EFFECT OF GLYCINE ON 5-FLUOROURACIL- INDUCED ORAL MUCOSITIS IN ADULT MALE RATS (*Rattus norvegicus*)

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ABSTRACT

The study designed to assess the impact of glycine on 5-FU induced oral mucositis. Twenty five adult male rats were used in this study, and randomly distributed in to three groups: the first group (G1) served as control (5 rats). The other two experimental groups (G2 & G3) (10 rats for each) were received IP injected (60 mg/ kg B. W) of 5-FU one time / week for six weeks. The animals of G3 were received a (2 mg /g B.W.) of 5% glycine once time / day from the first day of experiment till the finish of experiment at 6 weeks. After that, the rats were sacrificed, and the cheek mucosa was removed and processed for light microscope. Histological examination of cheek sections of G2, revealed reduce in thickness of epithelium with secession of keratin, perinuclear vacuolation cells with pyknotic nuclei, intense infiltration of inflammatory cells and the areas of degeneration. Light microscopic analysis of G3 treated with glycine, showed improvement of most histological changes described before as a degree of reepithelization with keratin layer, more proliferation of basal cells, mild inflammatory features with numerous blood capillaries. Glycine shows more fast epithelial differentiation and wound healing.

INTRODUCTION

Oral mucositis (OM) is damage and popular problem of the oral mucosa either induced through irradiation or due to chemotherapy in patients with cancer (1). The prevalence of OM differs depending on the treatment managed and individual patient features. This adverse

reaction is recurrently encountered within a week next chemotherapy and is characterized in its mildest shape by erythema, erosions and ulcerative lesions in the oral cavity. The severity of mucositis may be highly aching for the patients and may require important influence on their quality of lifestyles death (2). Furthermore, mucositis can have important economic effect because of raised hospitalization, the requirement of parenteral nutrients and the use of opioids (3). 5-fuorouracil (5-FU) is one of the most popular reasons of OM. Mucositis grade (3-4), which outcomes from postponement, dosage lowering or stoppage of chemotherapy, happens in more than 20% of cases through administration of 5-FU (4).

Sonis suggested a theory for the pathogenesis of OM (5), based on new proof and developing realization of molecular and cellular procedures in mucositis. This concept comprising, the initial phase of mucositis contains of an inflammatory response to radiation chemotherapy induced production of reactive oxygen species (ROS). Several programs have been employed to manage mucositis in patients getting chemotherapy or radiotherapy. So, novel remedy protocols are of great attention. Glycine is the most vital and simple non-essential amino acids, and it exhibits important biological actions via performing as a modulator of the general inflammatory cascade by getting better of the microcirculation and supporting in the suppression of TNF-a and IL-1b (6,7). Some studies have demonstrated that glycine have beneficial remedy for several types of inflammatory procedures (8), 5% glycine has supplied useful impacts against toxicity of liver and inflammation in rats (9). Administration of glycine to be helpful in the barring of mucosal alteration subsequent in a rat model of ischemia / reperfusion damage by increasing mucosal viability and thickness, probably mediated through a down-regulation of cellular apoptosis (10). The promising antioxidant properties exhibited by glycine in earlier studies (11, 12) propose the possible for curative advantage in chemotherapy- induced mucositis. The study amid to evaluate the effect of glycine on 5-FU induced oral mucositis.

MATERIALS AND METHODS

Twenty five male rats (*Rattus norvegicus*), about (250-300)g and aged (10-12) weeks were used. The rats were provided and care in the isolated polycarbonate cages in the animal's house of the biological department, college of Science, Basra University under strict clean and standard managing conditions at temperature 20-25°C and a 12 hour light\ dark cycle. The animals were distributed randomly in to three groups, G1: 5 rats served as rat's control, the two experimental

groups G2 and G3 (each of 10 rats), the two experimental groups received intraperitoneal injected of 5-FU (Sigma-Aldrich,UK) (60 mg/ kg B.W) one time / week for six weeks (13). The animals in G3 were received a 2mg/ g of body weight IP injection of glycine diluted in saline 5%, treated with glycine was initiated in the first day of the experiment with usage once time\ day in the morning until the end of experiment at 6 weeks. After that, the animals were scarified; cheek mucosa specimens were removed for histopathological analysis. The samples were fixed in 10% formalin for at least 24h, and then the specimens were regularly treated for embedding in paraffin and cut into 5 micron thick and stained with hematoxylin and eosin, and then examined the sections under light microscope.

