inflammatory, anticancer and antiviral[8]. The obvious mucosal protective effect of Licorice is due to its saponin content[9].

Glycyrrhiza glabra is used widely for its antioxidant activity. This powerful antioxidant activity is probably due to the phenolic content[10]. Different authors described that glycyrrhizin is primarily responsible for the anti-inflammatory action of licorice. In *vitro*, it inhibits factors responsible for inflammation and promotes healing of stomach and mouth ulcers[11].

Glycyrrhiza glabra extract is widely used as an anti-ulcerative. In the gastrointestinal system, it is used for ulcers of the stomach and duodenum while as a treatment for spasmodic pains caused by chronic gastritis it is used as an adjuvant[12]. So, this work aimed to evaluate the role of licorice root extract on the tongue mucositis induced experimentally by CPA.

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MATERIALS AND METHODS

Animals

Forty adult male Sprague-Dawley rats with an average weight of 180-220 g were acclimated for one week in stainless steel cages before initiating the experiments under standard laboratory conditions of illumination with a 12 h light/dark cycles at (20 ± 5°C) and allowed free access to food and tap water. They were bought from the Egyptian Organization for Biological Products and Vaccines (Cairo, Egypt). All experimental procedures and animal maintenance were conducted according to the roles and the guidelines of the Research Ethics Committee, Menoufia University, Faculty of Medicine with the ethical approval number (5/2021 ANAT 1).

Chemicals

- Anhydrous cyclophosphamide one-gram vial with trade name; Endoxan from Baxter Company. The powder was dissolved in distilled water in a ratio of (1: 10; one gram of cyclophosphamide powder in 10 ml distilled water for preparation of reconstituted solution).
- Glycyrrhiza glabra root extract was provided from Nature's Answer Company with trade name; licorice in the form of aqueous solution ready to be used. The number of servings per container are 15; the serving size is 2 ml. The amount of Glycyrrhiza glabra root extract per serving is 2 g.

Experimental design

Animals were weighed and randomly divided into four groups, (each included 10 rats):

Group I (Control group): each rat received daily intraperitoneal injection of distilled water.

Group II (Licorice group): each rat received licorice root extract by an oral daily dose of 200 mg/kg/day [9] for 10 days[13].

Group III (CPA group): each rat received CPA in a single intraperitoneal dose of 300 mg/kg on the second day of experiment[14].

Group IV (Licorice CPA group): each rat received licorice root extract by an oral daily dose of 200 mg/kg/day for ten days. On the second day of experiment, each rat received CPA a single intraperitoneal dose of 300 mg/kg.

At the end of the experiment, body weight for each rat was recorded. The rats were anesthetized by inhalation of overdose of pentobarbital (200 mg/kg) and then sacrificed, then immediately the tongue was excised and washed with saline.

Measurement of Body Weight

In order to assess weight gain or loss, initial and final body weights of each experimental rat were recorded using a digital weighing balance (Hanson, China).

Histopathological study

For the preparation of the paraffin blocks, tissue samples from the tongue were fixed in 10% neutral buffered formaldehyde, prepared fresh and changed every day, for a period of at least 24 hours. Dehydrated through a graded alcohol series, cleared in xylene, then embedded in paraffin wax. Serial sections of 5µm were deparaffinized, hydrated, and stained with hematoxylin and eosin (H&E), for routine histological assessment[15] and Masson trichrome, for detection of collagen deposition[16].

Immunohistochemical study

Paraffin sections on poly-L-lysin coated slides were deparaffinized and rehydrated. Endogenous peroxidase was blocked by inserting the sections in 3% hydrogen peroxide (H_2O_2). The microwave antigen retrieval procedure was performed. The sections were incubated with primary anti-E-cadherin rabbit polyclonal antibodies, intercellular marker, (abcam 15148), anti-Ki-67 rabbit polyclonal antibodies, proliferative marker, (abcam 833) and anti-P53 rabbit polyclonal antibodies, apoptotic marker, (abcam 13142). Then, biotinylated goat-polyvalent secondary antibody was applied. The sections were then incubated in preformed streptavidin peroxidase, and finally the prepared DAB substrate chromogen (3,3′-diaminobenzidine tetrahydrochloride) was applied and the slides were counterstained with hematoxylin[17].

Morphometric study: Ten non-overlapping fields (x 40) for every specimen from five different rats/experimental group were examined at Anatomy Department, Faculty of Medicine, Menoufia University. All measurements were carried out by the same investigator to avoid interobserver errors. All morphometric measurements were performed by using ImageJ 1.47 v software (National Institutes of Health, USA)[18], for quantification of the followings:

1. The parameters of H & E-stained filiform lingual papillae (The length of filiform papillae of the tongue was measured from the tip to the base of the papillae, the width was measured at its base)[18].
2. The percentage of Masson's-stained surface area.
3. The percentage of E-cadherin-stained surface area.
4. The number of Ki-stained cells.
5. The percentage of P53-stained surface area.

Scanning electron microscopic study

The specimens were taken from the rats' dorsal surface of tongue mucosa and fixed in 2.5% gluteraldhyde in 0.1M phosphate buffer (pH 7.4). The samples were treated with 8N hydrochloric acid at 60° for 30 minutes removing mucus from the surface of the tongue[19]. The specimens were photographed with scanning electron microscope (JSM- 6510 LV) (JEOL, Japan) at the electron microscope unit in the faculty of Agriculture, EL-Mansoura University, El-Mansoura, Egypt.

Statistical analysis

The collected data of the rat weight and the morphometric parameters were recorded and expressed as mean ± standard deviation of mean (SD) and analyzed using SPSS software version 20 (SPSS, Inc., Chicago, IL, USA). Differences among the study groups were detected by using Mann Whitney U test. The results were considered statistically significant with p *value* < 0.05. (Graph Pad Software, San Diego, California, USA)[20].

