Histological description for the protective effect of glycine supplement on oral mucositis induced by 5-Flourouracil in male rats (*Rattus norvegicus*)

A thesis

Submitted to the council of college of Science, University of Basrah in partial fulfillment of the requirements for the degree of Doctor of Philosophy of Science in Biology/Histology

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MSc. Histology 2013

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2021


بسم الله الرحمن الرحيم
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صدق الله العظيم الاسراء (85)

Certification

I certify that this thesis has been prepared under my supervision at the Biology department/ College of science, university of Basra, as a partial requirement for the degree of Doctor of Philosophy (PhD) in Histology.

Signature

Prof. Dr. Kareem Halal Al-Derawi (Supervisor)

Recommendation of head of the department

In view of the available recommendation, I forward this thesis for debate by examining committee.

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Date:

Dedication

“All commendation is due to Allah” for the guidance and support, who has given to me to attain this work. Thanks most to my family, for their support and encouragement.

Fatin

Acknowledgement

Praise is to “Allah”, who gives me the strength to achieve this study. I would like to express my thanks to my supervisor Professor Dr. Karim Al-Derawi for his advice and support me along the study period. I am exceedingly thankful to Professor Dr. Maha Khalil, who has given me the time, follow and aided I wanted to complete this thesis. I would like to deeply thank the dean of the college of science and the head of department of biology/university of Basra for all facilities. Special thanks to prof. Dr. Assad yahea in college of Agriculture for help me in the statistical analysis of the current study, and to prof. Dr. Alaa A. Sawad in college of Veterinary Medicine for his help in histomorphometrical study.

My great thanks and deepest appreciation are to all members of the department of biology/College of Science, particularly for Prof. Dr. Muslim Abdurrahman, Assist. Prof. Dr. Sanaa Jameel, Dhahir Alaale. My deep thanks extend to Dr. Huda in College of Veterinary Medicine for her help in biochemical study.

Fatin

Summary

The current study was carried out to investigate the toxicity effects of 5-fluorouracil drug (5-FU) administration on the oral mucositis (OM) induced as experimental model and to clarify the possible protective role of glycine supplementation on(OM) in male rats (*Rattus norvegicus*).

In this study healthy and adult male rats , total number (96), age (10-12) weeks and average weight (200-250) gm, were used after breeding in animal house / University of Basra / College of Science then isolated and divided into two equal main groups, each group subdivided into three sub groups for two periods (21, 45 days), with (48) male at each period as follow , Group I (control group) ,Group II (mucositis group); Group III (mucositis /glycine).

At the end of experiments, all the animals were anaesthetized with chloroform ,body weight measured ,blood was collected , serum was separated and stored for biochemical analysis included malondialdehyde (MDA), glutathione peroxidase (GPx), Superoxide dismutase (SOD) and hematological parameters ,also samples from cheek , tongue and lips were collected from each rat of control group and treated groups at each period. The study determined all histomorphometrical changes on oral mucosa regions (tongue, lip and cheek), also histopathological observations associated with (OM) was evaluated. In addition immunohistochemistry technique for PCNA and BCL-2 proteins expression was established.

The study showed that the animals body weight was significantly decreased at ($P \leq 0.05$) in mucositis group in comparison to the other group, the weight returned gradually to the normal in treated group after glycine used. The study recorded the alterations of biochemical parameters through the study periods, It showed that the

effect of (5-FU) on (MDA) level caused a significant increased at (P≤ 0.05) post(21 and 45) days, while the anti-oxidant enzymes (GPx and SOD) level was significantly decreased at (P≤ 0.05) at each period, moreover glycine supplementation in combination with (5-FU) showed significantly decreased in (MDA) concentration and the level of antioxidant enzymes were increased significantly at (P≤ 0.05).

Results from hematological observation revealed to significant decreased at (P≤ 0.05) in WBCs count in all rats with (OM) after both doses of (5-FU) particularly post (45 days). The result recorded significant variations in lymphocytes, neutrophils and monocytes counts in mucositis group, It was shown when glycine used in combination with (5-FU) significantly inhibited the decline in WBCs count at (P≤ 0.05) and results return to the normal limit particularly post (21) days, also the mean counts of lymphocyte, neutrophil and monocytes return to normal value compared with 5-FU group. RBCs count and Hb decreased significantly at (P≤ 0.05) in mucositis group, while the concentration of Hb and RBCs count on each subgroups that treated with (5-FU) and glycine was significantly increased.

Histomophometrical measurements indicated that (OM) caused significant decrease of epithelial layer thickness in each section of rat tongue, cheek and lip in (5-FU) treated group after 21days of induction and more significant reduction after 45 days compared with control group. While mean thickness of epithelial layers was gradually increased at (P≤ 0.05) in all rats treated with (5-FU + glycine) during both doses of 5-FU (30, 60 mg/kg) for both periods, but it was still lower than the control group.

Macroscopically, oral mucosa of (tongue, cheek and lip) of control rat appeared normal without any abnormal signs. Observations on oral mucosa of rats with (OM) appeared with inflammatory signs as redness, hyperemia and hemorrhage after (14) days. These signs caused clear epithelial ulceration of the surface layer at the end of experimental periods approximately in all rats treated with (5-FU) post 45days with both doses. When rats treated with (5-FU+ glycine) showed no ulcers, less redness, no hyperemia and morphologically the oral mucosa nearest to the normal especially post 21 days.

Variable histopathological changes were noticed in oral mucosa regions (tongue, lip and cheek) of (OM) group as compared with control group, mild loss of normal appearance and shape of dorsal surface papillae, secession of keratin layer , perinuclear cells and mild inflammatory cells in lamina propria in all rats treated with dose (30mg/kg) of 5-FU, while tongue sections related to rats treated with dose (60mg/kg) of 5-FU post (21) days showed hyperkeratosis, acanthosis, severe atrophied papillae, heavy infiltration of inflammatory cells, edema, highly vascularized with congested large blood vessels. While the treated period of 5-FU was increased, alterations became more obvious, so tongue sections of rats injected with (30mg/kg) of (5-FU) drug after (45) days showed sever atrophied papillae, reduced thickness of epithelium, heavy infiltration of mononuclear cells as compared to rats treated with (60mg/kg) of (5-FU) showed more severity alterations in dorsal and ventral surfaces revealed to loss of normal appearance and destruction papillae, , heavy infiltration of inflammatory cells. But when used combination of (5-FU) and glycine, restoration most normal structures , increase epithelial layer thickness (degree of re-epithelization), well develop papillae less inflammatory cells, increase proliferation of basal cells particularly after (21) days be more healing than (45) days. Moreover lip and

cheek sections in (OM) group were revealed to less thickness of epithelium, cells with vacuolated cytoplasm, vascular hyperemia and congested blood vessels with dose (30mg/kg) of 5-FU post (21) days, but the effect of dose (60 mg/kg) was more injurious in the same period, particularly in cheek sections. While changes on cheek and lip sections with both doses of (5-FU) post (45) days were revealed to sever ulceration with destruction of epithelial layer, inflammatory cells infiltration, edema and hemorrhage. Whereas results clarified that glycine with 5-flourouracil drug at different periods showed variable improved re-epithelization nearly similar to control group especially with dose (30 mg\kg) with renewal of normal stratified squamous epithelium, keratin layer deposit, well-organized lamina propria compared to (OM) group.

Immunohistochemistry showed that 5-FU has effect on (PCNA and BCL-2) protein expression , there was significantly decrease post (21, 45) days compared with control group, but when used combination of (5-FU + glycine), the values was revealed to significant increase of proteins expression after 21 and 45 days comparing with (OM) group. The data from recent study referred to glycine as protective, bioactive factor that reduced (5-FU) complications especially (OM) and act on repairing, regeneration of oral mucosa.

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Symbol	Description
AT	Alimentary tract
5-FU	Flourouracil
BCL-2	B-cell lymphoma-2
BW	Body weight
CBC	Complete blood count
COX-2	Cyclooxygenase-2
DNA	Deoxyribo nucleic acid
ECM	Extracellular matrix
EGF	Epidermal growth factor
FGF-20	Fibroblast growth factor-20
GIT	Gastrointestinal tract
GLyR	Glycine receptor
GM-CSF	Granulocyte macrophage-colony stimulating factor
GPx	Glutathione peroxidase
HB	Hemoglobin
IHC	Immunohistochemistry
IL	Interleukin
LV	Leucovorin
MDA	Malondialdehyde
MMPs	Metalloproteinase enzymes
NCI	National cancer institute

NF- κB	Nuclear factor-kappa
NO	Nitric oxide
OM	Oral mucositis
OMAS	Oral mucositis assessment scale
PCNA	Proliferating cell nuclear antigen
RBCs	Red blood cell count
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TGF	Transforming growth factor
TNF--α	Tumor necrosis factor alpha
TS	Thymidylate synthase
VEGF	Vascular endothelial growth factor
WBCs	White blood cell count
WHO	World health organization

Chapter One

Introduction & Literature Review

Chapter One

Introduction and Literature Review

1.1 Introduction

Mucositis is aching inflammation and ulceration of the mucous membrane inside layer of the alimentary tract is mainly declined and seen about perpetually in cancer chemotherapy and following radiotherapy (Mead, 2002). Also it is amongst the most important main dose-limiting toxicities of sever cancer therapy and in the case previous to bone marrow transplantation-hemopoietic stem cell transplantation (HSCT) (Stiff, 2001).

Oral mucositis (OM) is described as mucosal barrier damage as a result of desolation of its mucosal epithelium or suppression of its growth leading to change of the safety and function of oral cavity. It may become visible anyplace within the mouth but are regularly determine on cheeks, interior of lower lip or on the sides or base of the tongue (Mahdi *et al*., 2016).

(OM) recognized by erythematous, erosive and ulcerative injury within oral cavity, so it can induce many complications via extremely painful to patients and limiting their ability to eat, drink, talk, swallow problems and sever pain of ulcers more than infection which can progress into systemic infection. Moreover affect the patient's life style by lower their dietary status and exacting to their hospitalization (Alvarino and Sarrion, 2014).

Most of patients who taken chemotherapy and approximately all those undergoing radiotherapy are usually affected by (OM), the incidence and its severity depend on period, form and dose of chemotherapy drug used (Hernández *et al*., 2012; Da *et al*., 2014), it occurs in 40-60% of patients getting chemotherapy, about 60% of patients getting radiotherapy and 90% of patients receiving together chemotherapy and radiotherapy (Barasch *et al*., 2009).

Histopathological estimation of oral mucositis lesions shows reduction in epithelial layer thickness because of cells apoptosis and exhaustion of the basal epithelial layer following erosion; it starts in the epithelium and then extends to influence the connective tissue (Maria *et al*., 2013).

There are several methods to induce oral mucositis as animal models, among these chemical 5-flourouracil (5-FU) and cisplatin …etc (Sonis *et al*., 1990). Also, irradiation model (Dorr and Kummermehr, 1990; Hwang *et al*., 2005), together chemotherapy and radiation model and less frequent model involve mucosal damage with acetic acid.

Previous studies used the aspect of drugs-induced (OM), for example 5-flourouracil (5-FU), cisplatin and methotrexate, at the same time other studies used chemical drugs and plant extracts to minimize the effect of (OM) or reduce its effect like melatonin, green tea, Chamomile, *Nigella satival*, Cur cumin and Olive leaf extract (Abdurrahman *et al*., 2012; Al- Bahtiti, 2012; Al-Refai *et al*., 2014).

Sonis (2009) considered that 5-FU the best chemotherapeutic agents associated with oxidative stress and (OM). It is an effective antineoplastic drug used for treating diversity of malignancies, mainly of the breast, colon or rectum and in the remedy of gastric, pancreatic, uterine, ovarian and bladder carcinomas (Longley *et al*., 2003). Also apparent that 5-FU is a toxic to the Gastrointestinal tract (GIT) and one of the most popular causes of OM, 5-FU is considered as secure drug with effective anti-mitotic action, though it labors on malignant tumor cells as well as ordinary cells displaying unbalanced mitotic action as numerous glandular epithelial cells and hematopoietic cells generating in bone marrow (lima *et al*., 2004). Pathogenesis of OM toxicity caused by 5-FU was initiated by the production of reactive oxygen species (ROS) which is injurious to the DNA of mucosal layer (Generoso *et al*., 2015).

The managing for the remedy of (OM) instituted by several techniques such as herbal remedy, laser therapies, topical anesthetics, anti-ulcer, anti-inflammatory and anti-oxidant agents like glycine (Watanabe *et al*., 2013). Glycine, a simple nonessential amino acid, is providing a new selection in the treatment of mucositis; it has been demonstrated to have anti-inflammatory, immunomodulatory and cyto-protective impacts in various experimental models (Wang *et al*., 2013). Protective impact of glycine on inflammatory lesions has been shown in different models; glycine is a potent restraint of resident liver macrophages and acts through a glycine-gated chloride channel. Previous studies have shown that glycine supplementation triggered a fast remodeling of tissue, may be a useful healing assistance for persons with inflammatory damages of tendons like Achilles tendon damages (Wheeler *et al.*, 2000). Also, other studies elucidated the anti-inflammatory role of glycine in oral tissues of animal experimental model. These researchers were able to prove important decrease of insincerely triggered periodontal infection subsequent a specified glycine intake (Breivik *et al*., 2005).

Furthermore, administration of glycine in a rat model of ischemic-reperfusion damage resulted in down regulation of cell apoptosis and the expression of pro-apoptotic genes (Den Hartog, 2009). Thus, glycine has shown to have affirmative impacts on numerous of the paths concerned in the pathogenesis of OM.

1.2. Aims of the study:

Since oral mucositis (OM) is one of the most debilitating side effects in oral mucosa caused by antineoplastic drugs that afflicted high rate of patients undergoing chemotherapy and lead to severe complications so the study was designed to achieve the following objectives:

1-Induce oral mucositis in rats by (5-FU) as animal model stimulates (OM) in human and determine the suitable dose.

2- Investigating the efficacy of glycine supplementation on the pathogenesis of (OM) induced by (5-FU) and the protective effect of this agent against (OM) complications post different periods of exposure.

3-Determined the hematological parameters, malondialdehyde (MDA), oxidative enzymes (GPx & SOD).

4- Describe the histomorphometrical and histopathological changes on (tongue, lip and cheek) oral mucosa regions post (21 and 45 days) period of (OM) induction.

5-Clarify the (PCNA& BCL-2) proteins expression in tongue mucosa by immunohistochemically study and determine the mechanism of these expressions.

1.3. Mucositis

Mucositis was a major trouble for patients undergoing chemotherapy and radiotherapy remedy. It is a problem that occurs throughout the alimentary tract (AT) (Keefe *et al*., 2000; Keefe, 2004) and causes a spectrum of medical signs and symptoms which range from intractable and debilitating oral pain because of mucosa ulceration to gastrointestinal symptoms like abdominal bloating, vomiting and diarrhea (Pico, 1998). In addition different mucosal surfaces through the body including the genitourinary and respiratory mucosa can also be affected (Keefe and Gibson, 2007).

Generally, mucositis the painful infection and ulceration of the mucous membranes lining the digestive tract as a result of the most common harmful reactions encountered in radiation therapy for head and neck cancers as well as in chemotherapy especially with drugs affecting DNA synthesis (S-phase-specific agents such as fluorouracil, methotrexate, and cytarabine) (Wolfgng *et al*., 2001). However, mucositis remains a crucial dose-restricting factor in patients' cancer treatment, the debilitating effects of mucositis can result in unplanned interruptions or may be untimely stopping of remedy, the threat of systemic infections and even death is elevated in patients with mucositis (Parulekar *et al*., 1998; Bellmet *et al*., 2000). Though with recognize to chemotherapy the dynamic remedy or prevention of mucositis can also allow an increase within the maximum tolerated doses of remedy and progressed best of life styles for cancers patients in the course of and after remedy, this will also be translate to an extended chance for cancer reduction, mucositis also cause an increase in the morbidity of patients undergoing cancer remedy effects in prolonged hospital stays and similarly re-admission rates also are increased (Pico *et al*.,1998; Elting *et al*., 2003).

The prevalence of mucositis is changeable and appeared to be dependent on the kind of treatment and to the disorder that is being treated, for example, happens in 80-100 % of patients known as (high mucositis risk) including radiotherapy to the head and neck or high dose chemotherapy and stem cell or bone marrow transplantation (Keefe *et al*., 2ooo). Moreover, cytotoxic chemotherapy agents such as 5-fluorouracil (5-FU) are associated with more intense mucositis in regimens taken into consideration to be (low-risk) for the development of mucosal toxicity, so the prevalence of mucositis can be as low as 10-15% but given the numbers of people receiving of chemotherapy that is nevertheless represents a great number of patients which are affected by mucositis (Rubenstein *et al*., 2004).

1.4. Oral mucositis (OM)

It was described as oral mucosa membranes damage in most cancer patients either caused through irradiation of patients who have head and neck carcinoma or due to chemotherapy and lead to particular inflammation and ulceration of the mucosa lining the oral cavity, it takes place as common side effects of chemotherapy due to the cytotoxic therapy effect dividing epithelial cells, which can be most susceptible that explaining why (GIT) disturbance. Immunosuppressant and alopecia are the most obvious restricting side effects, in the mouth, the killing of epithelial progenitor cells inhibits the potential of the mucosal layer to restore micro lesions leading to ulceration exacerbated via infection (Montejo *et al*., 1999; Fong, 2007).This adverse reaction is recurrently encountered within a week next to-chemotherapy and is characterized in its mildest shape by erythema, after that, the mucosal changes progress to increase in intensity and appear as erosions or ulcers which can be covered with a white pseudo membrane that usually solve within two weeks.

However, the healing period can be longer when secondary infections associated with microorganisms like virus or fungi arise (Peterson *et al*., 2011; Chaveli-Lopez, 2014).

The ulcerations have a tendency to be abnormal, shallow and may be developed to larger wounds. The mucosa lining oral cavity and oropharynx seem on the identical time in systemic chemotherapy brought on mucositis, the oral mucosa extends from inner lining of the buccal mucosae, gingival sulcus, labial folds, ventral surface of tongue, floor of the mouth, soft-palate, pharynx, dorsal surface of the tongue, gingival ridges and hard palate, hard palate and tongue dorsum are keratinizing and are constant while relax mucosa are movable and these parts preferentially manifest mucositis induced by chemotherapy (Sequier and Kremer, 2001). The prevalence of (OM) condition varies depending on the treatment administered and individual patient characteristics , incidences as high percentage 100% had been suggested in patients receiving radiotherapy for head and neck cancers (Petrson, 2009).

(OM) may be extremely painful for the patients and have a significant impact on their quality of life styles by limiting their ability to eat, drink, talk, swallow and sleep also it may be presented in mixture with a variety of debilitating symptoms that could comprise the ability of the patients to preserve oral hygiene practices (Gandhi *et al*., 2017).