RESULTS

Histological observation of cheek mucosa

The specimens of control group (G1) revealed that the cheek mucosa composed of normal keratinized as mild deposition at surface layer, stratified squamous epithelium exhibiting well-develop of rete ridges extend down to the regular arrangement of the lamina propria formed of connective tissue with numerous small capillaries (Fig.1). Microscopic observation of (G2), revealed different histological changes as decrease in thickness of epithelium, and most stratified squamous epithelial cells with perinuclear vacuolated cells and degeneration other cells, partially flattening of rete ridges, inflamed lamina propria, atrophied muscle fibers with numerous congested capillaries (Fig. 2). Furthermore, diminished in the epithelium thickness with secession of keratin, the inflammation process constituted by extended of moderate inflammatory cells to the connective tissue in lamina propria with areas of degeneration (Fig. 3). While microscopic examination in cheek sections from rats injected with 5-FU and treated with glycine of (G3), showed keratin layer and seemingly degree of re-epithelization with restoration the normal shape of rete ridges, more proliferation of basal cells, still perinuclear vacuolated in some epithelial cells, mild inflammatory features were still notable in lamina propria with numerous blood capillaries were detect (Fig. 4).

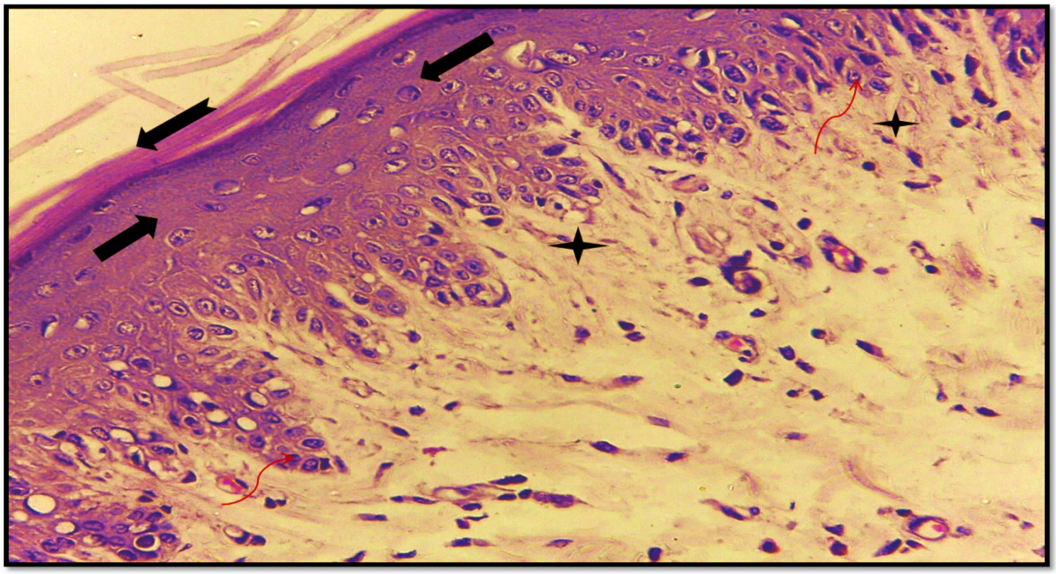

Figure (1): section of cheek in G1 showed normal stratified squamus epithelium (➡), mild deposition of keratin (➡) on surface layer and rete ridges (↗) well develop extend down to the lamina propria (✦). (H & E. stain 40 x)