RESULTS

Body weight

Figure 1 showed the body weight changes among the studied groups. There was a statistically significant increase in the final body weight in control group when compared to their initial one (p <0.05). There was a statistically significant decrease in the final body weight in CPA group when compared to their initial one (p <0.05). On the other hand, Licorice CPA group showed non-significant decrease in their final body weight (p >0.05) when compared to their initial one.

Histopathological findings

All sections of both control and licorice groups showed nearly similar histological picture throughout all the examined parameters with no statistically significant differences between them.

H&E stain

Sections of control group stained with H&E revealed sharp conical projections of filiform papillae covered by keratinized epithelium. The underlaying lamina propria was formed of connective tissue. The skeletal muscle fibers appeared running in different directions (Figure 2a). On the other hand, sections of CPA group revealed thin keratin layer with short, blunted ends filiform papillae. Dilated congested blood vessel was observed in lamina propria. The lingual muscle fibers appeared running in different directions with accumulation of adipocytes (Figure 2b). Compared with the CPA group, H&E sections of licorice CPA group revealed marked improvement; most filiform papillae appeared normal, flattening of the dorsal surface with a focal area of atrophied filiform papillae were noted. Normal appearance of muscles in different directions were clearly demonstrated (Figure 2c). There was a statistically significant decrease in the mean length and width of the filiform papillae in CPA group compared to control group (p <0.05), While there is a statistically significant increase in licorice CPA group compared to CPA group (p <0.05) (Figure 2d).

Control group revealed regular orientation of the filiform papillae. The lamina propria contained well prominent blood vessels. The skeletal muscle fibers appeared running in different directions (Figure 3a). CPA group revealed atrophied short filiform papillae, very thin filiform papillae and separated keratin layer. Loosely disorganized connective tissue lamina propria was seen (Figure 3b). Thin separated keratin layer and an area that has lost its normal covering epithelium were seen. Muscle fibers appeared atrophied with increase spacing between them (Figure 3c). Licorice CPA group revealed normal orientation of filiform papillae. The lamina propria was apparently normal as normal blood vessel were seen (arrowhead). However, few dilated and congested vessels are encountered (yellow arrow). Some atrophied muscles fibers were still noted (Figure 3d).

Masson trichrome stain

In control group, the connective tissue fibers revealed normal distribution with strong positive staining reaction to Masson trichrome stain in the lamina propria and thin rims in-between muscle fibers (Figure 4a). On contrast, the underlying lamina propria of CPA group showed dissociation of connective tissue fibers that revealed weakly positive staining reactivity to Masson trichrome stain. On the other side, thick bands of connective tissue appeared in-between the muscle fibers (Figure 4b). In Licorice CPA group, the connective tissue fibers restore their normal distribution with strongly positive staining within the lamina propria and thin rims of connective tissue fibers in-between the muscle fibers (Figure 4c). There was a statistically significant increase (p <0.05) in the percentage of stained surface area of Masson trichrome stain in CPA group compared to control group, while there is a statistically significant decrease in Licorice CPA group compared to CPA group (p <0.05) (Figure 4d).

Immunohistochemical findings

Control group showed strong cytoplasmic E-cadherin immunoreaction in almost all layers of the epithelium (Figure 5a), while CPA group showed mild immunoreaction (Figure 5b). In licorice CPA group, there was moderate immunoreaction (Figure 5c). There was a statistically significant decrease (p <0.05) in the percentage of stained surface area of E-cadherin in CPA group than control one, while there was a statistically significant increase in licorice CPA group compared to CPA group (p<0.05) (Figure 5d); Regarding Ki-67 immunoreaction, control group showed strong nuclear Ki-67 immunoreaction in the basal layer of the epithelium (Figure 6a), while CPA group showed negative nuclear Ki-67 immunoreaction (Figure 6b). Licorice CPA group showed mild nuclear Ki-67 immunoreaction in the basal layer of the epithelium (Figure 6c). There was a statistically significant decrease (p<0.05) in the number of Ki-stained nuclei in CPA group than control group, while there is a statistically significant increase in Licorice CPA group compared to CPA group (p <0.05) (Figure 6d). Regarding p53 immunoreaction, control group showed negative nuclear P53 immunoreaction in the basal layer of the epithelium (Figure 7a), CPA group showed strong P53 immunoreaction in basal nuclei (Figure 7b). In licorice CPA group there was mild P53 immunoreaction in the basal layer of the epithelium (Figure 7c). There was a statistically significant increase (p<0.05) in the percentage of stained surface area

of p53 in CPA group compared to control group, while there was a statistically significant decrease in licorice CPA group compared to CPA group (p<0.05) (Figure 7d).

Scanning electron microscope result

The dorsal surface of the anterior part and the body of control tongue showed the midline groove dividing it into two halves. Less numerous fungiform papillae scattered between numerous filiform papillae which appeared also on the root of it (Figures 8a,b). The circumvallate papillae appeared to be marginated by a normal wide groove and the foliate papillae appeared on the margins of the root (Figure 8c). The dorsal surface of the anterior part and the body of the tongue of CPA group showed distorted filiform and fungiform papillae together with collapsed circumvallate papillae (Figures 8d,e,f). The dorsal surface of the anterior part and body of the tongue of Licorice CPA group showed preservation of normal midline groove, fungiform, foliate, and filiform papillae (Figures 8g,h,i).

The filiform papillae of control tongue are tall conical in shape having 2 pointed processes and the fungiform papillae contain taste pores of taste buds. Caudally, both circumvallate and foliate papillae can be seen (Figures 9a,b,c). The lingual papillae of the tongue of CPA group including filiform papillae, fungiform papillae and circumvallate papillae appeared to be distorted, desquamated with loss of their characteristic features (Figures 9d,e,f). The normal fungiform papillae of the tongue of Licorice CPA group preserved the taste pores of taste buds and the filiform papillae also preserved their normal characteristics. The circumvallate papillae are surrounded by narrow groove (Figures 9g,h,i).