Furthermore (OM) is associated with malnutrition, weight loss and used of feeding tubes or total parenteral nutrients and it may represent a portal of entry for systemic infections that may cause sepsis and loss of life. These signs along with their associated sequel can result in hospitalization and might in cure increase costs for health care systems (Biswal, 2008 Epstein *et al*., 2012; Al-Refai, 2017).

Sonis in (1998) was suggested four stages by which mucositis develops and resolves, a new model that has been accepted as the illustrative for the condition producing with new evidence, however , revisions being made to the initial theory (Sonis, 2004, 2007, 2009). This model describes particularly the pathological events that arise inside the oral mucosa after chemotherapy.

1.4.1. OM Incidence and development

The prevalence of (OM) varies relating to treatment type for patient gets and impacts about (40-60%) of patients receiving standard dose of cytotoxic chemotherapy (Rubenstein *et al*., 2004). Greater than 80% of patients undergoing high dose of cytotoxic chemotherapy will be affected by OM at variable level (Logan, 2009; Peterson *et al*., 2012). Some associated researchers have mentioned 100% morbidity of (OM) with excessive dose cytotoxic chemotherapy (Rodríguez-Caballero *et al*., 2011). Also (OM) has been reported between 60% and 100% in patients undergoing head and neck cancer getting radiotherapy (Nottage *et al*., 2003; Trotti *et al*., 2003), but the incidence of OM in patients submit to bone marrow or stem cell transplants is much higher. It reached 76% and 100% of patients rising some degree of mucositis (Castagna *et al*., 2001; Blazar *et al*., 2006; Lilleby *et al*., 2006).

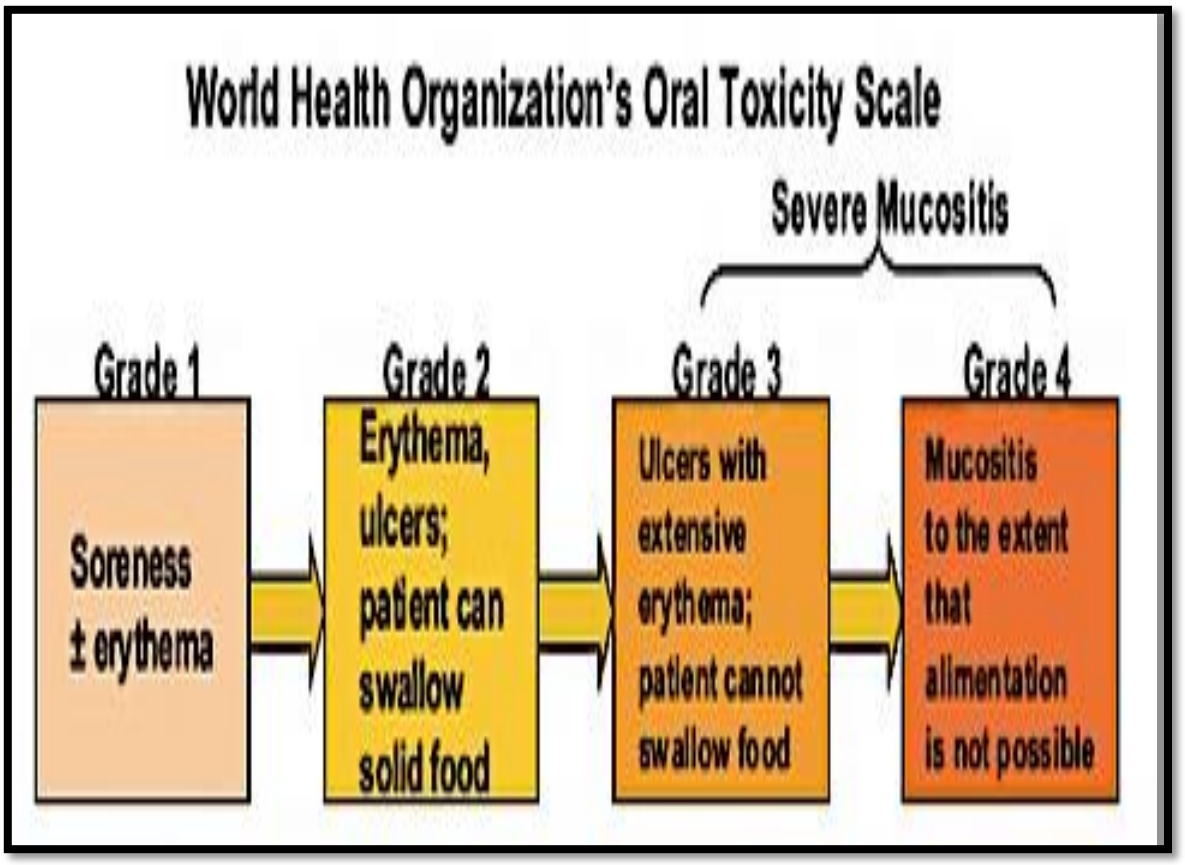
Commonly, the toxic effects of chemotherapy which begin after 4 or 5 days submit therapy with reddening of the mucosae such case is called erythema (Scully *et al*., 2006) whereas ulceration begins between 7 to 14 days post chemotherapy and usually takes place on non-keratinized mucosal surface inside the mouth including buccal mucosa, the soft palate, floor of the mouth and tongue. OM usually starts to solve among days 14 and 17 without the presence of infection (Scully *et al*., 2003; Ramirez-Amador *et al*., 2010).

Several factors had been credited to both remedy and affected person characteristics that could impact the grade, occurrence and intensity of OM, those included intrinsic related to the patients and those associated with the therapy course (Lalla *et al.*, 2008; Allen *et al.*, 2018). Many factors include the type, dose and program of whole cytotoxic drugs delivered like 5-FU conduct are related with OM in concerning 40% of patients by way of affecting DNA synthesis are mainly stoma toxic (Popescu and Norman, 1999). Those treatment factors which can affect the incidence and (OM) severity will increase when the intensity of therapy increases, whereas patient's related factors included age has more effect in children and younger patients revel in much recurrent and debilitate mucositis because they have got excessive cellular turnover. Also old age affected person additionally increase higher mucositis because of deficient DNA repair (Balducci and Exterrman, 2000). Moreover gender, nutritional state, variation in salivary output and composition, bad oral health, mucosal trauma (Kwitkowski *et al*., 2010), pre-existing medical conditions, Poor dental health mainly periodontal disorder has been recognized as an environmental aspects which could raise the severity of OM as well as other factors related to patients such as genetics, the kind of malignancy, pharmacogenetics, smoking and oral hygienic (Keefe *et al*., 2007).

1.4.2. Diagnosis of OM

OM is identification is primarily based on its appearance, clinical characteristics, debut of signs and localization. There are several mucositis assessment scales which also established instruments for recording the rang and severity of OM, World health organization (WHO) used a common and most popular scale for chemotherapy, common place toxicity criteria of national cancer institute "NCI" for chemotherapy and radiotherapy, which contains grades 0-4 (Grade 0 point to "no changes" while Grade 4 points to "ulcers", so alimentation is not possible due to OM (Fig1.1).

Oral mucositis assessment scale "OMAS" is a recent device evolved to evaluate details anatomical sites of mucositis and can be suitable for multi-disciplinary health care groups and is a goal scale appropriate for academic research which measures erythema and ulceration under scales of 0–3 for ulcers and 0–2 for erythema (Grade 0 agrees to normal, grades 2 and 3 are related to sever erythema and ulcers more than 3cm respectively) (WHO, 1979; Lalla *et al.*, 2008; Maria *et al.*, 2017).



(Figure1.1) World health organization oral toxicity scale for oral mucositis (Maria *et al*., 2017)

1.4.3. Induction of oral mucositis in animal models

Various animal studies have been widely used in research to investigate oral mucositis and gastrointestinal mucosal toxicity triggered by cancer remedy to get sensible image of human disease (Bowen et al., 2011). Some of animal species (rats, hamster and mice) have been provided as useful equipment to study the numerous mechanisms under chemotherapy and radiotherapy induced mucosal damage, because of their applicability and the great sample size that can be used. Sonis *et al*., (1990) have published the first chemotherapy mucositis animal model concerned the use of golden hamster and reiterated intraperitoneal injections of 5-fluorouracil (5-FU) followed by mechanical scratching to the buccal mucosa to induce ulceration. Histological and clinical assessment confirmed that the changes that take place in the mucosae were similar to that visible in the human with mucositis. Actually, popular chemotherapeutic agents used to set off mucositis include (cisplatin) and 5-FU (Dorr *et al*., 2005) other than a less popular model includes mucosal injury with acetic acid.

Early researches using hamster as a model for mucositis examined the biology of mucositis and the capability of mucosa to damage whereas other number of researches used hamster model to investigate the roles of cytokines as epidermal growth factor (EGF) and transforming growth factor- β3 (TGF-β3). Sonis *et al*., (1992) demonstrated that the (EGF) is a protein that stimulates the growth and differential of epithelial cells that susceptibility to mucosal damage may be related to the rate of epithelial cell duplication by way of stimulating epithelial basal cell transformation rate through management of (EGF) and mucosal injury was elevated, so assisting the idea that the epithelial transformation impacts the susceptibility of the mucosae to chemotherapy .

At the same time (TGF-β) is a regular growth factor that has capability to reversible catch proliferating cells in G1 phase of the cellular cycle, Sonis *et al*., (1994) showed that the topical application of TGF-β3 to the OM of hamster previous to conduct of 5-FU minimize different parameters associated with mucositis such as intensity, occurrence, duration and decrease of weight assigned to chemotherapy. Thereafter, other study showed the proliferation in basal epithelial cells by measuring the proliferating cell nuclear antigen (PCNA) confident that the topical applications of (TGF-β3) considerably decrease replication of basal.

Different studies have employed the animal model to demonstrate complications of mucositis pathology together. Leitão *et al*., (2008) examined the role of nitric oxide (NO) at the pathogenesis of (OM) Sonis *et al*., (2004) were examined (COX-2) expression in experimental (OM) triggered by radiation. Other studies explained the role of interleukin-1 β (IL-1β), interleukin-6 (IL-6) as well as tumor necrosis factor (TNF) in (OM) brought on by radiation alongside with evolution of mucositis (Moller and Villiger, 2006). Additionally Cho *et al*., (2006) used topical granulocyte-macrophage colony stimulating factor (GM-CSF) to evaluate the role of pro-inflammatory cytokines in the progress of mucositis.

Subsequent studies have described possible remedy techniques for OM as the usage of fibroblast growth factor-20 (FGF-20) or velafermine to keep mucosal safety of experimental radiation and chemotherapy triggered mucositis (Alvarez *et al*., 2003).

1.4.4. Pathogenesis of OM

Cytotoxic chemotherapeutic agents were affecting directly on the highly mitotic basal epithelial cells, injured additional epithelial cells leading to atrophied and ulceration of mucosal layers (Sonis, 2010). Furthermore the anti-neoplastic agents not just impact the cancer cells but also the normal cells of the body and induced cell death by apoptosis. However, the idea of OM was simplified since renowned there after involving premature activation of genes and endothelial cells injure or early over-expression of pro-inflammatory cytokines subsequent chemotherapy (Logan *et al*., 2008; Sonis, 2011). The mucositis is a complex procedure within the epithelium and sub-epithelial connective tissues so the (OM) pathogenesis summarized in 5 phases (fig1.2) Sonis, (2009):

1.4.4.1. Initiation phase

The initiation stage of tissue damage appears immediately after the administration of chemotherapy or the exposure to the radiation trigger –direct DNA damage resulting harm and death within the cells of basal epithelial layer, sub mucosal layer and to endothelial cells, then break of DNA strand overlap with normal cellular functions of cells, also endogenous damage and related molecular patterns released by damaged cells join to particular receptors and begging inflammation and toxicity (Blijlevens and Sonis, 2007). Moreover oxidative stress forming reactive oxygen species (ROS) within injured cells can later straight damage cells, other tissues as well as blood vessels, (ROS) also affects on tissues to induce transcription factors in the subsequent phase. However the mucosa seems normal in this stage, a cascade of actions initiate in sub mucosa that finally led to mucosal damage (Sonis *et al*., 1995).

1.4.4.2. Prime injure response "Up-regulation and activation"

Several events arise through this phase of OM pathogenicity , radiation and chemotherapy with (ROS) together activate numerous transcription factors following DNA injure and lead to apoptosis (Boers-Doets *et al*., 2012). Nuclear factor kappa- (NF-κB) is one of the most significant factors due to the fact once activated and will up regulated numerous genes which affect on injure and mucosal toxicity that cause an increase pro inflammatory cytokines involving tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) (Boers-Doets *et al*., 2013; Castellani *et al*., 2014).

The injury which is extended to connective tissue and endothelium, subsequently give rise to damage and apoptosis of basal epithelial layer. Also the activation of metalloproteinase enzymes (MMPs) led to injuries in fibroblasts as a result of chemotherapeutic agents which leads to the damage of collagenous sub epithelial matrix and destruction basement membrane of epithelial cells. Thereafter, macrophages are activated by fibronectin fragments which lead to stimulation of matrix (MMPs) in turn cause raised of TNF-α (Bamba *et al*., 2003).

1.4.4.3. Signal enlargement phase

The events of previous two stages are overlapped so the pro inflammatory cytokines expand the mucosal injure that is started via chemotherapy and radiation by direct effect and additionally indirectly by enlargement and signaling mucosal damage, for instance, TNF-α is highly capable stimulator of numerous pathways that caused tissue damage, comprising the ceramide and caspase paths and activates the transcription pathway mediated via NF-κB (Sonis, 2007). This signaling procedure may give rise to excess production of additional pro inflammatory cytokines like TNF-α, IL-1β and IL-6 through plus feedback loop, (MMPs) enzymes also activated by the above signaling

pathways in the epithelial cells and connective tissue that cause injures tissue and enlargement of above indicators.

1.4.4.4. Ulcerative phase

The ulcerative phase of OM is the most important phase which is characterized by whole epithelial destruction in the affected person after the chemotherapy and radiotherapy remedy, is comprise damage of mucosal safety results highly painful sores and microbiological colony via oral microbes, ulcerated mucosa provide entrance for microorganisms to initiate secondary infection inside the mouth into systemic flow causing life-minatory. Moreover, the product of bacteria attack sub mucosal tissues and stimulates macrophages to release more pro inflammatory cytokines that cause more inflammation and apoptosis (Sonis, 2004).

1.4.4.5. Healing phase

The final stage of (OM), ulcers restore to health spontaneously, even though this technique is else biologically energetic, indicators from sub mucosal extracellular matrix region (ECM) contribute the average of squamous epithelial cell migration, the rate of proliferation and differentiation, leading to mucosal restoration (Jensen *et al*., 2013), re- establishment of microorganism flora of oral cavity, return normal number of white blood cell count and lack factors that intervene with injury cure like inflammation and mechanical scratching. The tissue appears normal in shape and function, but epithelial tissue modifications secondary to cancer remedy stay as it is and there's remaining angiogenesis raising the patient's threat of evolving OM for next courses of anticancer remedy (Hensley *et al*., 2009).

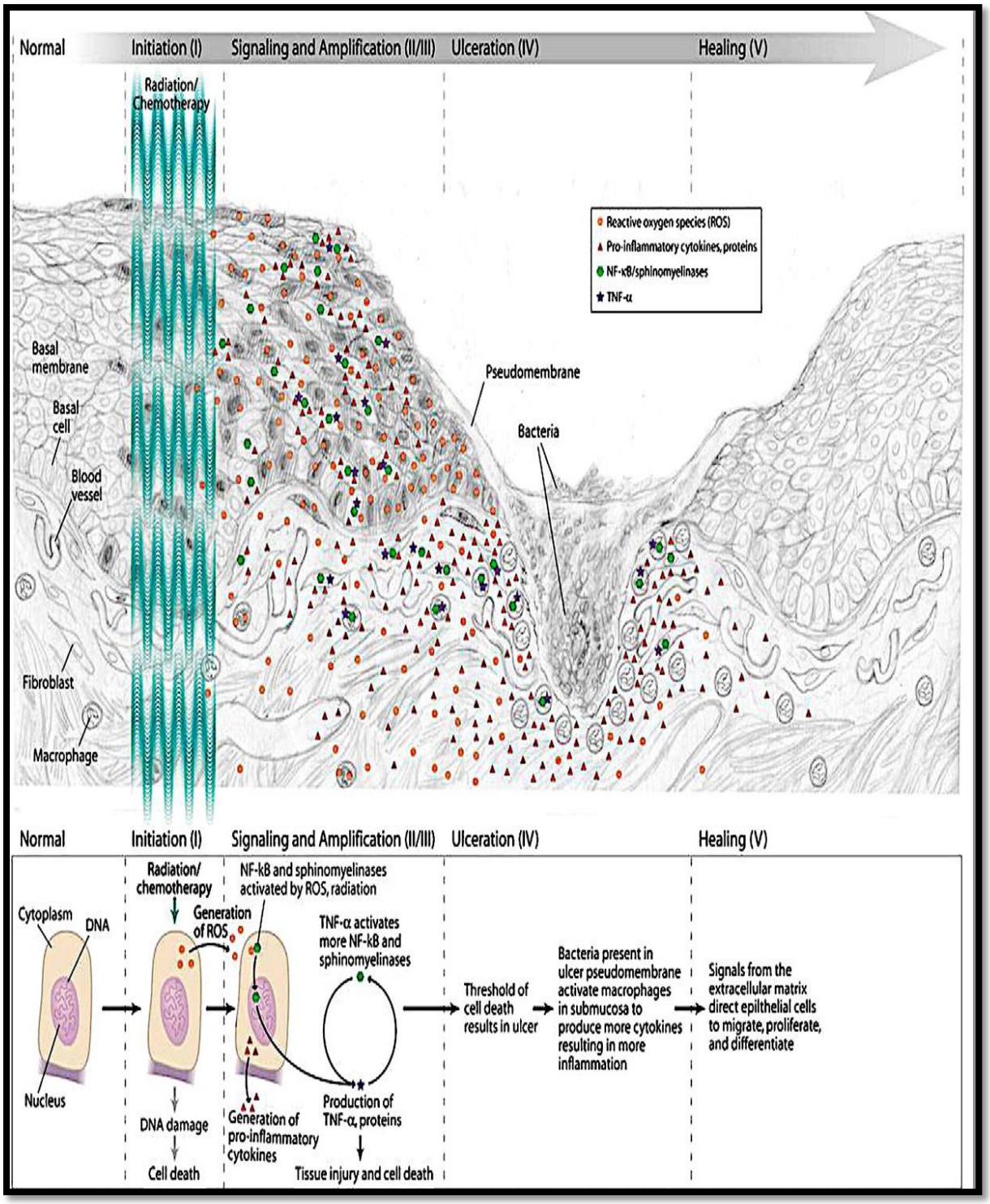


Figure (1. 2) the pathogenesis of OM comprising five phases.