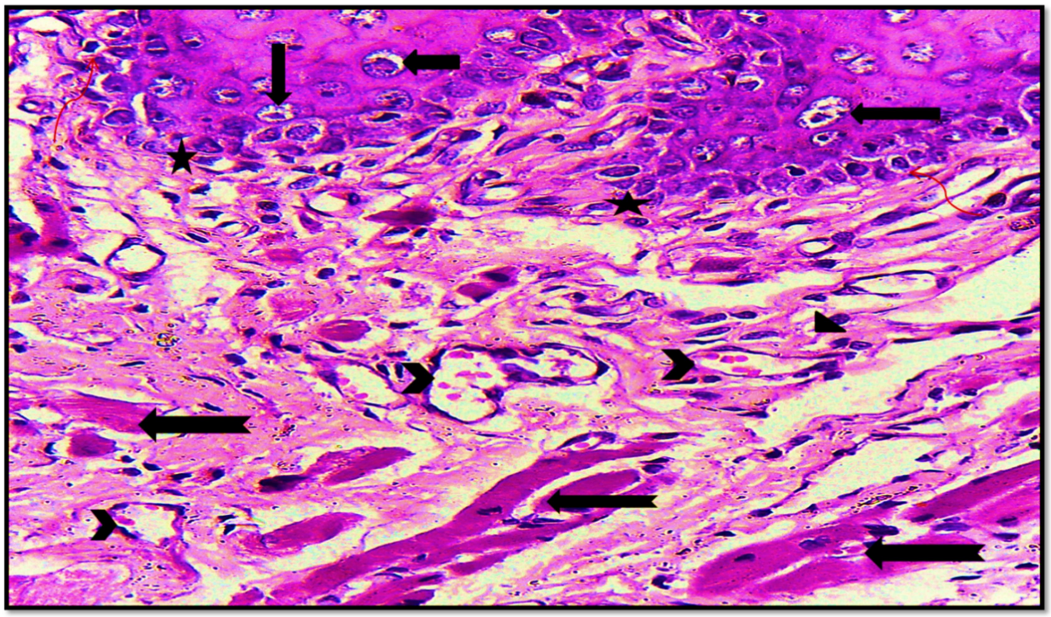

Figure (2): section of cheek in G2 showing the cells with vacuolar degeneration (➡), most cells of basal cells with perinuclear (↗), flattening rete ridges (★), mild inflammation (◣), congested capillaries (➤), atrophied muscle fibers (➡), (H & E. stain 40 x).