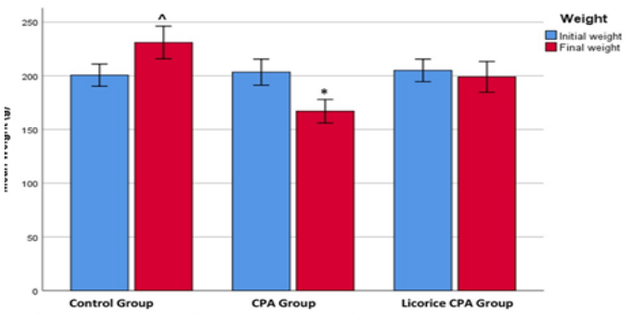

Fig. 1: Mean initial and final body weight of all rats (gm). Significant increase (^ p) from the initial body weight in control group, significant decrease (*p) from the initial body weight in CPA group. (Foot note (^ p &*p <0.05).

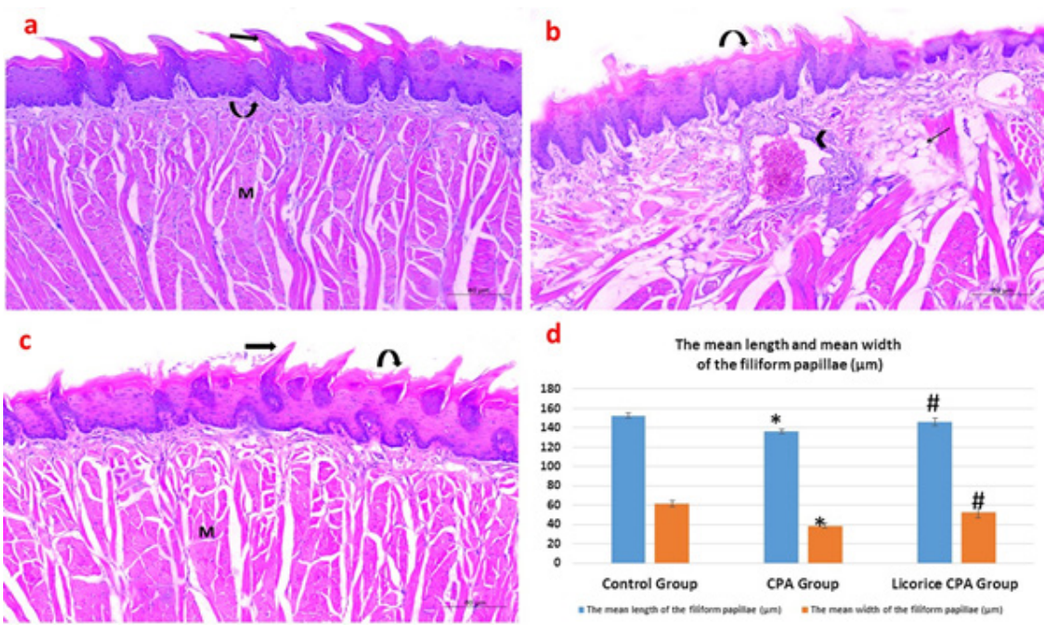

Fig. 2: Photomicrographs of dorsal surface of tongue sections in all experimental groups; a. Control group: sharp conical projections of filiform papillae are covered by keratinized epithelium (arrow). The underlaying lamina propria is formed of connective tissue (curved arrow). The skeletal muscle fibers are clearly noted (M). b. CPA group: thin keratin layer with short, blunted ends filiform papillae (curved arrow). Dilated congested blood vessel is observed in lamina propria (arrowhead). Accumulation of adipocytes is clearly evident (thin arrow). c. Licorice CPA group: presence of normal filiform papillae (arrow), flattening of the dorsal surface with a focal area of atrophied filiform papillae are noted (curved arrow). Normal appearance of muscles in different directions is clearly demonstrated (M) (H&E, X100). d. Comparison between groups as regards the mean length and width of the filiform papillae (µm): There is a statistically significant decrease (*p) in the mean length and width of the filiform papillae in CPA group compared to control group, while there is a statistically significant increase in licorice CPA group compared to CPA group (#p). (Foot note *p & #p <0.05).