(Sonis, 2009)

1.5. Oral mucosa toxicity following chemotherapy:

Generally, mucositis toxicity is a result of two main mechanisms including, direct toxicity because of the treatment and myelosuppression that outcomes from remedy (Sonis *et al*., 1989). Also, the epithelial cells in oral mucosa submit fast turnover, cells regeneration and epithelial maturing averages, which make these cells liable to the side effect of cytotoxic chemotherapy (McCarthy *et al*., 1998). Together radiation and chemotherapy can reduce the average of epithelial cells regeneration, causing mucosal atrophied, localized or widespread inflammation and mucosal ulcers (Chan *et al*., 2003). Indirect toxicity not just changed mucosal integrity, but also alter microbial flora of the mouth by the indirect infestation of microorganisms in the mouth, it also changes the amount and structure of saliva, epithelial maturing and raising the risk of infection which is preserved via impervious unbalances particularly leucopenia (Turhal *et al*., 2000).

Early studies by Lockhart and Sonis, (1980) comprising post-mortem checking buccal mucosae of human notified important epithelial atrophy in affected person who had get cytotoxic chemotherapy at the same time as they had been contrasted to uncured individuals. Gibson *et al*., (2006) demonstrated that apoptosis was rise following chemotherapy in human oral epithelium. This was correlated with additional histological alterations involving cytoplasmic vacuolation, damage of membranes; connect with adjacent cells, multi-nucleation of supra basal cells, loss of cellular cytoplasm and hemorrhage.

1.6. Fluorouracil (5-FU)

Fluoropyrimidine is a synthetic anti-metabolites agent that is still used in cancer therapy, it is a cytotoxic drug, which recognized to possess immunosuppressive actions, 5-FU is broadly indicated for remedy of malignant tumors, mainly of colon or rectum, breast and in the remedy of gastric, primary hepatic, uterine, pancreatic, bladder and ovarian carcinomas (Mead, 2002; Inoue and Horii, 2002). (5-FU) is the first fluoropyrimidine drug was intended reasonably, its main effect is to inhibit nucleotide metabolism, Rutman *et al*., (1954) showed that uracil was taken up and united to a much larger amount in most cancerous tissues than in normal tissues.

1.6.1. Chemical structure for (5-FU)

5- FU is an analog of uracil with a fluorine atom at the C-5 location in place of hydrogen (fig1.3), the Vander Waals radius of the atom likes that of hydrogen which permits the molecule to imitate uracil biochemically (Chabner *et al*., 2005). 5-FU quickly gets in the cell employing the similar facilitated transportation method as uracil and intracellularly submits to the same anabolic and catabolic responses like uracil , with the exemption of the methylation at location 5, stimulated by thymidylate synthase (TS), it is most significant goal of 5-FU in tumor cells.

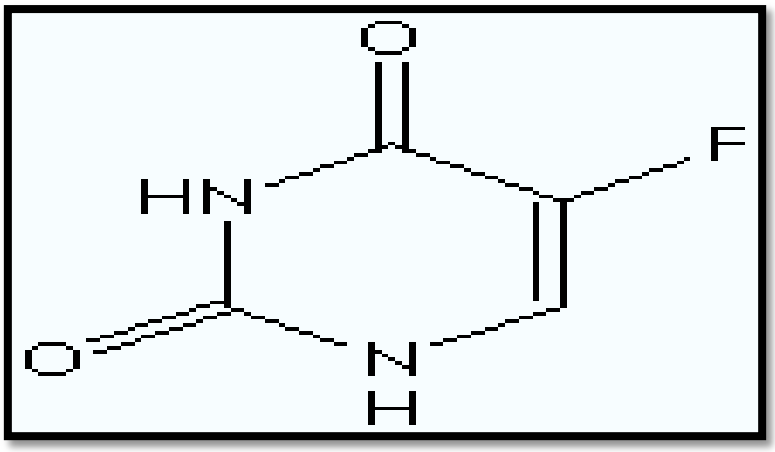


Figure (1.3) The Chemical structure of 5-FU

(Chabner *et al*., 2001)

1.6. 2. Biological activity of (5-FU):

cytotoxic impact of 5-FU resulted by transformed one of its nucleotides, this activation is stimulated by the enzymes contributing in the normal pyrimidine metabolism of cells, the existent differences among cancer cells and normal cells in the quantity, activity of these enzymes and their substrates give 5-FU tumor eclectic cytotoxic vitality. Furthermore, the level vitality of metabolizing enzymes in the tumor cells may well expect the reply to fluoropyriemidine remedy, which would be a main support to the individualization of chemotherapy (Akiyama *et al*., 2004; Kobayashi *et al*., 2005). Several modification have been suggested to increase the 5-FU anticancer activity for clinical resistance and to save normal cells from 5-FU triggered damage, the main one consists of decrease the degradation or increasing the activation of it and enhancing the binding of 5-fluoro-2'-deoxyuridine-5'- monophosphate (FdUMP) the main active metabolite of 5-FU to thymidylate synthase (TS) the principle goal of fluoropyrimidines, the final technique has led to clinical applications of leucovorin (LV) like a biochemical

modulator of 5-FU (Andre *et al*., 2004; Noordhuis *et al*., 2004). Regarding to the selective defense of normal tissues from 5-FU damage, uridine was investigated due to the association among the integration of 5-FU into RNA and cytotoxicity (Connolly and Duley, 1999).

1.6.3. The Pharmacokinetics of 5-FU

5-FU is a weekly absorbed from GIT after oral administration but it is well absorbed after intravenous injection (I.V) either like bolus or a continuous infusion and intraperitoneal (I. P), the highest peak in plasma concentration was (0.1 to 1.0 mM) occurs after (IV) administration, while the lowest concentration occurs after continuous (IV) infusion for 24 to 120 hours (Pratt *et al*., 1994). The drug is rapidly dispersed to tissues and extracellular fluid, incorporating GIT and oral mucosa, bone marrow, liver, brain and neoplastic tissues (Danesi *et al*., 2001).

5-FU elimination was fast with obvious half-life approximately 5 to 30 minutes, because primarily of catabolism by the initial and rate-restricting step which is stimulated via dihydropyrimidine dehydrogenase enzyme (DPD) found mainly in the liver and several other tissues such as tumor cells and GIT mucosa, so 5-FU reduces to 5, 6-dihydro-fluorouracil by DPD, a therapeutically inactive resulting eventually degraded to α-fluoro-β-alanin, renal eliminated of unaffected 5-FU in 24 hours quantity to just 5 to 20% of a single intravenous dosage (Chabner *et al*., 2001).

1.6.4. Mechanism action of 5-FU

Oral mucositis correlated with the generation of ROS and induced DNA injure in both normal tissues and cancer cells, debilitates the metabolism in progenitor cells and lead to inhibition of mitosis and increase of apoptosis (Ahmed *et al*., 2013; Al-Refai, 2014; Koizumi *et al*., 2017). The mechanism of

5-FU cytotoxicity has been summarized that it obtains its vitality in the body by first transformation into fluorouridylate (FUMP) and then transforming into a kind of 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP). This metabolite forms the trio-complex, correlating with thymidylate synthase (TS) and N5-10 methylene tetrahydrofolate, a cofactor with folate then inhibit (TS) enzyme, ultimately, thymine production is disordered and DNA synthesis blocks after TS enzyme production decreases in a cell (Correale *et al*., 2003).

Furthermore, imbalance between FUMP- figure from fluorouracil in the body and inhibition of DNA production and mend will outcome breaks in single or doubled strands in the DNA molecule, triggering of DNA injure will activate downstream genes and protein synthesis that start the processes of cell cycle arrest and cell death (Kuilenburg, 2004; Chabner *et al*., 2005; Milano *et al*., 2005).

Though, inhibition of TS is the main significant mechanism of the cytotoxicity of 5-FU which have the ability to be integrated with RNA and DNA and causes cell death (Lönn and Lönn, 1988; Peters *et al*., 1995). Integration of FUMP into RNA impacts the normal RNA processing and functions, in addition to important associations among 5-FU misincorporation into RNA and loss of clonogenic possible in human colon and breast cancer cell lines (Miura *et al*., 2010). Additionally, the metabolite structures overlaps with central dogma of the hereditary materials (RNA and DNA), which is critical for cellular homeostasis and finally results in cell toxicity (Chibber *et al*., 2011). Inflammation and oxidative stress are the significant factors correlated with poisonous appearance and syndrome pathogenesis of chemotherapeutic drugs (Conklin, 2000; Conklin, 2004). Moreover, excessive product of ROS and inflammatory mediators causes injure or alteration of cell macromolecules performing in low or up expression of organizers of ordinary cell physiology (Chang *et al*., 2012; Yoshino *et al*., 2013).

1.6.5. Side effects of 5-FU

5-FU can be used with radiotherapy in the primary remedy of head and neck cancer, lung and esophageal tumors (Kayaalp, 2000). Additionally, the employ of 5-FU is one of the majority common causes of OM and ulceration, which outcomes in delay dose decrease or stopping of chemotherapy, that occurs in more than 40% of states (Sonis *et al*., 2004). Other common adverse effects of 5-FU are gastrointestinal disturbances like nausea and vomiting, anorexia, weight loss and bone marrow decline, whereas dose restrictive side effects are stomatitis, esophagopharyngitis and diarrhea (Loury *et al*., 1999; Bowen and Keefe, 2008).

Leucopenia is the most important side effects noticed through chemotherapy, immediately affecting the permanence of the chemotherapeutic remedy (Mehta and Malik, 2006). Numerous authors believe that leucopenia secondary to chemotherapy because one of the main causes of the anticancer remedies stopping that confirm the require to check the immune capability of the patients during a complete blood count (CBC) through the course of remedy (Sonis, 1998; Lima *et al*., 2004).

1.6.6. Toxicity of 5-FU

5-FU is able to cause cell injure separate from its affect or inhibits together DNA and RNA production causing obvious cell death and apoptosis, like inducing lipid peroxidation as increase malondi-aldehyde (MDA) is ultimate product of lipids oxidation (Haque *et al*., 2011), results elucidate that elevated of MDA activity after administration of 5-FU proposes its role in gastrointestinal injure and shows its powerful oxidative stress and inflammation (Davies *et al*., 2008; Durak *et al*., 2009).

The toxicity of 5-FU has occurred in proliferative tissues by suppression of DNA and RNA synthesis and creation of apoptosis, reduced propagation of mucosa cells , injury their cellular regeneration and led to important reduction of (PCNA) immunoexpression of mucosal cells (Lotfy and Zayed, 2009; Won *et al*., 2013; Al-Refai, 2017). In addition, 5-FU was joined in a significant decrease of anti-apoptotic immunoexpression of (Bcl-2) positive cells, because that inhibition of DNA production, DNA injure and production of (ROS) through chemotherapy harm the metabolism in progenitor cells as well as inhibition of mitosis and raise of apoptosis (Bhaskaran *et al*., 2012; Curra *et al*., 2013).

Moreover, various agents support the participation of oxidative species for OM managing and can prevent the injury or decrease its severity, or infection manager included anti-microbial agents, magnesium hydroxide, hydroxypropyl cellulose, complementary amino acids, growth factors, topical anesthetic, vitamins, antioxidants, corticosteroids and recently low level of laser therapy (Lima *et al*., 2005; Davarmanesh *et al*., 2013). So, increasing new remedies for OM with larger activity and lower side adverse is extremely ensured.

1.7. Glycine supplementation

Generally, glycine is the most vital and simple non-essential amino acids in animals, human and several mammals, it was isolated from acid hydrolysates of protein in 1820 by H. Braconnot (Wang *et al*., 2013). Glycine is created by alkaline hydrolysis of meat and gelatin with potassium hydroxide; chemically it is synthesized from monochloroacetic acid and ammonia and instituted the formation of glycine (Wu *et al*., 2013).

Glycine has very important roles in metabolism and nourishment of several mammals and humans, usually about 80% of the whole body glycine is used for protein synthesis for growing individual body (Yan and Sun, 1997). It plays a critical role in central nervous system as neurotransmitter, and therefore controlling ingestion of food manner and entire body homeostasis. Also, glycine controls the immune function, by output of superoxide and synthesis of cytokines through changing the intracellular Ca2+ levels. It is simplified the conjunction of bile acids in human, so it does not directly play a critical role in assimilation and digestion of lipids and lipid soluble vitamins (Rajendra *et al*., 1997). Moreover, to do its actions, glycine binds to diverse receptors but the most studied is anion channel receptor (GlyR), which is a (Cl-) choosy channel for bind glycine to (GlyR), a mechanism broadly assumed to elucidate the beneficial impacts of glycine by activation of the glycine-gated (Cl-) channel (Pan et al., 2005).

Communally, glycine has critical action in cyto-protection, growth, immune reaction, metabolism, evolution and existence of individuals and several other mammals. In remedy of patients, glycine has been described to have more positive impacts comprising defense against toxicity triggered via oxidative stress, anoxia with numerous toxic factors of entire body levels at the cells and tissues (Gundersen *et al*., 2005).

1.7.1. Synthesis of glycine

Glycine is synthesized in humans, pigs and other mammals while the biochemical researches on rats demonstrated that glycine is synthesized from threonine (by threonine dehydrogenase way), choline (through forming of sarcosine) and serine (via serine hydroxymethyltransferase). After that, in additional investigations, it was demonstrated that the glycine formation in humans and other mammals is during the three ways aforementioned (Ballevre *et al*., 1990).

Within current studies, it was stated that substrates intended for glycine formation are hydroxyl proline and glyoxylate in humans and mammals (Wu *et al.*, 2011).

1.7.2. Antioxidant and anti-inflammatory roles of glycine

Supplementation of glycine with suitable dose is so successful in lessening numerous metabolic disturbances in individuals such as several inflammatory diseases, diabetes, cancers, arthritis, ulcers and obesity; thus, glycine is antioxidant that suppresses the manufacture of (ROS) when fat succumbs oxidation and through the propagation of free radical reaction (Jacob *et al*., 2003). It is firstly located in the cell and organelle membranes where it can exert its maximum defensive effect, which acts as the primary line of defense against lipid peroxidation (Oku *et al*., 1995). Senthilkumar *et al*., (2004) reported that the glycine can as well fight against free radical intermediate oxidative tension in hepatocytes, plasma and erythrocyte membrane of individuals and animals with liver damage. Furthermore, glycine is a fundamental constituent of glutathione, which is required for detoxification; therefore, glycine has indirect impacts as scavenger of free radicals (Bilzer *et al*., 2002). Several recent studies have demonstrated the capability of glycine to decrease chemotherapy related damage, in vivo model of chemotherapy related liver damage in a clinically pertinent, glycine reduced liver injure, raised hepatic microcirculation and decreased micro vesicular steatosis (Mikalauskas *et al*., 2011).

Glycine plays an important role as anti-inflammatory agent by inhibiting the inflammatory intermediaries via decreasing the activation of (NF-κB) and (TNF-α), and reducing free radicals and toxic mediators (Stoffels *et al*., 2011). Supplementation of glycine exhibits significant biological actions by increase microcirculation and supporting in the suppression of (TNF- α and IL-1b); these

inflammatory molecules are concerned with the triggering of apoptosis (Figueiredo *et al*., 2009; Kim *et al*., 2010).

Other studies suggested glycine as a beneficial remedy for several types of inflammatory procedures (Weeler *et al*., 2009; Carmans *et al*., 2010).

Glycine has supplied useful impacts against toxicity of liver and inflammation in rats. 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 (Kallakuri *et al*., 2003; Zhong *et al*., 2003).

Additionally, glycine is employed in remedy of cancer diseases like melanomas; moreover safety supplementation of this simple amino acid in humans and other mammals was proved (Li and Wu, 2018).

Chapter Two

Materials & Methods

Chapter Two

Materials and Methods

2.1. Materials

2.1.1. Equipments and Instruments

The following equipment's and instruments were used with their sources indicated in table (2.1) below.

Table (2.1): The equipments and instruments used with their sources.

Equipments and instruments	Providers
BCL-2 Kit	Detection, Dako, Denmark
Centrifuge	Jenetzki, Germany
Digital camera	Sony, Japan
Disposable syringe (1, 5, 10 ml)	Changzhou, china
Drier Section	ISUZU, Seisakusho \ Japan-LTD
Electron sensitive balance	OHAUS CORP, USA
Eppendorf tubes	Beer giraffe, China
Glass slides, cover glass (0.13-0.18mm)	CE – China
Hematology analyzer	Genex-USA
Hot plate	Photax, UK
Microtome	Micro me-England
Imaging Microscope	Leica, Switzerland
Incubator	Fisher-Germany
Rotary microtome	Thermo \ England
Oven	Memmert- Germany
Scale Pocket	CE-China
Scissors, Forceps, Clamp, Scalpels	AFCO-Jordan

Spectrophotometer	CE-Cell French
Syringe with 18 gauge needle	Korea
PCNA Kit	Detection, Dako, Denmark
Water bath	Seisakusho\ Japan-LTD
Vacuum	UL, Korea

2.1.2 Chemicals

Table (2.2): The chemicals used in the present study and their providers

Chemical	**Providers**
Absolute ethanol	Scharlan, Spain
Formalaldehyde 40%	Memmert - Germany
5-flourouracil (5-FU)	Sigma-Aldrich, USA
Chloroform	Scharlan, Spain
Canada balsam	Merk, Germany
D. P. X	Merch chemical, Germany
Eosin stain	Scharlan, Spain
Haematotoxyline	Scharlan, Spain
Hydrochloride acid (HCL)	Merch chemical, Germany
Paraffin wax	Scharlan, Spain
Thiobarbituric acid (TBA)	Merch chemical, Germany
Thymol crystals	GCC, England
Xylene	Bio, Germany
Normal saline 0.9 Nacl	Adwic ,Egypt
Glycine	BDH England
Xylazine& Ketamine hydrochloride	Korea

2.2. Methods

2.2.1. Experimental Animals

About 96 adult males rats (*Rattus norvegicus*) weighting (200-250) gm and aged (10-12) weeks were used, the rats were housed and breed in isolated plastic cages at the animal's house of Biology department, Collage of Science, Basra University, under strict clean, hygienic and standard managing conditions at temperature (20-25°C), controlled room on a (12:12) hour light\ dark cycle with the humidity rate was 50%. All animals were kept for one week for acclimating with novel location before the beginning of the experiment, allowed palate common nutrition and drinking water *ad libitum.*