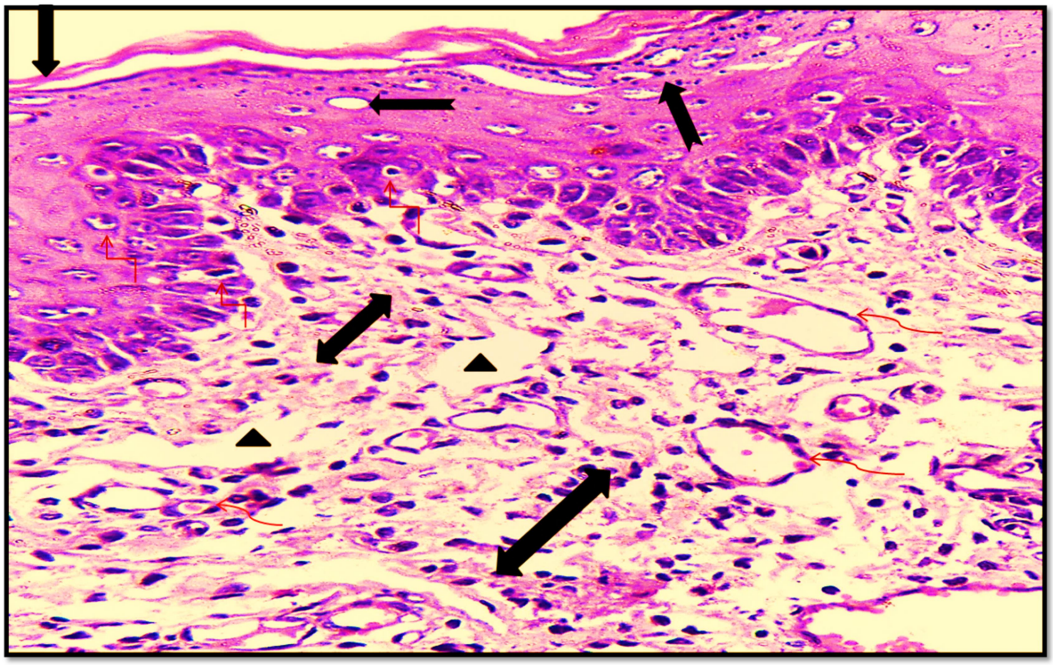

Figure (3): Section of cheek in G2 showing separation of keratin layer (⟶), vacuolated cells (⟶) of stratified squamous epithelium, most cells of pyknotic nuclei (↱), moderate inflammatory of lymphocytes in lanina propria (⟷) and appeared vascularized (↱) with areas of degeneration (▲). ((H & E. stain 40 X).

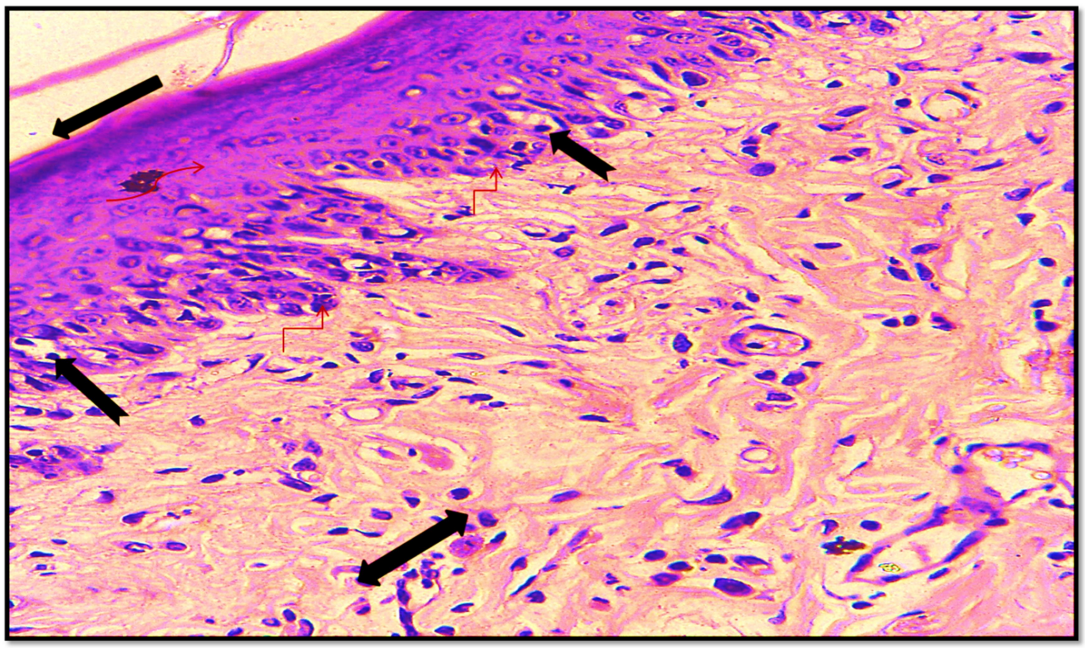

Figure (4): section of cheek in G3 showing mild keratin layer (⟶) with degree of re-epithelization (↱), still perinuclear vacuolation (⟶) , mild inflammatory cells (⟷) more proliferation of basal cells (↱). (H & E. stain 40 X).