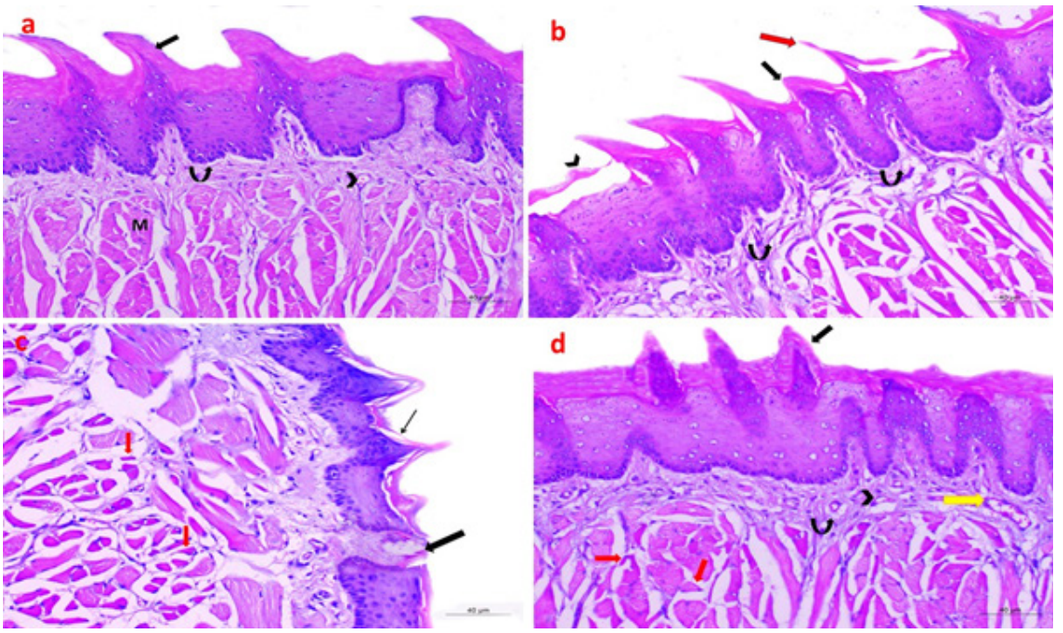

Fig. 3: Photomicrographs of dorsal surface of tongue sections in all experimental groups; a. Control group: regular orientation of the filiform papillae which are covered by keratinized epithelium (arrow). The lamina propria is formed of connective tissue (curved arrow) and contains well prominent blood vessels (arrowhead). The skeletal muscle fibers appear running in different directions (M). b. CPA group: atrophied short filiform papillae (black arrow), very thin filiform papillae (red arrow) are seen. Degenerated and separated keratin layer (arrow head) is observed. Loosely disorganized connective tissue lamina propria is seen (curved arrows). c. CPA group: thin separated keratin layer (thin arrow) and an area that has lost its normal covering epithelium (thick arrow). Muscle fibers appear atrophied with increase spacing between them (red arrows). d. Licorice CPA group: normal orientation of filiform papillae (black arrow). The lamina propria is apparently normal as normal blood vessel are seen (arrowhead). However, few dilated and congested vessels are encountered (yellow arrow). Some atrophied muscles fibers are still noted (red arrows) (H&E, X200).

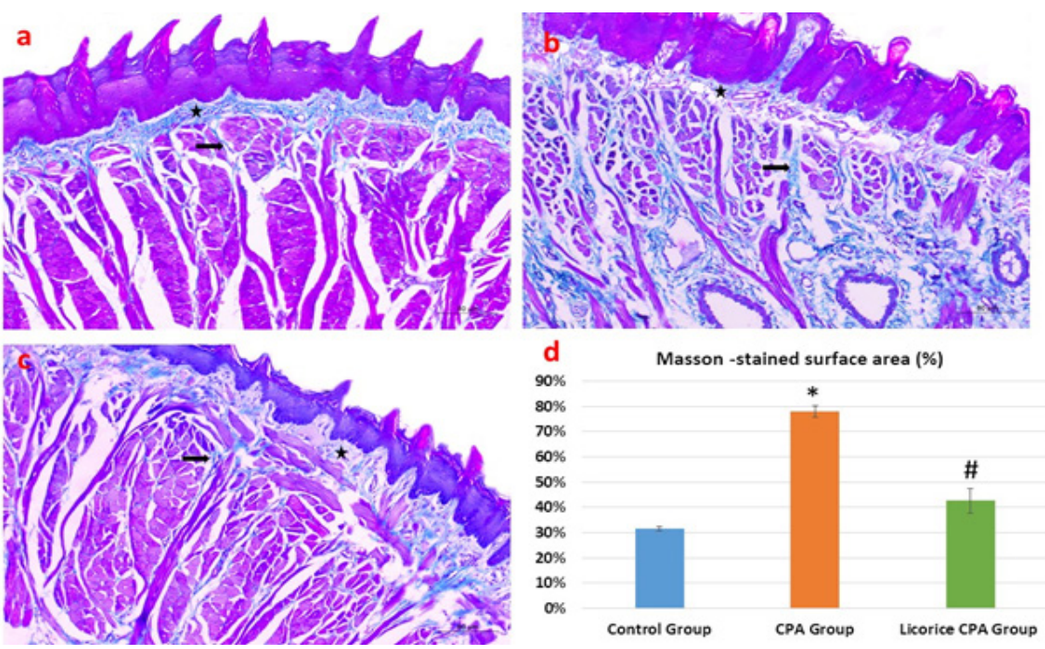

Fig. 4: Photomicrographs of the dorsal surface of the tongue from all examined groups; a. control group: normal distribution of thick connective tissue fibers with strong staining affinity to Masson trichrome stain mainly in the lamina propria (star) and thin rims of connective tissue fibers in between the muscle fibers (arrow). b. CPA group: degeneration of lamina propria with dissociation of connective tissue fibers that revealed weakly positive staining affinity (star) with thick bands of connective tissue fibers appear in-between the muscle fibers (arrow). c. Licorice CPA group: positive staining affinity to Masson trichrome in the lamina propria (star) and thin rims of connective tissue fibers in between the muscle fibers (arrow) (Masson trichrome stain, X100). d. Comparison between groups as regards the percentage of stained surface area of Masson trichrome stain: There is a statistically significant increase (*p) in the percentage of stained surface area of Masson trichrome stain in CPA group compared to control group, while there is a statistically significant decrease in licorice CPA group compared to CPA group (#p). (Foot note *p & #p <0.05).