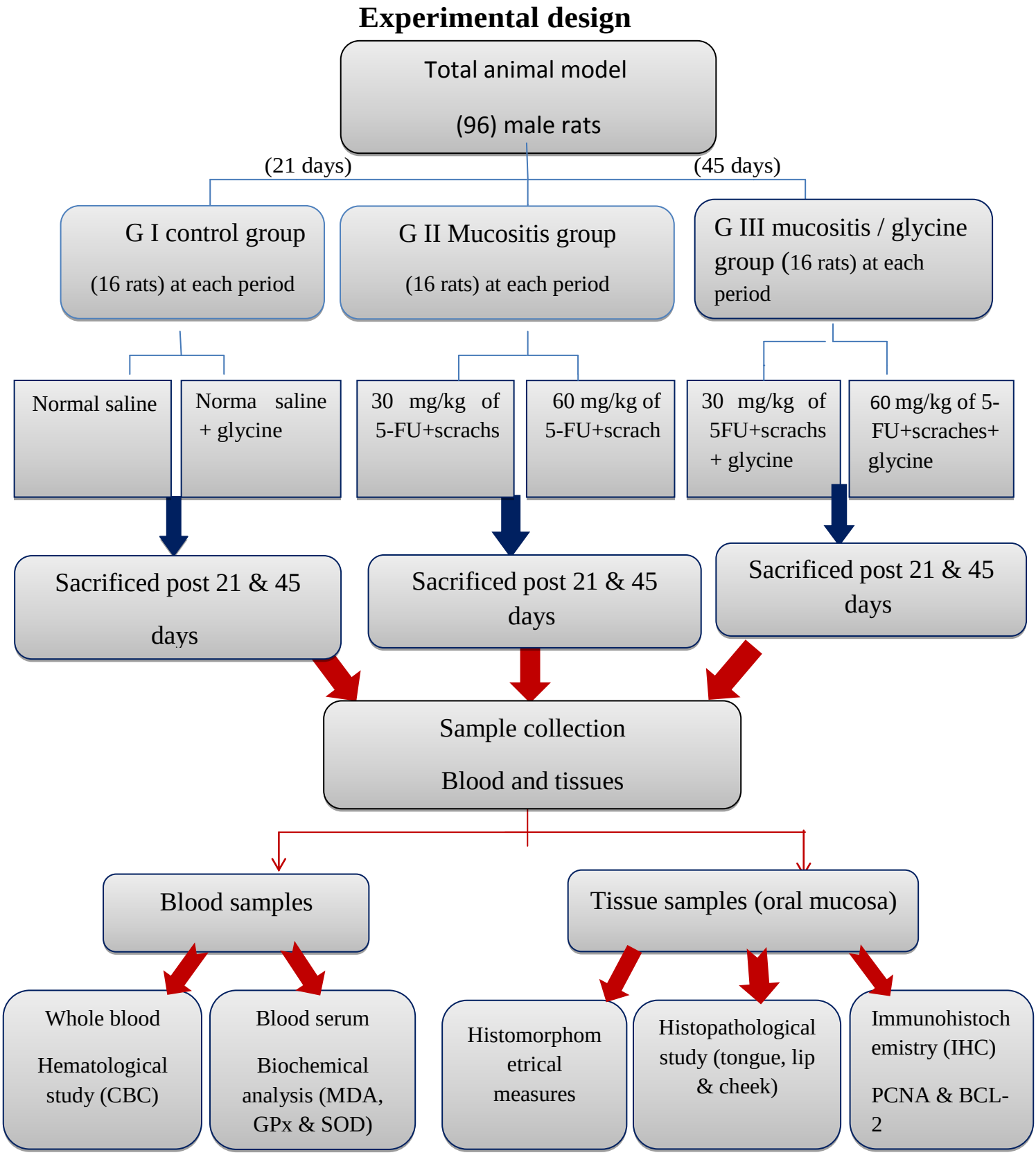


Figure (2.1): Flowchart representing the design of the study

2.2. 2. Experimental design

The animals were divided randomly into two main experimental groups, each group subdivided into three subgroups for two periods (21, 45 days) with (48) male at each period; each group was subdivided in 3 subgroups:

The subgroups of the first main group (16 animals each) were treated as the following:

Group I (Control): Rats of this group further subdivided into two subgroups:

Subgroup IA: each rat injected intraperitoneal (IP) with 0.5 ml normal saline on days (0, 5, 10, and 15).

Subgroup IB: each rat injected intraperitoneally with 0.5 ml saline on days (0, 5, 10, and 15) as the vehicle of 5-FU and glycine for 21 days.

Group II (Mucositis): Rats of this group also subdivided into two subgroups:

Subgroup IIA: OM in this group was induced according to protocol by Lima *et al* (2015) 30 mg\ kg of 5-FU was injected intraperitoneally to each animal on days 0, 5, 10 and 15. The tongue, cheek and lip mucosa were irritated by external superficial scratches with tip of an 18- G needle on days 3 and 4 after anesthetized the animals by xylazine (3mg/kg) and ketamine hydrochloride (90mg/kg) to trigger mucositis.

Subgroup IIB: OM was induced according to protocol suggested by Sonis *et al* (1990) and modified by Leitão *et al* (2007). In brief, 60 mg\kg of chemotherapy drug 5-FU was injected intraperitoneal (IP) to each animal on days (0, 5, 10 and 15) with same irritation on oral mucosa.

Group III (Mucositis\ glycine): Rats further divided into two subgroups:

Subgroup IIIA & subgroup IIIB: The animals received a 2mg\g of body weight IP injection of 5% glycine diluted in normal saline (Sa *et al*., 2013), which was followed by injection of 5-FU in same dose and for the same period as subgroup IIA and IIB, to determine the effect of glycine on OM, treated group with glycine started on day 0, with usage single dose daily at the morning until the end of experiment on day 21

The subgroups of the second main group (16 animals each) were also treated as the following:

Group I (control): Rats of this group further subdivided in to two subgroups:

Subgroup IA: each rat's injected IP 0.5 ml normal saline given one times weekly for 45 days.

Subgroup IB: each rats injected IP with 0.5 ml saline one time\ week as the vehicle of 5-FU and glycine for 45 days.

Group II (Mucositis): the animals were subdivided into two subgroups:

Subgroup IIA and subgroup IIB: OM was induced according to Chang *et al* (2015), 30mg\ kg and 60mg\ kg of body weight respectively were injected IP to each animal in those subgroups one time\ week for 45 days. Some irritation by external superficial scratches with tip of an 18- G needle made on the tongue, cheek and lips on days (14 and 15) after anesthetized the animals with xylazine and ketamine to induce OM.

Group III (Mucositis / glycine): Rats were subdivided into two subgroups:

Subgroup IIIA and Subgroup IIIB: The animals received 2mg /g of body weight by IP injection of 5% glycine diluted in saline, which was followed by the injection of 5-FU with same dose and for the same period as

subgroups IIA and IIB. Treated with glycine started on day 0, single dose / day at the morning for 45 days (the end of experiment periods), all experimental rats were anaesthetized with chloroform, the thoracic cavity was opened by midline incision for blood samples collection while tissue samples were taken from oral cavity (fig 2.1)

2.3.1. Collection of blood samples

Blood sample (5) ml was collected after anesthesia via cardiac puncture by using disposable syringe from each animal. Each sample was separated into two portions. The blood were poured into plan tubes to be centrifuged at (3000) cycle\min for 15 min, to get the serum which then transferred into several Eppendorf tubes. Serum may be used directly or may be stored at (-20°C) for biochemical analysis, and the blood of (EDTA) tubes was employed immediately for determination of hematological parameters.

2.3.2. Tissue specimens

Tissues of oral mucosa which included tongue, cheeks and lower lip were removed carefully from all groups post (21, 45) periods, washed with normal saline, prepared for macroscopic examination and recorded the morphological features. Specimens from each sample were fixed in 10% neutral formalin fixative for histological and immunohistochemically analysis.

2.4. Study of parameters:

2.4.1. Determination of animal's body weight

Body weight and food intake were observed frequently in each experimental groups during the different periods of the experiments, all animals were being weighted weekly starting from day 0 until the end of experiment, the average body weights for each group was recorded throughout the trail and just before killing the animals, results expressed as(mean ± SD).

2.4.2. Biochemical study

2.4.2.1 Estimation of serum Malon Dia Aldehyde (MDA)

MDA serum as one of the major product of lipid peroxidation was measured according to the method formerly performing by (Tanideh *et al*., 2014).

Procedure:

Sample (µl)	Thiobarturic acid (0.375 gm) diluted in Hydrochloric acid (0.25m) (µl)	Trichloro acetic acid (15%) (µl)
200	1000	4000

Set sample tubes at water bath 100ºC for 15 min, after that, cool sample tubes and centrifuge for 5 min, read the supernatant at a wave length in the 532nm rang, read the blank (0.2 ml distilled water instead of sample) at the similar wave length.

Calculation of MDA

Malondialdehyde (micromoles/L) = (AT-AB) × 1000000)/ (E × L)

MDA conc. (micromoles/L) = (AT-AB) ×1000000) / (1.56 × 10)

Where:

AT= absorbance for test at 532 nm.

AB= absorbance for blank at 532 nm.

L= cuvette length = 1cm

E= extinction coefficient= 1.56 × 10000 /*M. cm.*

2.4.2.2. Estimation of glutathione peroxidase (GPx)

Glutathione peroxidase as antioxidant enzymes was determined by manual GPx assay according to (Fairbanks and Klee, 1994) method.

Procedure:

Step 1

Sample µL	Phosphate buffer (0.1M,ph7.4) µL	GSH (2mM) µL	Sodium azide (10mM) µL	H2O2 (1mM) µL
300	300	200	100	100

Incubate reaction mixture for 15 min in a water bath at 37ºC.

Mixture µL	5% TCA µL
1000	500

Tubes were centrifuged at 1500 cycle/min for 5 min.

Step 2

Supernatant µL	Phosphate buffer (0.1M,ph 7.4) µL	DTNB(0.4mg/ml) µL
100	200	700

After mixing, absorbance was recorded at 420 nm after 3 min.

Calculation of enzyme activity:

One unit is the amount of enzyme that catalyses the reaction of 1 μmol of substrate per minute. The activity (units/ ml) is given by the following equation:

GPx activity (U/L) = {A/T/Ɛ) × (Vt/ Vs) × (1000000)

A= absorbance between zero time and after 3 minutes

T= time (3)

Ɛ= Extinction coefficient of DTNB (6.22× 1/M. cm)

Vt= total volume= 15ML

Vs= sample volume= 0.3ML

2.4.2.3 Estimation of Superoxide dismutase (SOD):

The principle of test based on the superoxide dismutase ability to inhibit the oxidation of epinephrine to adrenochrome. Test reaction was performed at 37ºC in air.

Procedure:

1- Samples (0.1ml) were diluted in 1.8ml of 50mM carbonate buffer (PH=10.2)

2- Then mixed with epinephrine (0.1ml) and 1ml of 10mM EDTA (PH=8.0)

The absorbance was read at 480nm wavelength directly (A1) after the addition of epinephrine, after 5 minutes (A2).

Sample μl	Carbonate buffer (50mM, ph=10.2) μl	EDTA buffer (10mM, ph=8.0) μl	Epinephrine Ml
100	1800	1000	100

Calculation of enzyme activity:

One unit of SOD was defined as the amount of enzyme that inhibits the oxidation 50%. Blank sample was used in order to exclude different spontaneous degree of oxidation. The absorbance of blank sample was subtracted from the absorbance of the sample to calculate the real absorbance of each sample.

The activity (unit/ml) is given by the following equation, one unit is the amount of enzyme that catalyses the reaction of 1 μmol of substrate per minute.

Inhibition %= (A1-A2) /A1

Superoxide dismutase activity (U/ ML) = (1%/ 2/ t) × D

Superoxide dismutase activity (Micromole/ min/ ML) = (1%/ 2/ 5) × 300

1% = Inhibition %

t= time= (5)

D= Diluted factor= 300

2.4.3. Hematological study including W.B.Cs count, W.B.Cs differential count, R.B.Cs count and Hb concentration.

Procedures:

All hematological elements were determined automatically by using automated hematology analyzer in Veterinary blood cell counter (Genex, USA, Veterinary medicine/ university of Basra) (Haen, 1995). The idea in this scientific technique of counting blood components is a device including two quarters; the first is to measure white blood cells and analyzing hemoglobin which was measured via the optical density by spectrophotometer and secondly the RBCs and platelets were measured.

2.4.4. Histomorphometrical study

Estimation of epithelial thickness for tongue, cheek and lower lip, parameters were used as the following:

_The thickness of epithelium of tongue for all three layers of epithelium which comprise stratum basal, stratum spinosum and stratum granulosum was measured (the main epithelial thickness) from every tongue mucosal epithelium section.

_The thickness of epithelium of cheek and lip were measured and registered between the external surface of epithelial and epithelial crista (the main epithelial thickness) (Naidu et al., 2004) from both cheek and lip mucosal epithelial section.

Image analysis of tongue, cheek and lip tissues from all animals were examined under light microscopy employing an objective micrometer at high power magnification 40 via using the interactive measuring menu of image analyzer (Lecia, Switzerland analyzer computer system). Present in biology department of college science, Basra University. These measurements were achieved in 10 fields of each specimen.

2.4.5. Pathological study of oral mucosa

2.4.5.1. Macroscopically examination

Macroscopic evaluation of the oral mucositis severity was scored according to a previous study by Leitão *et al* (2007), with the regular points were evaluated and recorded, The criterion used to examine the macroscopic injure were as follow; 0 (normal oral cavity) ; 1(presence of erythema and hyperemia) ; 2(presence hemorrhagic areas or small ulceration) ; 3(extensive ulcerations).

2.4.5.2. Histopathological examination

Samples taken from the different parts of oral cavity as tongue, cheek and lower lip mucosa, these samples were related to control and experimental animals at the end of experiments. All specimens were collected and prepared for light microscopic study according to (Bancroft and Layton, 2013) with the following steps:

_Fixation: the example fixated overnight at room temperature, 10% formalin buffered neutral used as fixative.

-Formalin solution (10% buffered natural)

-Formaldehyde (40%) 100 ml

-Distal water 900 ml

-NaH2PO4 4.0 g

-Na2HPO4 (anhydrous) 6.5 g

_Washing and dehydration: Next fixation the tissue washed with tap water, the water extracted the fixative in order to avoid the interaction between fixative and staining materials used later, then dehydrated through 70%, 80%, 90% and 100% alcohol for 1 hour each time.

_Clearing: Subsequently, the tissue fragments were treated with a mixture of alcohol: chloroform as (2:1, 1:1 and 1:2) for one hour each followed by chloroform for 12 hours.

_Infiltration: the tissue fractions were place in fusion paraffin wax in (60^0C) overnight on auto processor Ritable-Jung.

_Embedding: all specimens were embedded in paraffin with special blocks after appropriate orientation and absent for 2 hours at room temperature, and stored in the freezer.

_Sectioning: the paraffin tissue blocks were sectioned at (3-5) μm micron thickness, by rotary microtome and raft in a (45^0C) water bath, and then sections transported on slides coated with albumin, and dehydrated overnight on a hot plate.

_Staining: the histological sections of the studying specimens were stained with routine hematoxylin-eosin stain. Later; all slides were examined with light microscopy.

-Hematoxylin and Eosin preparation

-Procedure of staining

The sections were de-paraffinized with xylene for 10 minutes, rehydration in series graded of alcohol (100%, 90% and 70%) for 2 minutes in each change, stained with hematoxylin for 10 minutes, washed with tap water, at this point the slide examined microscopically to corresponding degree of staining, increase stain was removed by de-colorizing in 1% hydrochloric acid in 70% alcohol for 5 seconds, then by tap water for few minutes, staining with alcoholic eosin for (3-5) minutes. All the sections were dehydrated in (70%, 90% and 100%) for 2 minutes in each change, then placed in pure xylene for 5 minutes, dry and mounted in D.P.X, covered and left on hot plate at 37^0C for one day.

2.4.6. Immunohistochemistry study

The paraffin blocks of tongue rats tissue were sectioned of (3-5) µm thickness were cut by microtome and float in a 40ºC water bath containing, later, sections were transferred on to glass slides (chargeable slides) appropriate for immunohistochemistry detection of anti-proliferating cell nuclear antigen (**PCNA**) and anti-apoptosis (**BCL-2)**, also allowed to dry overnight at 37ºC (Jackson and Blythe, 2013).

Staging method

Slides were dewaxed in xylene two times, 5min each time, subsequently, and transferred to ethanol alcohol (100%, 90%, 80%, 70% and 50%) for 3 min every. After that, block endogenous peroxidase activity was incubated in 3% H2O2 in methanol for 10min, then sections were washed with PBS two times, for each 5 min. Antigen epitope was expose by antigen retrieval, which commonly used antigen retrieval is a citrate buffer method. After that, the slides were coordinated in a staining container. 300 m cast of 10 mM citrate buffer, the staining container and incubate it 96-100c for 10 min into PH 6.0, then, staining container was taken to room temperature, and allowed to cool for 20 min, washed the slides with PBS for 2 times, 4 min each time.

Added 100 µl blocking buffer (fetal bovine serum 10% in PBS) on the slides and incubated in a humidified chamber for 1 h. The primary antibody (monoclonal antibody used as anti-PCNA IgG-antibody) while the primary antibody (monoclonal BCL-2-antibody) for BCL-2 antigen, both of them diluted 100µl for 1h, washed with PBS for 2 time, every 5 min. the secondary antibody employing (antibody buffer) diluted 100 µl for 30 min, after that, two washed with PBS , for 5 min every. Sav-HRP conjugates (using the antibody dilution buffer) dilution 100 µl applied to the sections and incubated in a humidified chamber at room temperature for 30 min.

The solution dilution antibody buffer (100 µl) freshly composed: (0.015 H2O2-0.05 DAB in PBS), utilized to the section to disclose the color of antibody staining about 5 min to obtain required color intensity, then it was washed with PBS for 3 times each for 2 min. The side was completed by hematoxylin for 2 min, and was rinsed in running tap water for 10 min. Slides were dehydrated of alcohol series (95%, 95%, 100% and 100%) for 5 min each, then clear the slides 3 times with xylene.

In PCNA immunostaining, the cellular place of reaction was brown color in the nuclear of the cells lining of tongue mucosa while in BCL-2 immunostaining, the cellular place of reaction was revealed brown cytoplasmic staining, then the level of PCNA and BCL-2 immunohistochemistry was evaluated using a semi quantitative immune reactive score (0-3) accorded to the percentage of positive stain cells (PS) (0-3) points, and the intensity of staining (SI) (0-3) points, total score (TS) was calculated by adding the PS and SI scores, shown in table (3), and the mean of (TS) was used for the statistical analysis (Seleit *et al*., 2010).

Table (2.3): Illustrate the immunoreactive score for PCNA and BCL-2 immunohistochemistry.

Score	**Percentage of immunopositive cells (PS)%**	**Staining Intensity (SI)**
0	< 1%	Negative
1	1- 10%	Mild
2	10-50%	Moderate
3	> 50%	Strong

2.4.7. Statistical Analysis

All data of the current study were analyzed via ANOVA one way test. The analysis was achieved by using (SPSS) program; the data were expressed as Mean±Standard deviation (Mean±SD).