DISCUSSION

Chemotherapy brought about OM may cause sizable injury or cessation of remedy in cancer patients. Several studies are examining diverse remedy forms for mucositis, creating this one of the most investigated subjects inside the area of helpful cancer care. The pathogenesis of mucositis isn't always wholly understood; however each direct and indirect technique is regarded to be involved in mucositis (2). Factors used to deal with cancer can also reason epithelial atrophy, making tissue greater liable to traumatic or spontaneous ulceration. Different issues may additionally participate to the pathogenesis of mucositis, which comprises the endothelium, extracellular matrix and cytokines (14). ROS are considered to play a vital role in the inflammatory element of mucositis. 5-FU has been extensively used to cure diverse kinds of cancer; it suppresses both the synthesis of DNA and RNA and producing apoptosis (15). Numerous anti-cancer agents, which include 5-FU, were revealed to promote ROS production in both ordinary tissue and cancer cells, and mucosal damage resulted from overproduction of ROS (16, 17).

In the current study, glycine had influence on the healing method of mucositis; there were signs that the degree of retrieval from mucositis could be improved. The histopathological changes that showed in G2 as a reduction the thickness of epithelium with secession of keratin layer, perinuclear vacuolation of most epithelial cells was observed, also moderate inflammation that extend in to deeper layer of lamina propria compared with control group. These effects had been in agreement with some investigators (18, 19), who proposed that 5-FU became powerful chemotherapeutic agents inducing oral mucosal damage. While histological analysis in G3 treated with glycine, revealed a degree of reepithelization with restoration of normal rete ridges. It appears that the effect of glycine accelerate of healing action with time the mucosa has entirely recovered. Also, mild infiltrate of inflammatory cells, because of its anti-inflammatory and cytoprotective effect of glycine in oral mucosa. These results were elucidated by some scientists (20, 21), were described that nutritional supplementation of 5% glycine has improved the process of healing, antioxidant ability and resistance.

تاثير الكلايسين على التهاب الغشاء المخاطي للفم المحدث بواسطة الفلوريوراسيل في ذكور الجرذان البالغة نوع *Rattus norvegicus*

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الخلاصة

تم تصميم هذه الدراسة لتقييم تاثير الكلايسين عل التهاب الغشاء المخاطي للفم المحدث بواسطة الفلوريوراسيل. (٢٥) جرذا وزعت بصورة عشوائية الى ثلاثة مجاميع وكلاتي: اعتبرت المجموعة الاولى (G1)كمجموعة سيطرة (٥) جرذ ، بينما المجموعتين الاخرتين (G2 , G3) ١٠ جرذ لكل منهما حقنت عن طريق الصفاق بمادة ٥-الفلويوراسيل ٦٠ ملغم/ كغم من وزن الجسم مرة واحدة في الاسبوع لمدة ستة اسابيع. اما الحيوانات في المجموعة الثالثة عولجت بلكلايسين ٢ ملغم/ غم من وزن الجسم بتركيز ٥% حقنت بلصفاق أيضا يوميا ابتداء من اول يوم بالتجربة الى نهاية التجربة (٦ اسابيع). بعد انتهاء فترة المعاملة تم تخدير الحيوانات والحصول على الطبقة المخاطية للخد. اظهر الفحص النسيجي لمقاطع الخدين في المجموعة الثانية تناقص في سماكة طبقة الظهارة مع انفصال في طبقة الكيراتين، ظهرت معظم خلايا الطبقة الظهارية بتنكس فجوي وفراغ حول النوى مع تغلظ الانوية، احتقان بالأوعية الدموية مع ارتشاح معتدل للخلايا الالتهابية ومناطق من الانحلال. اما التحليل النسيجي لمقاطع المجموعة الثالثة اظهر تحسن ملموس في معظم التغيرات النسيجية التي ذكرت سابقا متمثلة بدرجة من اعادة الطبقة الطلائية مع طبقة الكيراتين، المزيد من انتشار الخلايا القاعدية، ملامح التهابية خفيفة جدا مع العديد من الاوعية الدموية . من هذا نستنتج ان العلاج بلكلايسين يظهر تمايز ظهاري اسرع و يحسن شفاء الجروح.

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