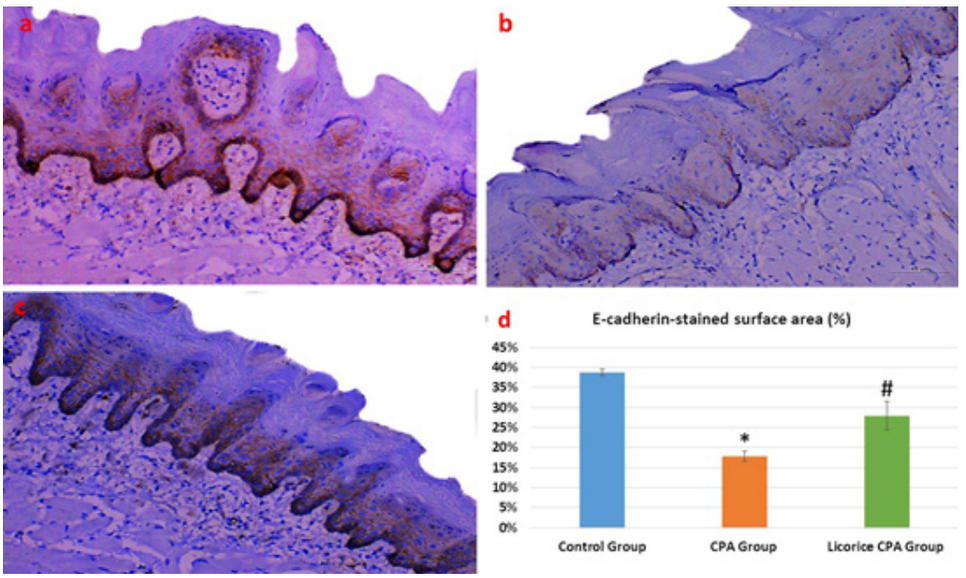

Fig. 5: Photomicrographs of E-cadherin immune-stained tongue sections of all groups: a.; Control group: strong cytoplasmic E-cadherin immunoreaction in almost all layers of the epithelium. b. CPA group: weak immunoreaction c. Licorice CPA group: There is moderate cytoplasmic E-cadherin immunoreaction as compared with CPA group (anti E- cadherin, X200). d. Comparison between groups as regards the percentage of stained surface area of E-cadherin: There is a statistically significant decrease (*p) in the percentage of stained surface area of E-cadherin in CPA group than control group, while there is a statistically significant increase in licorice CPA group compared to CPA group (#p). (Foot note *p & #p <0.05).

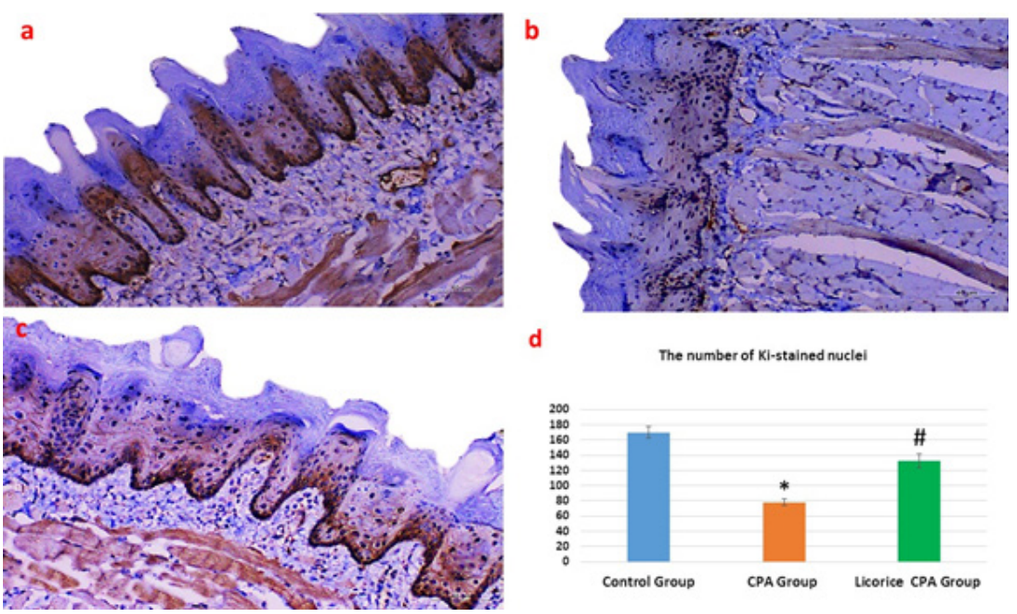

Fig. 6: Photomicrographs of Ki-67 immuno-stained tongue sections of all groups: a. Control group: strong nuclear Ki-67 immunoreaction in the basal layer of the epithelium. b. CPA group: negative nuclear Ki-67 immunoreaction in the basal layer of the epithelium. c. Licorice CPA group: mild nuclear Ki-67 immunoreaction in the basal layer of the epithelium (Ki-67, X200). d. Comparison between groups as regards the number of Ki-stained nuclei: There is a statistically significant decrease (*p) in the number of Ki-stained nuclei in CPA group than control group, while there is a statistically significant increase in licorice CPA group compared to CPA group (#p) (Foot note *p & #p <0.05).

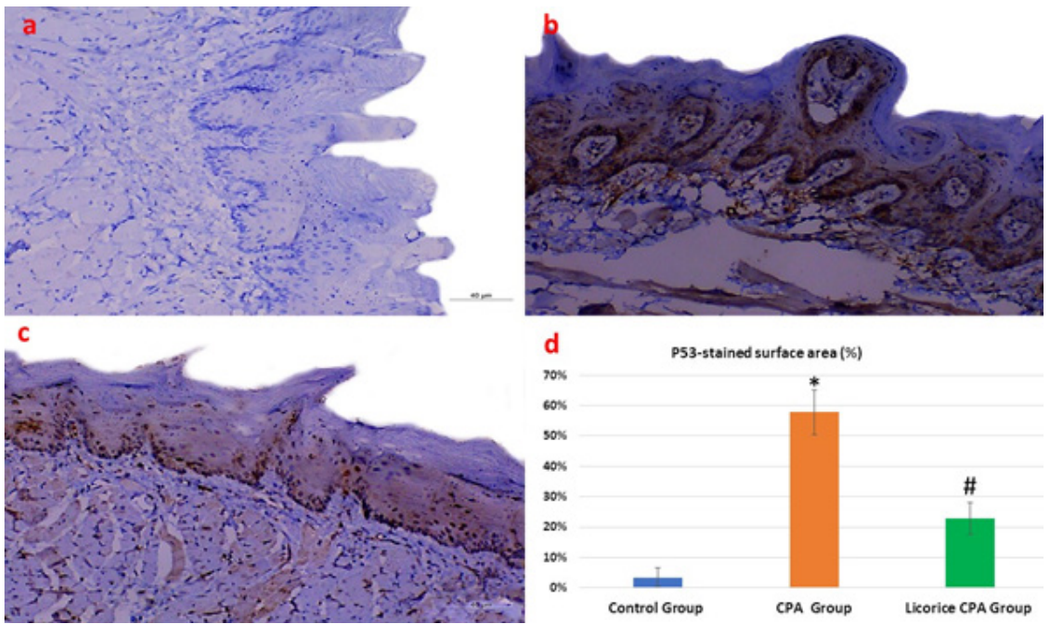

Fig. 7: Photomicrographs of P53 immunostained tongue sections of all groups: a. Control group: negative nuclear P53 immunoreaction in the basal cell layer of the epithelium. b. CPA group: strong P53 immunoreaction in basal cell nuclei. c. Licorice CPA group: mild P53 immunoreaction in the basal cell layer of the epithelium, compared with CPA group (p53, X200). d. Comparison between groups as regards the percentage of stained surface area of p53: There is a statistically significant increase (*p) in the percentage of stained surface area of p53 in CPA group compared to control group, While there is a statistically significant decrease in licorice CPA group compared to CPA group (#p). (Foot note *p & #p<0.05).