The results in all of the above were accepted as statistical level of significant when the p value was ($P \leq 0.05$).

Chapter Three

Results

Chapter three

Results

3.1. Animals' body weight

Results revealed that treated with dose (30, 60) mg/kg of 5-FU in mucositis groups was caused significant decrease at ($P \leq 0.05$) in the body weight post (21, 45) days during experimental periods compared to the body weight of control groups. While glycine was offered as treatment agent in 5-FU/ glycine groups lead to elevation in the body weight significantly ($P \leq 0.05$) in each subgroup treated with glycine (table 3.1).

Table (3.1): Average of animal's body weight in experimental groups. All values expressed as (mean ± SD).

Periods	Groups	Body weight (g)
21 days	IA Control	199.9 ± 35.7 a
	IB Control	205.0 ± 28.6 a
	IIA (5-FU 30mg/ kg)	188.5 ± 23.6 b
	IIB (5-FU 60 mg/ kg)	175.7± 26.1 c
	IIIA (5-FU 30 mg/ kg+ glycine)	200.3± 27.6 d
	IIIB (5-FU 60 mg/ kg+ glycine)	196.7 ± 29.9 d
45 days	Control IA	246.5 ± 27.4 a
	Control IB	246.5 ± 26.4 a
	IIA (5-FU 30mg/ kg)	220.5 ± 31.5 b
	IIB (5-FU 60 mg/ kg)	201.7 ± 30.4 c
	IIIA (5-FU 30 mg/ kg+ glycine)	242.0 ± 24.6 d
	IIIB (5-FU 60 mg/ kg+ glycine)	235.0 ± 27.8 e

a,b,c,d the similar letter means no significant between groups while different letters means there was significant ($P \leq 0.05$).

3.2. Biochemical parameters

3.2.1. Malon Dia Aldehyde (MDA) enzyme level

Results showed that (5-FU) has effect on serum MDA enzyme concentration by causing significant increase at ($p \leq 0.05$) after (21, 45) days in all rats compared to control groups, the concentration was more significant in rats treated with high dose (60mg /kg) than that treated with low dose (30mg/kg) after both periods, whereas the rats treated with glycine and (5-FU) referred to significant decrease at ($P \leq 0.05$) in MDA concentration compared to group that treated with (5-FU) alone at two doses (table 3.2).

Table (3.2): Evaluation of MDA enzyme concentration in all experimental groups. Values represent as (mean± SD).

Periods	Groups	MDA(um/L)
21 days	IA Control	4.32± 0.06 a
	IB Control	4.32 ± 0.06 a
	IIA (5-FU 30mg/ kg)	5.43 ± 0.04 b
	IIB (5-FU 60mg/ kg)	6.41± 0.07 c
	IIIA (5-FU 30mg/ kg+ glycine)	4.23 ± 0.04 d
	IIIB (5-FU 60mg/ kg+ glycine)	4.77 ± 0.08 e
45 days	Control IA	4.34± 0.06 a
	Control IB	4.24± 0.07 a
	IIA (5-FU 30mg/ kg)	6.39± 0.03 b
	IIB (5-FU 60 mg/ kg)	8.26± 0.05 c
	IIIA (5-FU 30mg/ kg+ glycine)	5.05± 0.01 d
	IIIB (5-FU 60mg/ kg+ glycine)	6.09± 0.01 e

a,b,.e the similar letter means no significant between groups while different letters means there was significant ($P \leq 0.05$) between groups.

3.2.2. Glutathione peroxidase (GPx) activity

Glutathione activity was significantly decreased ($P \leq 0.05$) in the serum of 5-FU groups during experimental periods at 21, 45 days in comparison with control group. When (5-FU) was given at dose (60mg/kg), there was more significance decreasing than (30mg/kg) in both periods. When the animals treated with glycine was given with 5-FU, there was significance increasing ($P \leq 0.05$) of GPx enzyme activity compared with 5-FU group. However,

glycine plus (5-FU) subgroups raised glutathione activity, but still lower than normal value of the control group (table 3.3).

3.2.3. Superoxide dismutase (SOD) activity

Biochemical results revealed that the level of (SOD) activity was significantly decreased at ($P \leq 0.05$) in the 5-FU group during experimental periods compared with control group (table 3.3), but there was more significantly decreasing after 6 weeks, while rats in (glycine with 5-FU) group showed significantly increased in (SOD) activity at ($P \leq 0.05$) for each periods in comparison to mucositis induced group (table 3.3).

Table (3.3): Determination of **(**GPx) and (SOD) antioxidant enzymes activity in all experimental groups. Values represent as (mean± SD).

Periods	Groups	GPx (µmol/L)	SOD (µmol/L)
21 days	IA Control	75.2± 0.01 a	8.4± 0.01 a
	IB Control	75.3± 0.01 a	8.2± 0.01 a
	IIA (5-FU 30mg/ kg)	51.1± 0.03 b	6.7± 0.10 b
	IIB (5-FU 60 mg/ kg)	40.3± 0.03 c	6.1± 0.03 c
	IIIA (5-FU 30 mg/ kg+ glycine)	69.4± 0.02 d	8.2± 0.05 d
	IIIB (5-FU 60mg/ kg+ glycine)	65.8± 0.4 e	8.01± 0.07 d
45 days	IA Control	75.2± 0.02 a	8.52± 0.16 a
	IB Control	75.2± 0.02 a	8.4± 0.01 a
	IIA (5-FU 30mg/ kg)	46.4± 0.03 b	5.5± 0.01 b
	IIB (5-FU 60mg/ kg)	27.6± 0.03 b	4.2± 0.03 c
	IIIA (5-FU 30 mg/kg+ glycine)	62.3± 0.01 c	7.5± 0.01 d
	IIIB (5-FU 60 mg/kg+ glycine)	49.4± 0.03 d	6.8± 0.01 e

a, b, c,.. the similar letter means no significant between groups while different letters means there was significant ($P \leq 0.05$) between groups.

3.3. Hematological parameters

3.3.1. Estimation of White blood cells counts (WBCs) and differential WBCs count:

Our data revealed that WBCs count has significant decrease (P≤ 0.05) in rats treated with dose (30 and 60mg/kg) of 5-FU especially after (45) days in comparison with control group, when glycine used in combination with 5-FU, it significantly inhibited the decline in WBCs count at (P≤ 0.05) and results return to the normal limit particularly post (21) days. Also data recorded variations in neutrophils , lymphocytes and monocytes counts, such neutrophils counts was significantly increased at (21) days and significantly decreased (P≤ 0.05) post (45) days in both doses in 5-FU group compared with control group, moreover glycine with 5-FU showed restoration to the regular limits. As for lymphocytes count was significantly decreased at (21) days and significantly increased (P≤ 0.05) at (45) days in 5-FU group compared with control, while rats treated with glycine plus 5-FU, the mean counts return to normal value compared with 5-FU group, also monocytes count was significantly increased in both doses and periods, but more significantly (P≤ 0.05) post (45) days compared to control group, also glycine with 5-FU significantly deceased the monocytes count at (P≤ 0.05) table (3.4).

Table (3.4): Estimation of White blood cells (WBCs) counts and differential WBCs count in all experimental groups. Values represent as (mean ± SD).

Periods	Groups	WBCs Count x10^3 mean±SD	Neutrophil% mean±SD	Lymphocyte % Mean±SD	Monocyte % Mean±SD	Basophil % Mean±SD	Eosinophil % Mean±SD
21 days	IA	9.61±0.17 a	31.76±0.07 a	64.72±0.07 a	1.31±0.11 a	0.31±0.08 a	2.04±0.31 a
	IB	9.62±0.17 a	31.75±0.07 a	64.73±0.07 a	1.28±0.09 a	0.31±0.08 a	1.94±0.49 a
	IIA	7.61±0.02 b	34.47±0.55 b	60.65±0.50 b	2.56±0.12 b	0.80±0.03 b	1.70±0.20 a
	IIB	6.58±0.17 c	34.05±0.61 b	58.58±1.06 c	5.11±1.14 c	0.63±0.08 c	1.46±0.04 a
	IIIA	9.00±0.08 d	31.86±0.67 c	63.07±0.67 d	1.76±0.05 d	0.64±0.20 d	2.43±0.05 b
	IIIB	8.26±0.46 e	31.27±0.57 c	62.82±0.82 e	3.05±0.40 e	0.56±0.10 e	1.99±0.34 a
45 days	IA	9.61±0.16 a	31.93±0.05 a	64.50±0.05 a	1.36±0.06 a	0.35±0.05 a	1.87±0.13 a
	IB	9.63±0.17 a	31.98±0.06 a	64.45±0.05 a	1.33±0.05 a	0.40±0.05 a	1.82±0.16 a
	IIA	5.54±0.10 b	13.23±0.80 b	76.60±0.81 b	7.80±0.36 b	0.71±0.10 b	1.63±0.25 b
	IIB	4.10±0.45 c	7.08±0.89 c	82.40±0.96 c	8.26±0.12 c	0.83±0.01 c	1.48±0.21 b
	IIIA	7.74±0.13 d	28.62±1.45 d	64.16±1.51 d	4.76±0.22 d	0.47±0.04 d	1.58±0.22 c
	IIIB	6.75±0.15 e	25.18±1.08 e	67.97±0.69 e	4.65±0.34 d	0.51±0.0 e	1.54±0.09 c

a,b,c.. the similar letter means no significant between groups while different letters means there was significant ($P \leq 0.05$) between groups.

3.3.2. Estimation of red blood cells (RBCs) counts and Hemoglobin (HB)

The results showed that both doses (30 and 60 mg/kg) of (5-FU) drug significantly decreased (RBCs) count at ($P \leq 0.05$) in all rats post(21, 45) days in comparison with control, while (5-FU) used in combination with glycine showed that (RBCs) count was significantly elevated. Furthermore results referred that (HB) concentration was significantly decreased at ($P \leq 0.05$) in (5-FU) group compared to control, while the concentration of (HB) on each subgroups that treated with (5-FU) and glycine was significantly increased (table 3. 5).

Table (3.5): Estimation of Red blood cells count (RBCs) and hemoglobin concentration in experimental groups .Values represent as (mean ± SD).

Periods	Groups	RBCs countX10	Hemoglobin
21 days	IA control	8.94 ± 0.042 a	10.92± 0.037 a
	IB control	8.97 ± 0.050 a	10.91± 0.036 a
	IIA (5-FU 30mg /kg)	7.55± 0.035 b	9.68 ± 0.019 b
	IIB (5-FU 60 mg/kg)	6.46± 0.038 c	9.13 ± 0.033 c
	IIIA (5-FU 30mg/kg + glycine)	8.25± 0.043 d	10.50 ± 0.057 d
	IIIB (5-FU 60 mg/kg + glycine)	7.87± 0.026 e	10.05 ± 0.079 e
45 days	IA Control	8.83± 0.083 a	10.92 ± 0.042 a
	IB control	8.90± 0.033 a	10.91± 0.038 a
	IIA (5-FU 30mg /kg)	6.71± 0.040 b	8.35 ± 0.030 b
	IIB (5-FU 60 mg/kg)	6.13± 0.030 c	8.11 ± 0.086 c
	IIIA (5-FU 30mg/kg + glycine)	7.78± 0.032 d	9.61 ± 0.037 d
	IIIB (5-FU 60 mg/kg + glycine)	7.23± 0.027 e	9.14 ± 0.091 e

a,b,c.. the similar letter means no significant between groups while different letters means there was significant (P≤ 0.05) between groups.

3.4. Histomorphmetrical study

Statistical analysis referred to significant decrease of epithelial layer thickness in each section of rat tongue, cheek and lip related to (5-FU) treated groups with mean (49.8±0.60,26.6±0.48) um, (31.3±0.51, 23.6±0.70) um and (30.3±0.75, 24.8±0.55) um respectively in both doses of 5-FU post 21 days at (P≤ 0.05) compared with control rats. While rats of (5-FU) treated with glycine group showed significantly increased in the mean thickness of epithelial layer of tongue, cheek and lip (55.6±0.80,41.6±0.70) um, (33.5±0.75, 30.5±0.80) and (36.9±0.80, 32.2±0.36) um respectively post 21 days compared to rats of induced group table (3.6). Also analysis referred to the epithelial thickness of rats tongue, cheek and lip in (5-FU) treated groups with two doses at 45 days period, the mean thickness was more significantly lower (25.3±0.45, 11.6±0.81) um, (19.1±0.75,11.6±1.21), (20.8±0.65, 11.0±0.70) um respectively compared with control rats. While the mean epithelial thickness was significantly increased at (P≤ 0.05) in rats treated with (5-FU + glycine) with both doses of 5-FU (30, 60mg/kg) post 45 days than that of induced group, but still lower than control in both doses of 5-FU at the same period (table 3.6).

Table (3.6): Estimation of tongue, cheek and lip mean epithelial thickness in experimental groups. Values represent as (mean ± SD).

Periods	Groups	Epithelial thickness of tongue (um)	Epithelial thickness of cheek (um)	Epithelial thickness of lip (um)
21 days	IA control	58.2±0.88 a	35.2±0.60 a	39.1±0.80 a
	IB control	58.2±0.76 a	35.5±0.83 a	38.8±1.00 a
	IIA (5-FU 30mg /kg)	49.8±0.60 b	31.3±0.51 b	30.3±0.75 b
	IIB (5-FU 60mg /kg)	26.6±0.48 c	23.6±0.70 c	24.8±0.55 c
	IIIA (5-FU 30 mg + glycine)	55.6±0.80 d	33.5±0.75 d	36.9±0.80 d
	IIIB (5-FU 60 mg + glycine)	41.6±0.70 e	30.5±0.80 e	32.2±0.36 e
45 days	IA control	58.2±0.79 a	35.3±0.55 a	39.0±0.70 a
	IB control	57.2±0.77 a	35.2±0.51 a	39.1±0.70 a
	IIA (5-FU 30mg /kg)	25.3±0.45 b	19.1±0.75 b	20.8±0.65 b
	IIB (5-FU 60mg /kg)	11.6±0.81 c	11.6±1.21 c	11.0±0.70 c
	IIIA (5-FU 30 mg + glycine)	47.5±1.05 d	30.4±0.95 d	31.3±0.90 d
	IIIB (5-FU 60 mg + glycine)	38.3±0.90 e	25.6±0.91 e	28.6±1.06 e

a,b,.. the similar letter means no significant between groups while different letters means there was significant ($P \leq 0.05$) between groups.

3.5. Pathological study

3.5.1. Macroscopically examination

Macroscopically ,the oral mucosa regions (tongue, lip and cheek) of control rats appeared normal, smooth and pink without any abnormal signs of oral mucositis (fig.1A). Observation on oral mucosa of rats treated with induced mucositis appeared with inflammatory signs as redness, hyperemia and hemorrhage after (14) days from administration of (5-FU) drug followed by irritation of the mucosa in all rats at both periods (fig.1B). These signs caused clear epithelial ulceration of the surface layer at end of experimental periods approximately in all rats treated with dose (60 mg\kg) of (5-FU) post 21 days and both doses post 45 days (fig 2, 3), while rats treated with (5-FU+ glycine) showed mild redness, no ulcers on oral mucosa and morphologically nearest to the normal especially post 21 days of treatment compared to rats with induced mucositis (fig 4).

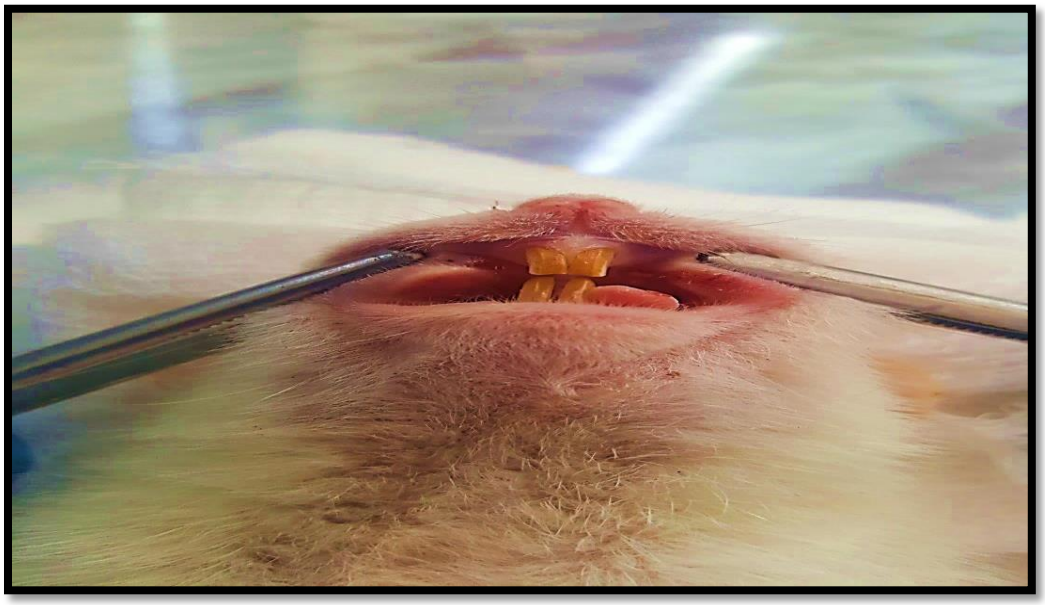


Figure (1): Image of healthy oral mucosa from control group

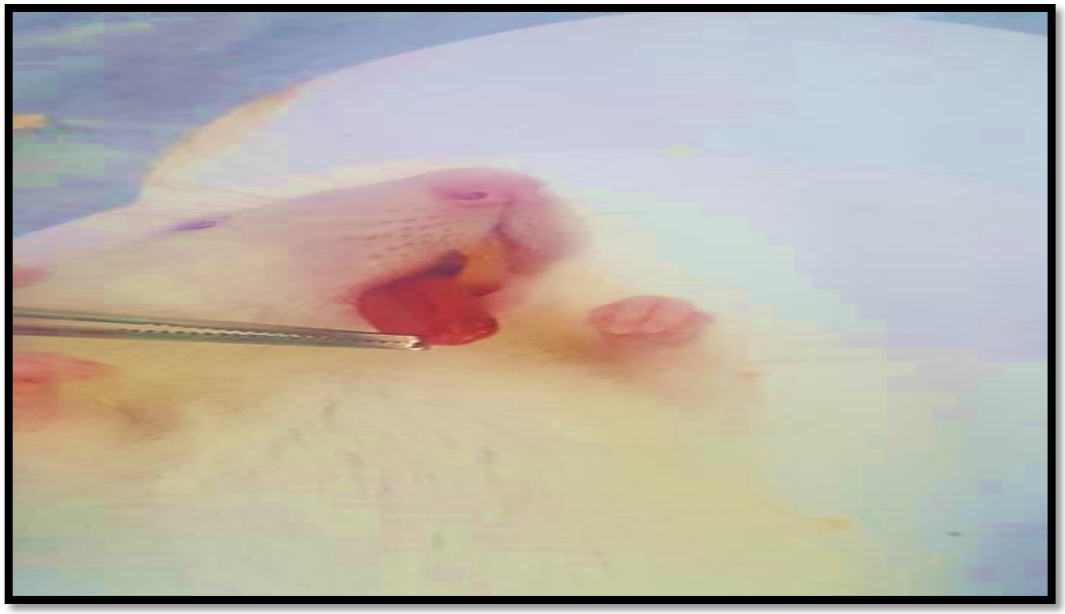


Figure (2): Image of the rat tongue treated with 5-FU showed redness of mucosa with hyperemia post (14) days at both periods.