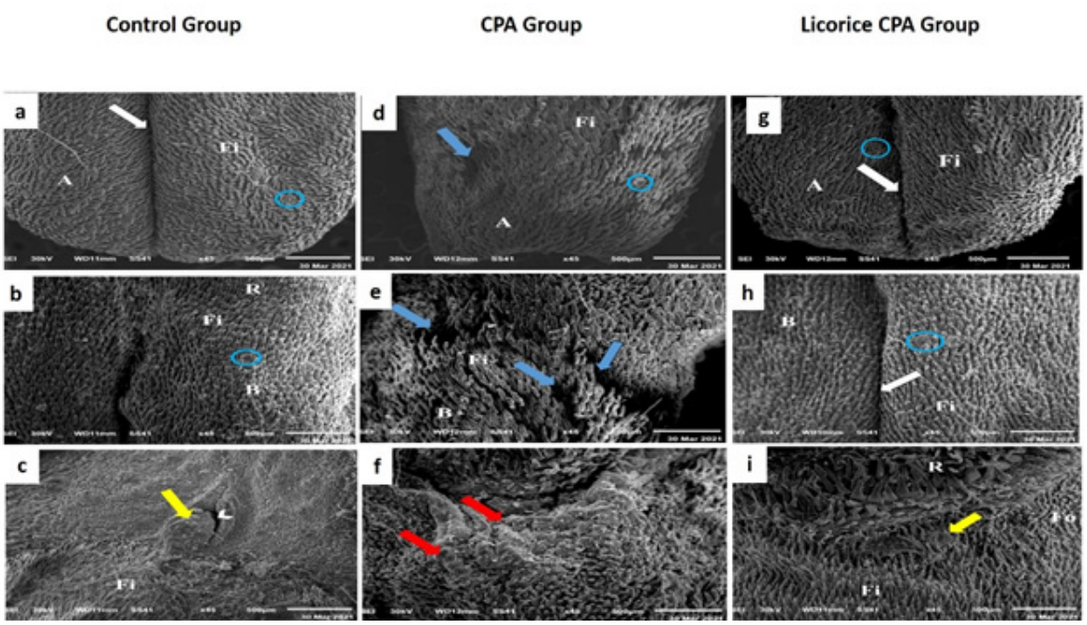

Fig. 8: Scanning electron micrographs of the rat tongue of experimental groups (SEM, X45); Control group (a,b,c): the dorsal surface of the anterior part (A) of control tongue showing the midline groove (white arrow) dividing it into two halves, fungiform (blue circle) papillae scattered between numerous filiform (Fi) papillae which appeared also on the body (B) and the root (R) of it. The circumvallate papillae (yellow arrow) appears to be marginated by a normal wide groove (white arrowhead). CPA group (d,e,f): the dorsal surface of the anterior part (A) and body (B) of the tongue of CPA group showing distorted (blue arrow) filiform (Fi) and fungiform (blue circle) papillae. Moreover, the collapsed circumvallate papillae (red arrow) losing their grooves can be noticed. Licorice CPA group (g,h,i): the dorsal surface of the anterior part (A) and body (B) of the tongue showing preservation of normal midline groove (white arrow), fungiform (Blue circle) and filiform (Fi) papillae. The circumvallate (yellow arrow) papillae are surrounded by narrow groove and the foliate papillae (Fo) appear on the margins of the root (R).

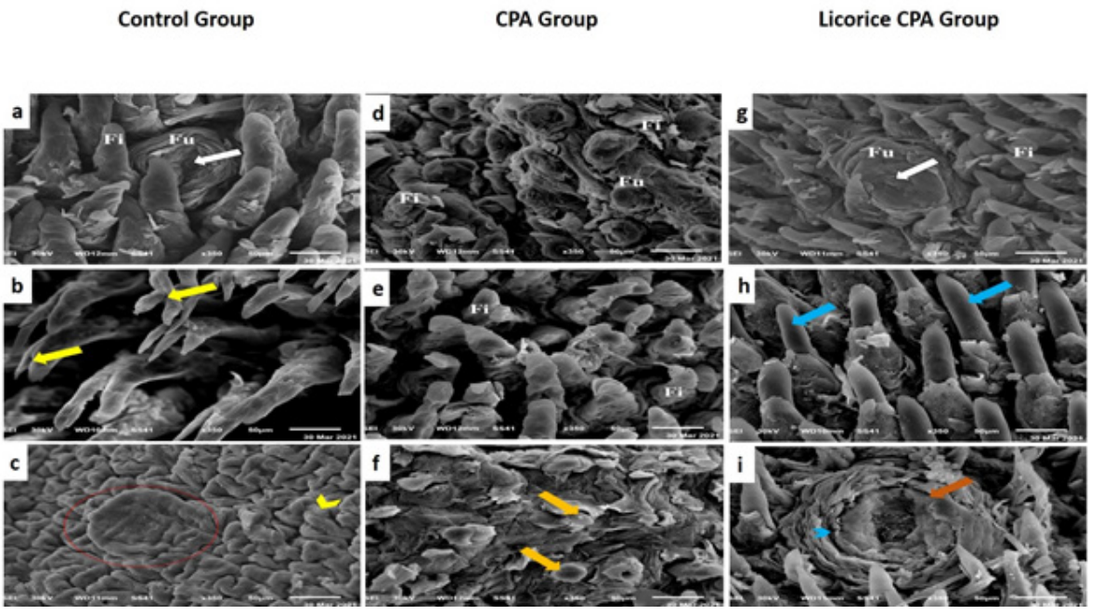

Fig. 9: Scanning electron micrographs of the rat tongue of all experimental groups (SEM, X350); Control group (a,b,c): numerous tall conical shaped filiform papillae (Fi) having 2 pointed processes (yellow arrow), less numerous fungiform papillae (Fu) contain taśte pores of taśte buds (white arrow), circumvallate (red circle) and foliate papillae (yellow arrowhead). CPA group (d,e,f): the lingual papillae including the filiform (Fi), the fungiform papillae (Fu) and the circumvallate papillae (orange arrow) appear diśtorted, desquamated with loss of their characteriśtic features. Licorice CPA group (g,h,i): normal fungiform (Fu) papillae having taśte pores of taśte buds (white arrow) and filiform (Fi) having normal characteriśtics (blue arrow) can be seen. The circumvallate (brown arrow) papillae are surrounded by narrow groove (blue arrowhead).

DISCUSSION

Cyclophosphamide is an immunosuppressive and anti-cancer chemotherapeutic agent[21]. Several studies have shown that cyclophosphamide is able to induce injuries in many organs including liver, lung, spleen, kidneys and testes[22-25]. High dose of CP is lethal within 10 days of its administration[26].

In cancer patients, mucositis is one of the most serious complications of antineoplastic treatment[27]. Oral mucositis induced by anticancer therapy can progressively reduce the quality of life[28].

The tongue, especially the filiform papillae, is thought to be a mirror of the general personal health. Since these papillae have a high metabolic function, nutritional deficiencies, vascular changes, or enzymatic disturbances can cause them to atrophy. Lingual atrophy may be caused by a variety of medications as antibiotics, or by diabetes, cancer, anti-cancer drugs, chemical or metal toxicity[29]. Subsequently tongue was selected in this study.

Nowadays, the herbal agents may have promising results in reducing the incidence or duration of oral mucositis. So, this work aimed to study the role of cyclophosphamide on rat's tongue mucosa and assess the effectiveness of licorice root extract as a protective agent against the induced tongue cytotoxic changes. We utilized histological, immunohistochemical and ultrastructural investigations to show its effect on cellular proliferation and apoptosis.

In this work, the body weight was significantly reduced in the CPA group as compared with the control group. Similar finding has been reported by Omole *et al*. (2018)[30] who reported decrease in the body weight of cyclophosphamide treated rats. They attributed this to the anorexia which was noticed in the cyclophosphamide treated group. In addition, they observed that the group treated with cyclophosphamide had a marked decrease in food intake when compared with the control group suggesting that cyclophosphamide has detrimental effects on the alimentary tract or hypothalamic center of appetite.

The histological findings of this study clearly demonstrated that cyclophosphamide usage led to obvious structural changes in the tongue mucosa of rats. These changes included thinning of the epithelium, degeneration of the epithelial cells, vascular hyperemia, inflammatory cell infiltration, atrophy and distortion of lingual papillae that was evident as a statistically significant decrease of their mean (length and width). Our results were in accordance with Al-Refai (2017)[14] who concluded that cyclophosphamide has a mischievous effect on the tongue mucosa resulting in marked histologic changes (decrease in the epithelial thickness and keratin layer, keratin separation, vacuolation of some epithelial cells displaying hyperchromatic nuclei, rete ridge flattening, infiltration of inflammatory cells, severe edema, and vascular congestion).

In the current study, examination of tongue sections of CPA group by scanning electron microscopy revealed

distorted filiform papillae with desquamation of epithelial cells. The distorted fungiform papillae lost their taste buds. This was in correlation with Ibrahim and Elwan (2019)[31] who evaluated the effect of irinotecan (anti-tumour drug) on the tongue mucosa of juvenile male albino rat. They reported by scanning electron microscopy several different signs of tongue papilla atrophy. Some filiform papillae showed desquamation of their surfaces, bisected tips, and thinning. An extravasation of red blood cells has been detected.

The pathophysiology of mucositis involves a complex mechanism. Chemotherapy generates reactive oxygen species which cause a series of biological events to occur and result in the production of numerous pro-inflammatory cytokines damaging the epithelial cells and connective tissue[32]. This damage may occur directly or through improving vascular permeability, thereby increasing cytotoxic drug uptake[33].

In this work, regarding control group, the Ki-67 expression in epithelial cells, suggesting the controlled proliferation rate but with a continuous proliferative capacity. In the CPA group (group III), a significant decrease in Ki-67 immune expression is observed following cyclophosphamide administration in comparison with the control group. Similar finding has been reported by Al-Refai (2017)[14] who reported a significant decrease in PCNA immuoreactivity in the tongue of cyclophosphamide treated rats. In addition, Al-Refai *et al*. (2014)[34] found that methotrexate-treated rats had mild positive Ki-67 immune reaction in some tongue basal epithelial cells. Sonis (2004)[35] demonstrated that chemotherapy inhibits DNA synthesis, damages DNA, produces reactive oxygen species which impede progenitor cells' metabolism leading to inhibition of mitosis and increase of apoptosis.

E-cadherin, an epithelial calcium dependent adhesion molecule, plays an important role in maintaining intercellular adhesion and epithelial cell-cell contact[36]. In the present study, decreased E-cadherin expression was noted in CPA group (group III). This finding is consistent with ElGhamrawy *et al*. (2014)[37] who reported markedly reduced cadherin immunoreaction in the testis of cyclophosphamide treated rats. Bruewer *et al*. (2003[38] suggested that E-cadherin plays an important role in tight junction defects, which is a pathological feature of mucositis. Chemotherapy-induced gut toxicity has been studied by Wardill *et al*. (2014)[39]. They reported significant changes in protein expression of tight junction proteins within the gut. Their findings strongly suggest that chemotherapy (irinotecan) leads to mucosal barrier dysfunction as a result of tight junction defects.