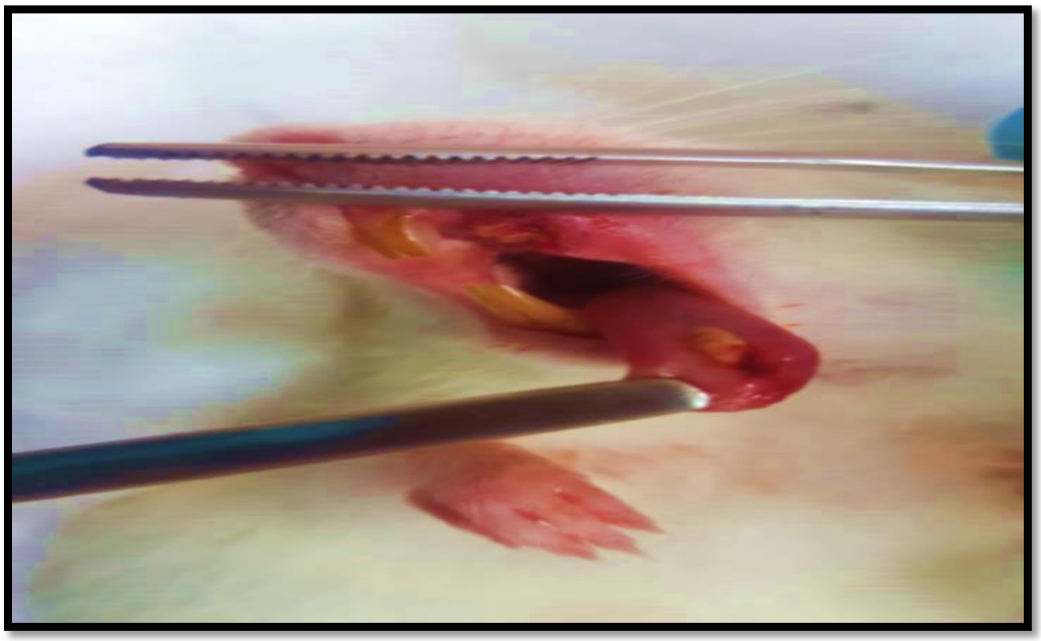


Figure (3): Image of the rat tongue treated with 5-FU showed epithelial ulcerated for both doses post (45) days.

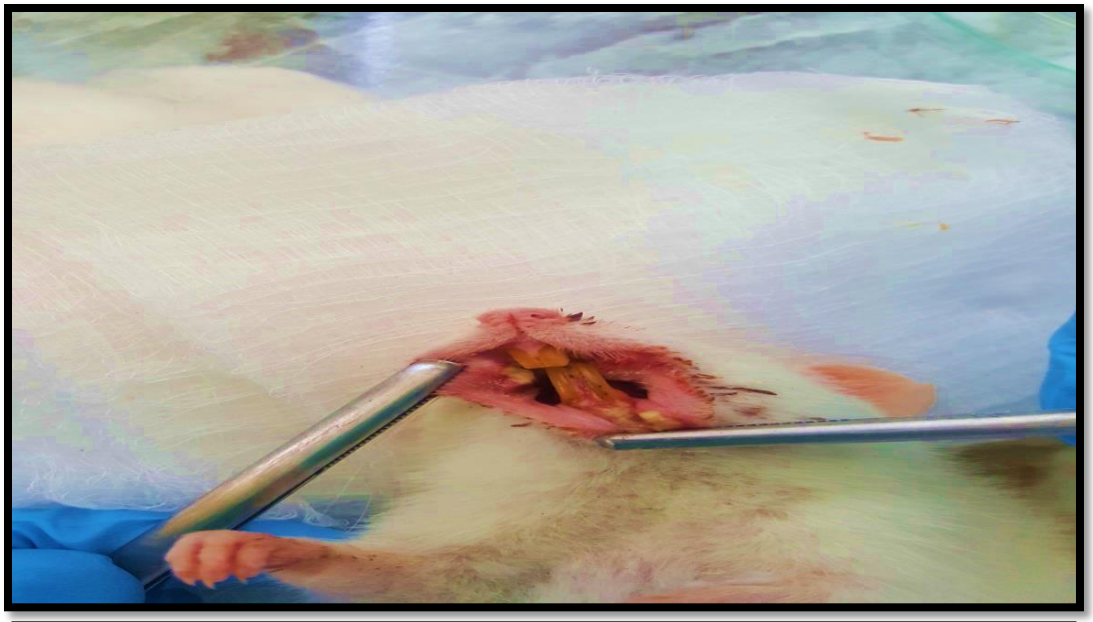


Figure (4): Image was showed epithelial ulcerated in rat cheek and lower lip tissue from (5-FU) group at both doses post (45) days.

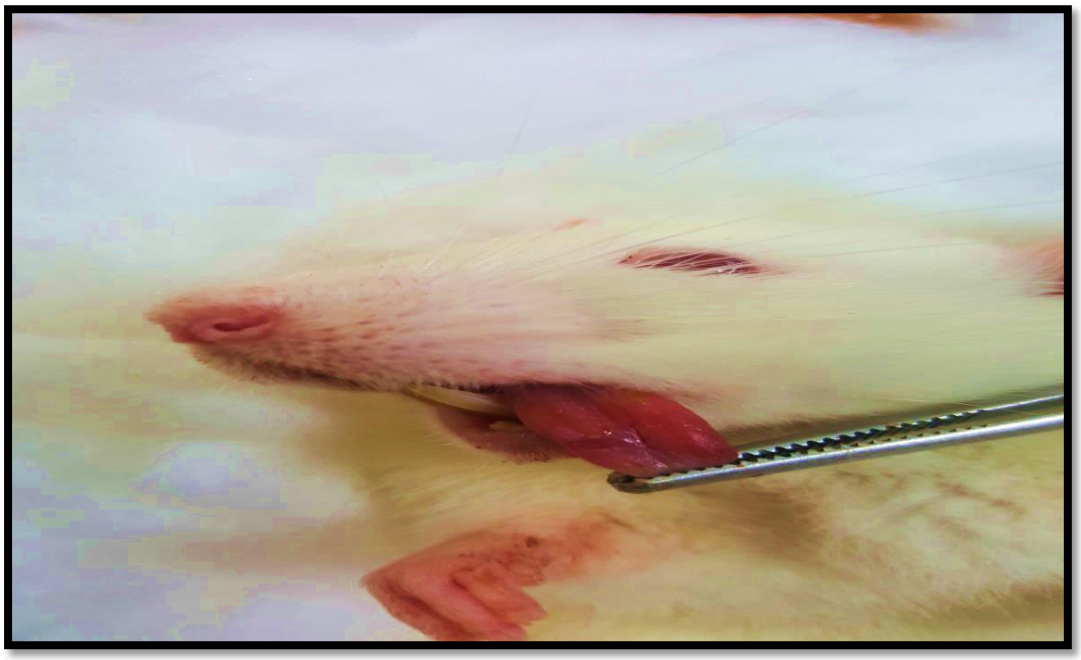


Figure (5): Image of the tongue tissue treated with (5-FU + glycine) was showed mild inflammation with redness and no ulcer.

3.5.2. Histopathological changes

3.5.2.1. Tongue

Light microscopic examination on transverse section of rat tongue related to control group showed normal oral mucosa covered the dorsal surface consist of keratinized stratified squamous epithelium and thin papillated lamina propria, the dorsal surface displayed conical, slender filiform papillae cornified with tapers tips and fewer fungiform papillae with rounded dilated surface, normal connective tissue within papillae, the section revealed to well define fungiform with test bud observed between the filiform papillae. The tongue composed mainly from interlacing bundles of striated muscles that run in different directions and cross one another (fig 6). While photograph of the ventral surface mucosa of normal rat tongue showed normal mucosal layer, less keratinized stratified squamous epithelium formed rete ridges projecting into the lamina propria which appeared as connective tissue with small blood vessels, absence of papillae and masses of skeletal muscles continuous with the lamina propria (fig 7).

When rats injected with 5-FU (30 mg/kg) after (21) days, the tongue sections showed variable changes included mild loss of normal appearance and shape of dorsal surface papillae, the filiform papillae loss their pointed on conical shape with less number and shorter, secession of keratin layer, mild flattening of the rete ridges, keratinocytes vacuolation, most cells with perinuclear or with vacuolated cytoplasm. Also mild inflammatory cells in the lamina propria, irregular masses of atrophied muscle fibers separated from each other by connective tissue which appeared vascularized with numerous congested blood vessels (fig 8, 9, 10). Whereas tongue sections related to rats

injected with (60mg/kg) of 5-FU after (21) days revealed to hyperkeratosis, acanthosis of keratin layer, sever atrophied of filiform papillae and degenerated fungiform papillae, most of sever inflammatory cells was the monocytes infiltrated in the lamina propria, few shallow epithelial rete ridges that invaginated deeply within the mucosa, basal and supra basal layer with vacuolated cells specially that surrounded the papillae and show no clear boundaries (fig 11, 12). Moreover the lamina propria showed degeneration, edema, highly vascularized with congested large blood vessels and atrophied muscle fibers separated by connective tissue (fig 13).

Results clarified the effect of glycine on (5-FU) induced mucositis in experimental rats, tongue sections from rats injected with (30mg/kg) of (5-FU) and treated with (2 mg/g) of glycine for (21) days revealed to an increase in the epithelial layer thickness , re-epithelization of stratified squamous epithelium, more filiform papillae with tapers tips and cornified, well developed fungiform, circumvallate papillae observed with test buds, restoration of rete ridges although inflammatory cells and vacuolated keratinocytes observed (fig 14). While the study pointed to the tongue sections from rats injected with (60mg/kg) dose of (5-FU) and treated with glycine for the same period, revealed to keratin layer formation, well developed papillae, large fungiform papillae, vascularized connective tissue with large congested blood vessels and irregular groups of striated muscles (fig 15).

As the treated period was increased, alterations became more developed, so tongue sections of rats injected with (30mg/kg) of (5-FU) drug after (45) days showed sever atrophied of filiform and fungiform papillae, reduced thickness of epithelium, heavy infiltration of mononuclear cells (lymphocyte and plasma cells), focal degeneration and hemorrhage, atrophied muscle fibers arranged as

groups separated by collagen fibers, the ventral surface of the tongue pointed to mild keratin deposition, inflamed lamina propria, vacuolated epithelial cells, aggregation of lymphocytes near the rete ridges and extend down to fill the connective tissues (fig16,17).Tongue sections from rats treated with (60mg/kg) of (5-FU) for (45) days showed more sever alterations in dorsal and ventral surfaces revealed to loss of normal appearance and destruction papillae, the filiform and fungiform papillae missing in most regions, heavy infiltration of inflammatory cells, congested large and small blood vessels, the ventral surface showed reduction of epithelial thickness with mild deposition of keratin, flat rete ridges, severe atrophied muscle fibers, severe inflammation, edema and vascularized connective tissue with thin septa of collagen fibers (fig 18,19).

Results elucidated the changes in the tongue sections of rats injected intraperitoneally with (30mg/kg) of (5-FU) and treated with glycine after (45) days showed moderate restoration of mucosal layer (degree of re-epithelization), increased epithelium thickness , increased proliferation of basal cells, restored normal shape of filiform and fungi form papillae, more rete ridges, closely attachment of keratin layer with the underlying epithelial cells, well developed connective tissue with bundles of striated muscle but mild inflammatory cells and mild congested capillaries were still observed (fig20, 21). Recent findings were observed in tongue sections of rats injected with (60mg/kg) of (5-FU) and treated with glycine post (45) days like normal filiform papillae with pointed tips, few fungiform papillae, increased thickness of epithelium, more shallow rete ridges with keratin layer, more collagen fibers, also still moderate inflammatory cells within lamina propria, dilated congested blood vessels and neoform of capillaries (fig 22).

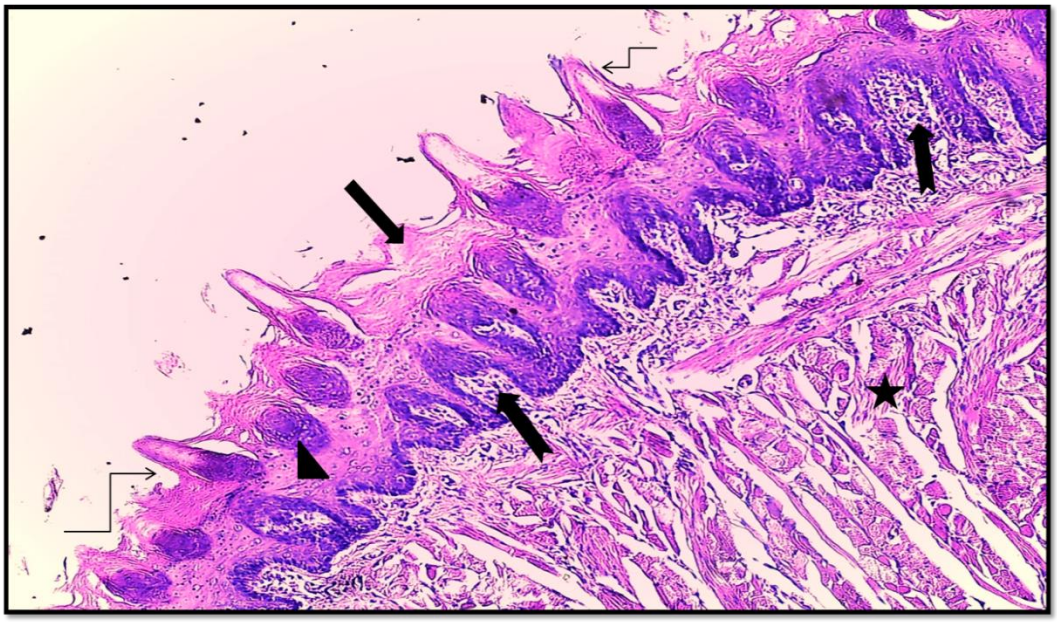


Figure (6) : section of dorsal surface rats tongue in control shows normal oral mucosa (➡) normal filiform papillae (⟶), normal fungi form papillae (◣), papillated lamina properia (➡) and bundles of stratified muscles (★). (H& E. 10 x)

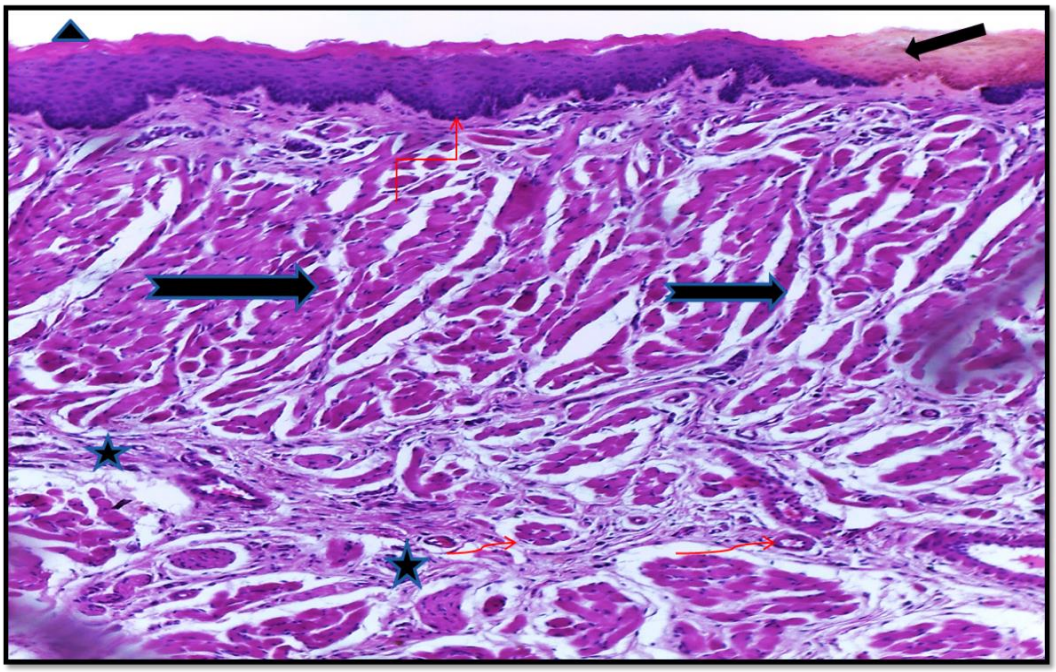
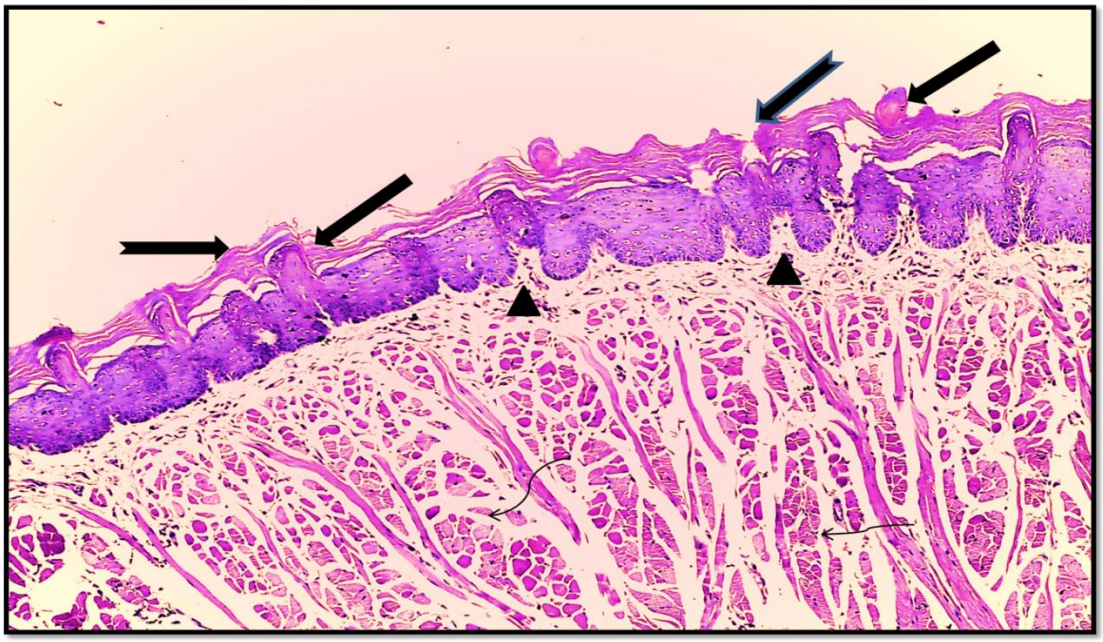


Figure (7) : Section of tongue in control shows the normal ventral surface (➡), keratinized layer (▲) , normal rete ridges (⟶), masses of striated muscle fibers (➡), capillaries (⟶) and collagen fibers (★) (H & E.10x).