In the current study, induced tongue mucositis was associated with overexpression of P53. P53 is a transcription factor and tumor suppressor protein that is involved in mediating chemotherapeutic-induced apoptotic cell death[40]. Within one hour of DNA damage, the levels of p53 are elevated dramatically, due to post-translational regulation[41]. Asker *et al*. (1999)[42] added that p53 is also involved in the transcriptional control of genes that mediate mitochondrial changes during chemotherapeutic induced apoptosis. Consistent with the current study, cyclophosphmide has previously been shown to induce apoptosis in a variety of cells and organs as the urinary bladder[43], testes[44], and liver[45].

For the first time, licorice root extract was utilized to battle against the cytotoxic effect of cyclophosphamide protecting the rat tongue mucosa. The combined treatment of cyclophosphamide and licorice root extract (group IV) improved the histological changes in the tongue caused by cyclophosphamide alone. The epithelium integrity was preserved, statistical analysis showed significant amelioration in E-cadherin, Ki-67 and P53 immune expression as compared to CPA group (group III). This was in correlation with Galal *et al*. (2012)[46] who reported that mixing licorice extract with other herbs extracts, such as acacia nilotica, improved the healing of minor aphthae. Haley *et al*. (2005)[47] also found that the use of oral patch treated with Glycyrrhiza extract significantly accelerated the resolution of minor aphthae.

The potassium and calcium salts of glycyrrhizic acid (glycyrrhizin); triterpenoid saponin which is the active ingredient of licorice root that may have anti-ulcer properties[48]. Through the anti-inflammatory effects of glycyrrhizin and its main metabolites, the production of reactive oxygen species was reduced in various animal models[49]. Glabridin is an isoflavonoid derivatives found in licorice. Glabridin also suppressed the development of reactive oxygen species (ROS) in various study[50]. In addition, it was found to be an effective antioxidant against low-density lipoprotein oxidation in both in *vitro* and in *vivo* studies[51]. Other components of licorice as hispaglabridin A, glyderinine, 4′-O-methylglabridin and hispaglabridin B showed an anti-inflammatory and antioxidant properties[52].

Moreover, antioxidant and free radical scavenging properties of Glycyrrhiza glabra extracts could protect tissues from damage caused by ROS and free radicals[53]. Anti-ulcer properties of flavonoids and tannins have been linked to antioxidant effects[54]. Lately, it was found that licorice could prevent the development and treat the mucosal ulceration efficiently[55,56]. All the above results of the other studies support our findings.

CONCLUSION

Cyclophosphamide is a detrimental chemotherapeutic agent to the mucus membrane of rat tongue causing serious microscopic changes. Our results demonstrate that systemic use of licorice root extract combat the cyclophosphamide induced cytotoxicity and oral mucositis through significant amelioration in the Ki-67, E-cadherin and P53 immunoreactivity. However, more studies are recommended to evaluate the therapeutic effect of this natural product against oral mucositis in patients undergoing cancer treatment.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

مستخلص جذر عرق السوس يخفف من التهاب الغشاء المخاطي للسان المستحث بالسيكلوفوسفاميد في ذكور الجرذان البالغه البيضاء

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مقدمة: سيكلوفوسفاميد (CPA) هو دواء مضاد للسرطان يستخدم في العلاج الكيميائي. في الآونة الأخيرة ، يُعتقد أن التهاب الغشاء المخاطي للفم هو أخطر الآثار الجانبية للعلاج المضاد للسرطان.

الهدف من الدراسه: دراسة دور مستخلص جذور العرقسوس في التهاب الغشاء المخاطي للسان الجرذ الناجم عن السيكلوفوسفاميد.

المواد والطرق: تم تقسيم أربعين فأرًا بالغًا من ذكور الجرذان البيضاء إلى أربع مجموعات: المجموعة الأولى (المجموعة الضابطة) ، المجموعة الثانية (مجموعة عرق السوس): تلقى كل فأر مستخلص جذر عرق السوس بجرعة يومية عن طريق الفم قدرها ٢٠٠ مجم / كجم / يوم لمدة ١٠ أيام ، المجموعة الثالثة (مجموعة السيكلوفوسفاميد :(تلقى كل فأر جرعة واحدة من السيكلوفوسفاميد (٣٠٠ ملغم / كجم) داخل الصفاق في اليوم الثاني من التجربة والمجموعة الرابعة (مجموعة عرق السوس و السيكلوفوسفاميد) تلقى كل فأر خلاصة جذر عرق السوس بجرعة يومية عن طريق الفم ٢٠٠ مجم / كجم / يوم لمدة عشرة أيام ، وفي اليوم الثاني من التجربة ، تلقت الفئران السيكلوفوسفاميد بجرعة واحدة داخل الصفاق تبلغ (٣٠٠ ملغم / كجم). في نهاية التجربة ، تم تخدير الفئران واستئصال اللسان للتحليل الهستولوجي والهستوكيميائي المناعي والبنية التحتية. كما تم إجراء التحليل القياسي والإحصائي.

النتائج: قلل مستخلص جذر عرق السوس من شدة التهاب الغشاء المخاطي للفم الناجم عن السيكلوفوسفاميد عن طريق زيادة سماكة الطبقه الطلائيه ، وتقليل احتقان الأوعية الدموية في الغشاء المخاطي للسان ، وتحسين كبير في التفاعل المناعى لمضاد Ki-٦٧ ، ومضاد E-cadherin ومضاد P٥٣.

الخلاصة: العلاج الكيميائي بالسيكلوفوسفاميد له تأثير ضار على الغشاء المخاطي للفم مما يؤدي إلى تغيرات قياسيه وميكروسكوبية ملحوظة. يمكن لمستخلص عرق السوس حماية الغشاء المخاطي للفم من السمية التي يسببها السيكلوفوسفاميد وتقليل الإصابة المصاحبة.