Figure(8) : Ssection in dorsal surface of rats tongue treated with 5-FU 30 mg\kg after (21) days showing shorter, less number of filiform papillae (➡), separation of keratin layer (➡) , moderate inflammation of lamina propria (▲), atrophied striated muscle fibers (⟶) . (H &E . 10 x).

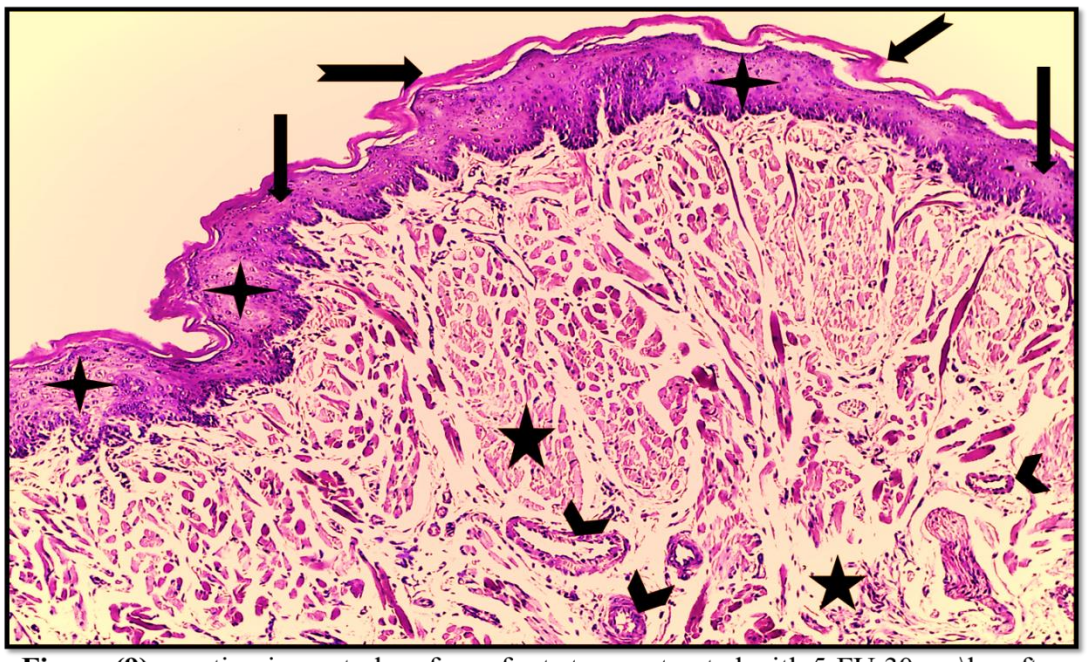


Figure (9) : section in ventral surface of rats tongue treated with 5-FU 30 mg\kg after (21) days showing reduced thickness of epithelium layer (➡), separation of keratin deposition (➡), vacuolation regions (✦) and vascularized connective tissue (★) with numerous capillaries (◡). (H & E . 10 x).

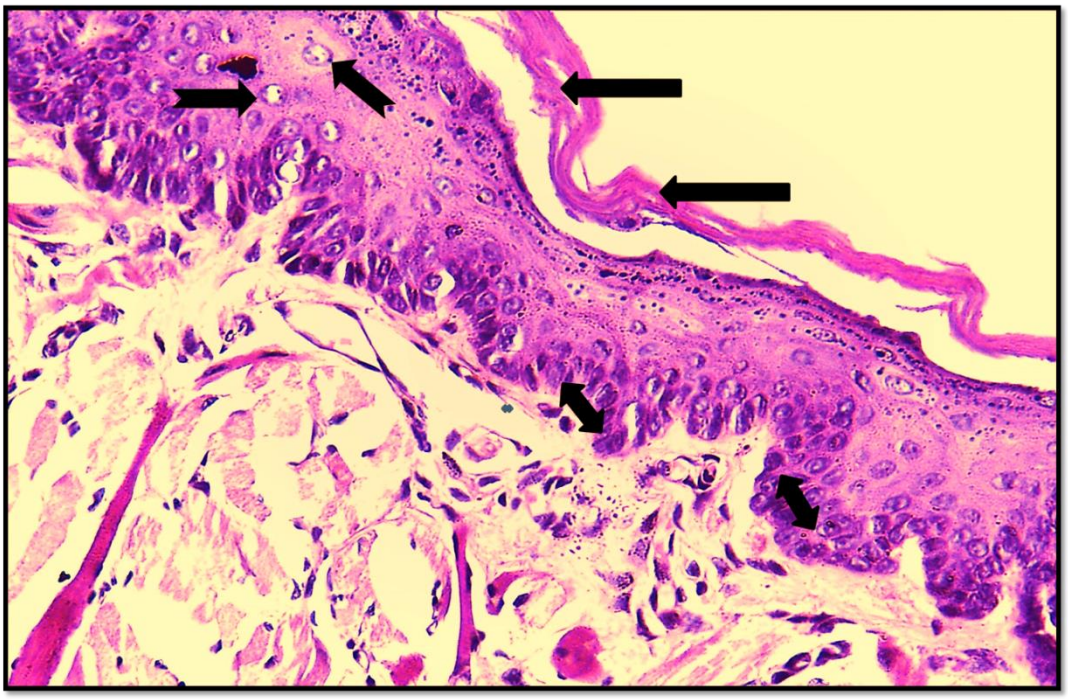


Figure (10) : Section in ventral surface of rats tongue treated with 30 mg\kg 5-FU after (21) days showing complete separation of keratin layer ( ), vacuolated keratinocytes ( ), irregular arrangement of basal cells( ). (H & E . 40 x).

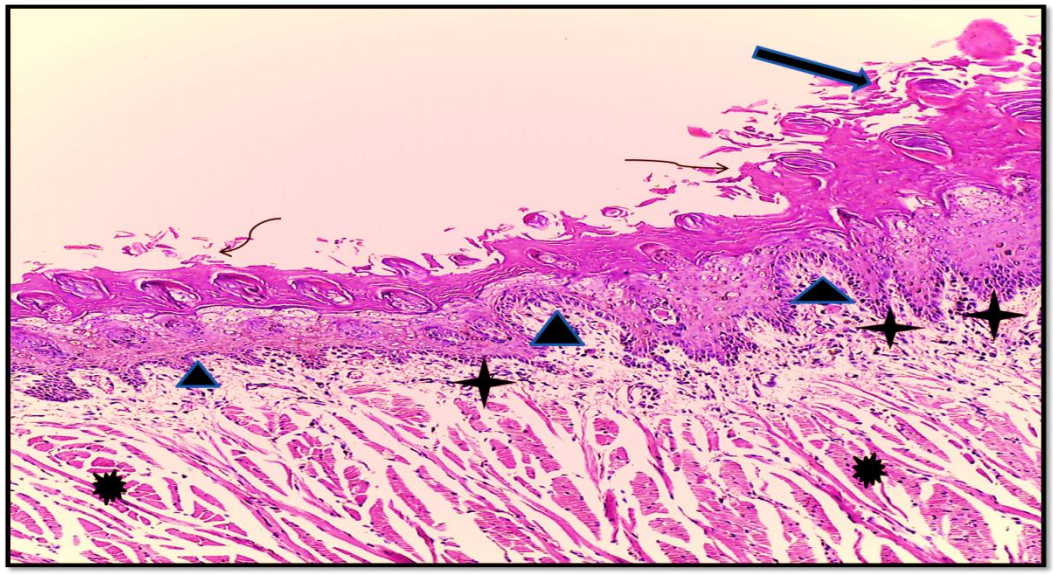


Figure (11) : Section in dorsal surface of rats tongue treated with 60 mg\kg 5-FU after (21) days showing hyperkeratosis ( ), sever damage of filliform papillae ( ), shallow rete ridges ( ), sever inflammation in lamina propria( ) and atrophied muscle fibers ( ). (H & E .10 x).

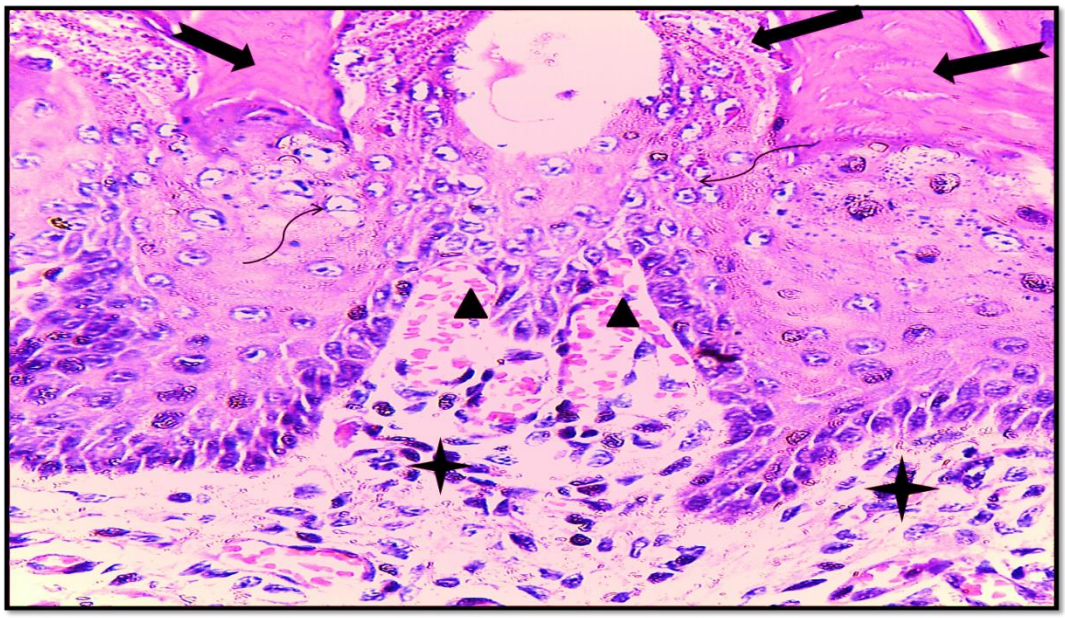


Figure (12) : Section in dorsal surface of rats tongue treated with 60 mg\kg 5-FU after (21) days showing degenerated fungiform papillae ( ), surrounding with vacuolated keratinocytes ( ), acanthosis ( ), hemorrhage ( ) and inflammatory cells within lamina propria ( ). (H & E .40 x).

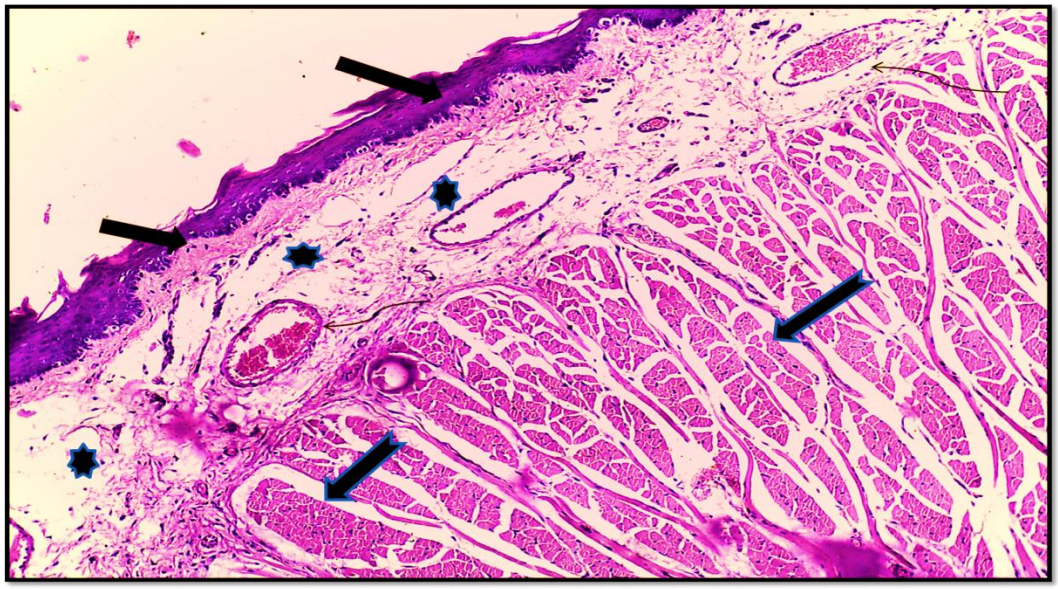


Figure (13) : section in ventral surface of rats tongue treated with 5-FU 60 mg\kg after (21) days showing decrease thickness of mucosal layer ( ), edema ( ), congested blood vessels ( ) and atrophied muscle fibers ( ). (H & E. 40 x).

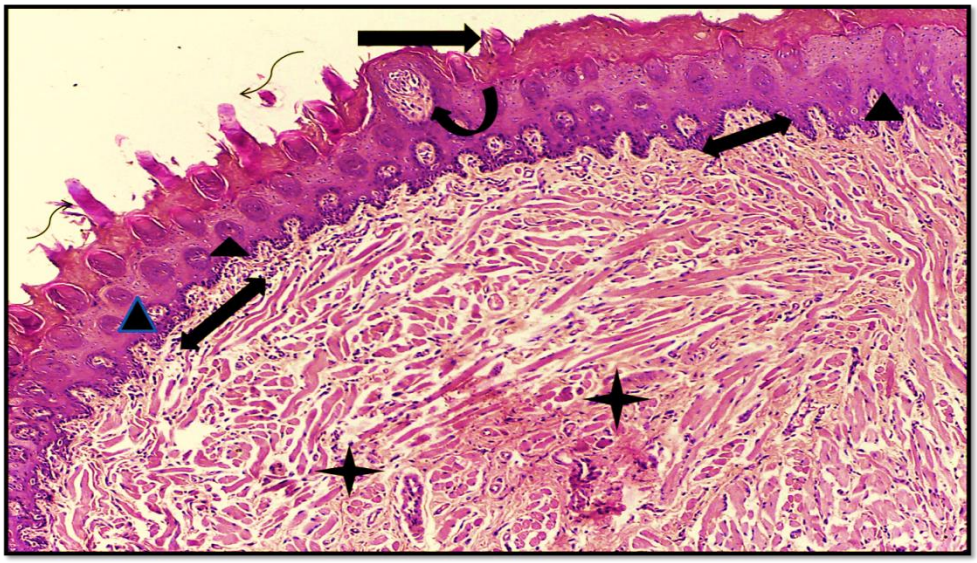


Figure (14): section in dorsal surface of rats tongue treated with (5-FU 30 mg\ kg + glycin) showing re-epithelization (➡), more normal filiform papillae(↗), few fongiform (▲), well develop circumvallate papillae (↻), still mild inflammatory cells in lamina propria (↔) with strand of irregular striated muscle fibers (✦). (H & E. 10 x).

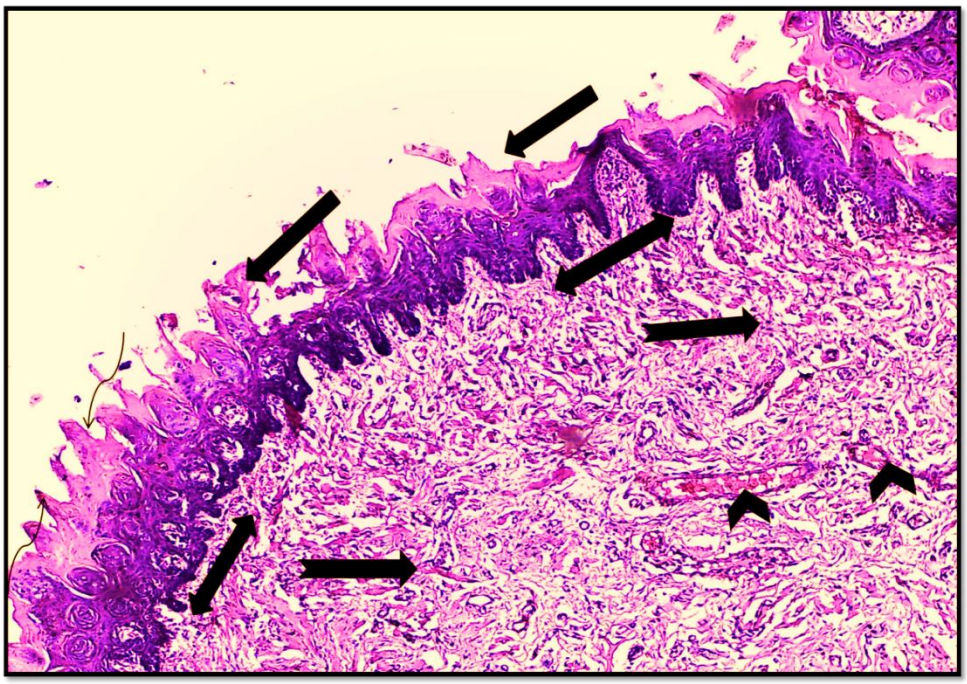


Figure (15): Section in dorsal surface of rats tongue treated with (5-FU 60 mg\ kg + glycin) showing more keratin deposition (➡), few normal papillae (⇝), congested blood vessels (^), mild inflammatory cells (↔) and irregular striated muscle fibers (➡). (H & E. 10 x).

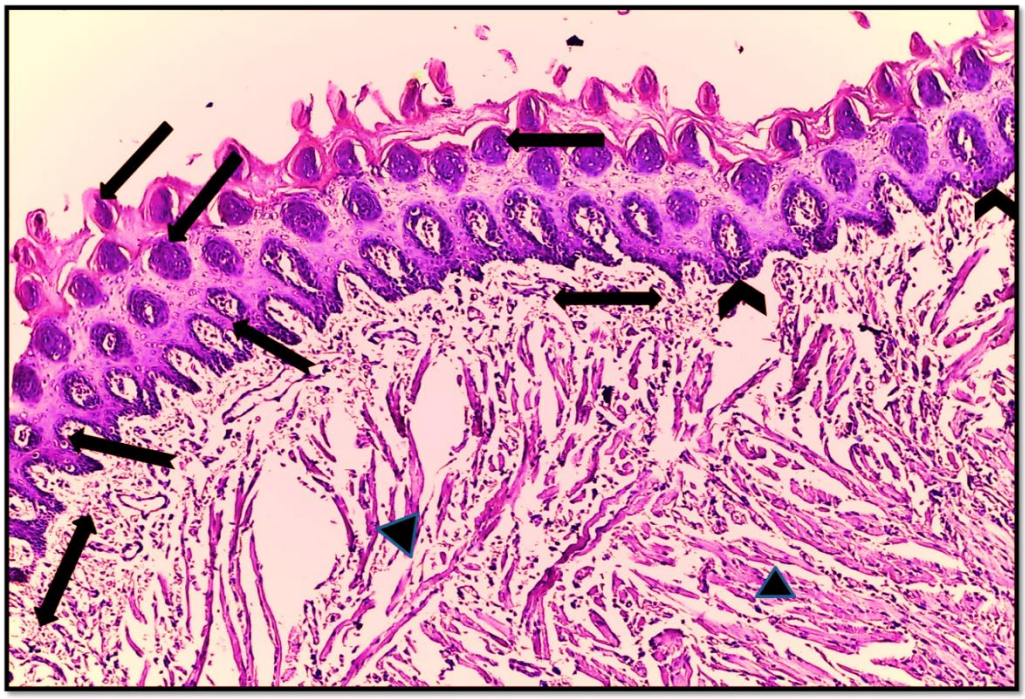


Figure (16): section in dorsal surface of rats tongue treated with 30 mg\ kg 5-FU post (45) days showing sever atrophied of filiform (⟶) and fungiform papillae (⟶), moderate infiltration of inflammatory cells (⟶), atrophied muscle fibers (▲) and flattening narrow rete ridges (❮). (H & E. 40 x).

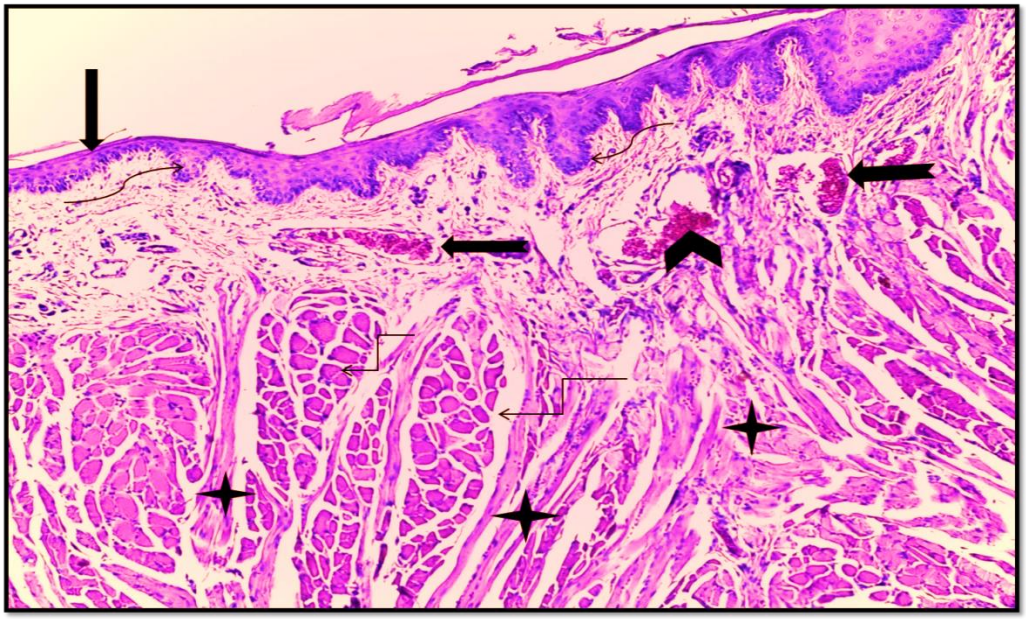


Figure (17): Section from ventral surface in rats tongue treated with 30 mg \kg of 5-FU post (45) days showing reduce thickness of epithelium(⟶), narrow rete ridges(⟶), hemorrhage (❮), congested blood vessels (⟶), groups of atrophied muscle fibers ⟶) separated by collagen fibers (✦). (H & E. 40x).

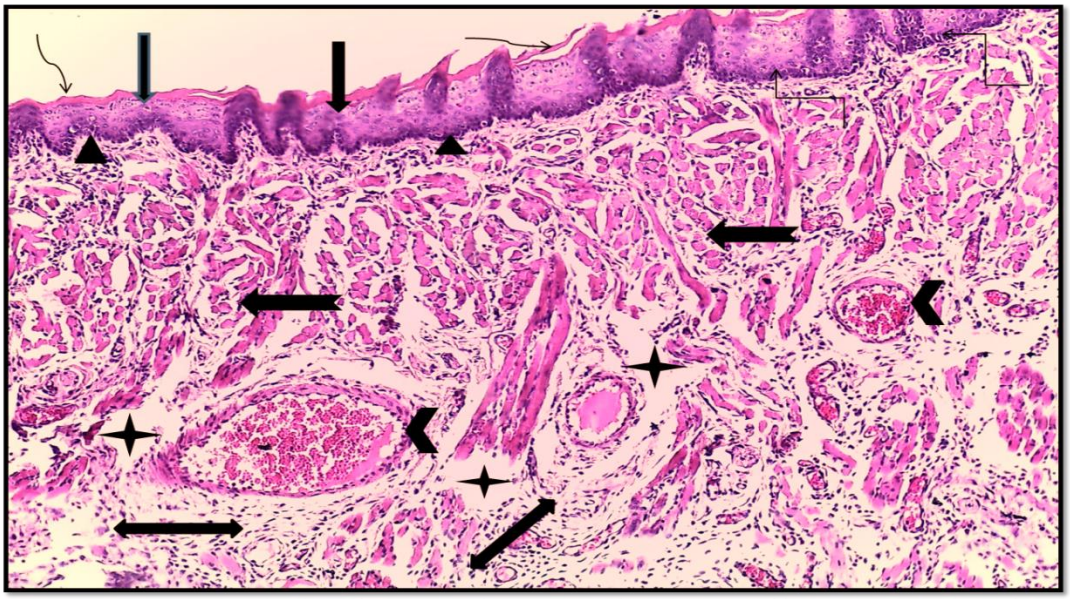


Figure (18): Section in dorsal tongue of rats treated with 60 mg \kg 5-FU post (45) days showing more decrease thickness of epithelial layer (⟶) with loss of most papillae, separation of keratin (⟶), flattening of rete ridges (⟶), thin lamina propria (▲), atrophied muscle fibers (⟶), heavy infiltration of inflammatory cells (⟷) and degeneration (✦) with congested blood vessels (❯). (H & E. 10 x).

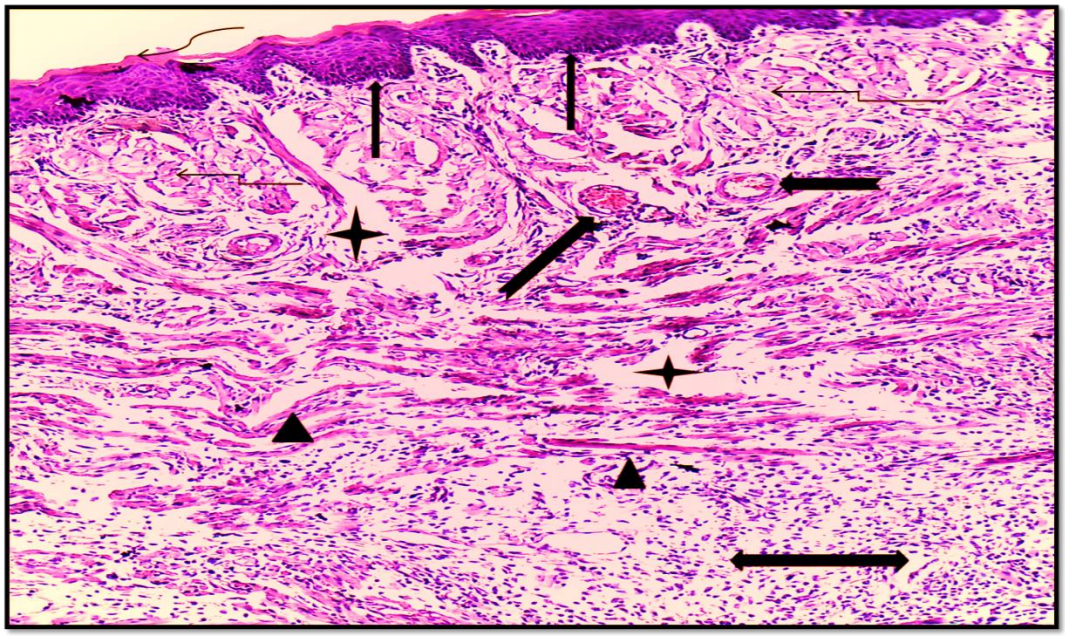


Figure (19): Section in ventral rats tongue treated with 60 mg \kg 5-FU post (45) days showing the ventral surface with keratin deposition (⟶), flat rete ridges (⟷), sever atrophied muscle fibers (⟶), sever inflammation (⟷), edema (✦) and vascular zed connective tissue (⟷) with thin septa of collagen fibers (▲).(H & E. 10 x).

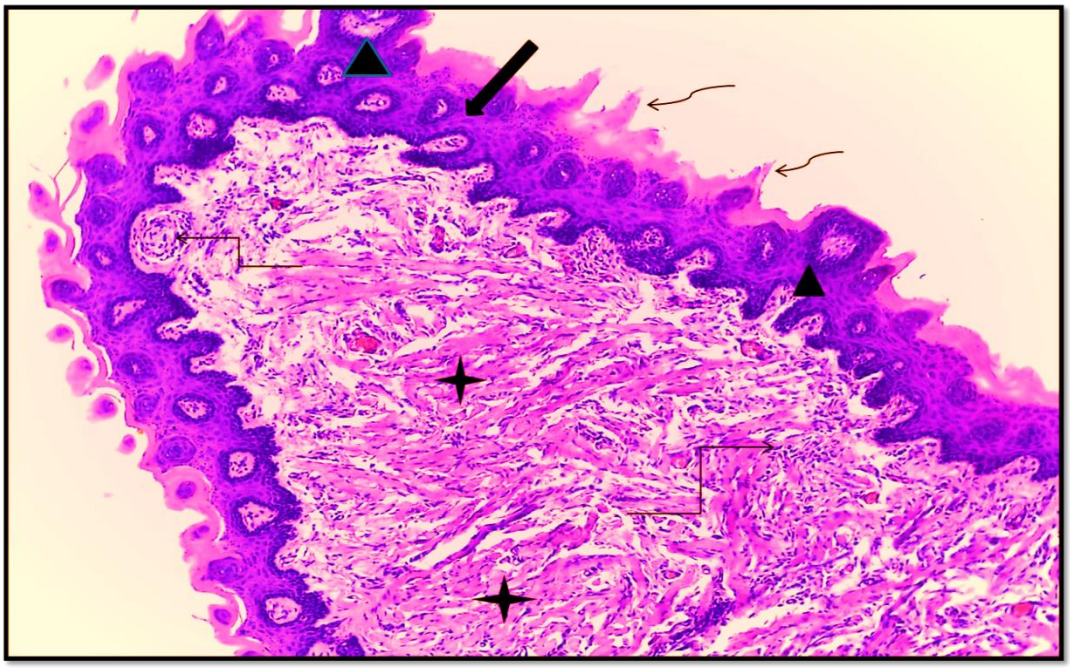


Figure (20): Section in dorsal surface of rats tongue treated with (30 mg \ kg 5-FU+ glycin) showing restoration of mucosal layer (⟶) and filiform papillae (⟶), well develop fungiform papillae (▲) and striated muscles (✦) in different directions and mild inflammatory cells (⌐) . (H & E. 100 x)

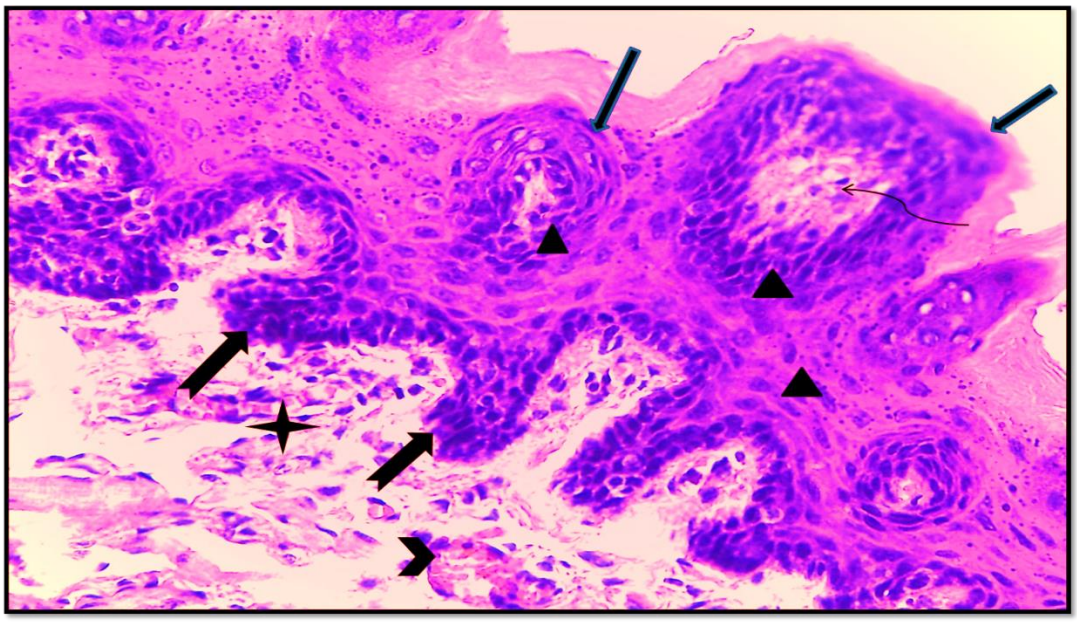


Figure (21): Section in dorsal surface of rats tongue treated with (30 mg \ kg 5-FU + glycin) showing restoration structure of fungiform papillae (⟶) with test buds (⟶), surrounding with normal keratinocytes (▲), normal basal and Para basal cells (➤), mild congested capillaries (❯) and mild inflammatory cells (✦). (H & E. 40 x)

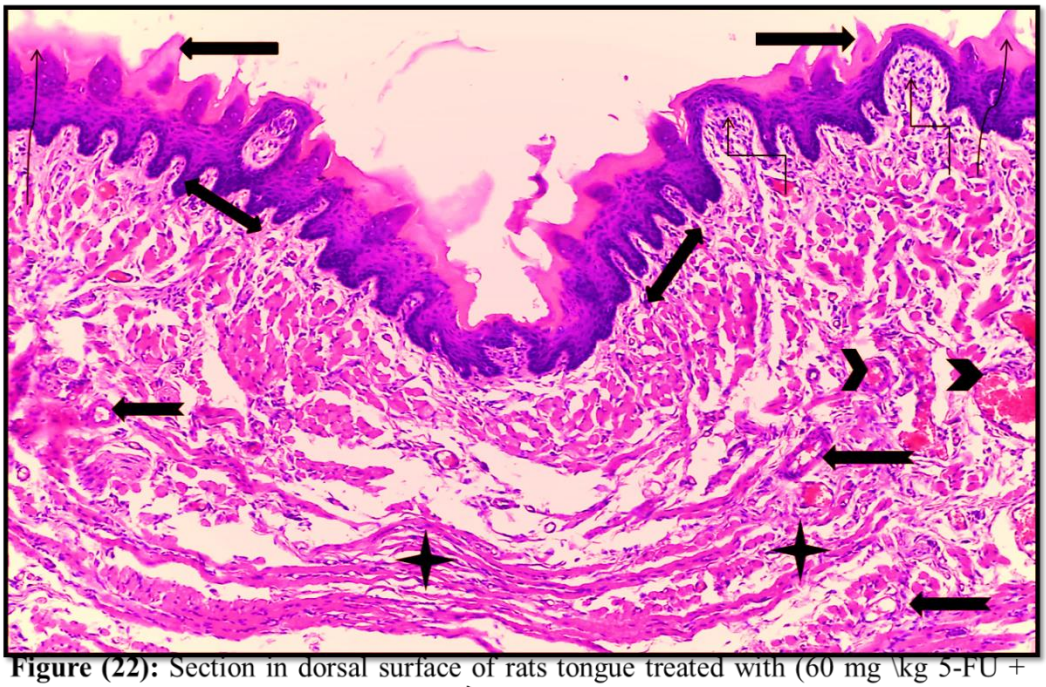


Figure (22): Section in dorsal surface of rats tongue treated with (60 mg \kg 5-FU + glycin) showing re-epithelization ( ), restoration shape of filiform papillae ( ), large dilated circumvallates papillae ( ), moderate inflammatory cells ( ), congested blood vessels ( ), dense collagen fibers ( ) and neoformed of capillaries ( ). (H & E. 10 x).

3.5.2.2. Lip

Light microscopic examination on the lip of control rats showed the central area composed of striated muscles, the upper surface revealed to the lining mucosa, mild keratinized stratified squamous epithelium, beneath it there was the lamina propria with groups of labial glands, the oral mucosa penetrate the underlying connective tissue with normal rete ridges, numerous and abundantly supplied with capillaries, the lower part of the lip was the skin so the dermis layer appeared with hair follicles and associated glands (fig 24, 25).

The lip sections from rats injected with (30mg/kg) of (5-FU) after (21) days showed decrease thickness of epithelial layer, keratin deposition, papillated lamina propria with mild inflammation, separation of keratin layer from the mucosa, most cells with vacuolated cytoplasm, groups of striated muscle fibers distributed in different directions, vascular hyperemia and congested blood vessels (fig 26, 27). Furthermore, sections from rat lips injected with dose (60mg/kg) of (5-FU) for (21) days revealed to destruction of oral mucosa, hyperkeratosis, irregular proliferation of basal cells and degenerative vacuolation of para basal cells, no boundaries between mucosa and lamina propria, severe inflammation extend to the deep layer and congested blood vessels intermingled with inflammatory cells most (lymphocytes and monocytes) (fig 28, 29).

Histological analysis on lip sections from rats injected with (30mg/kg) of (5-FU) and treated with glycine post (21) days revealed to well improvement as deposition of keratin on the surface layer, increase thickness of epithelium, obvious rete ridges, proliferation of basal layer cells, well developed lamina propria from collagen fibers and the connective tissue showed remodeling by increase the collagen fibers, fibrocytes and capillaries (fig 30). Also the study showed the effect of glycine on healing and remodeling of lips in rats injected with (60mg/kg) of (5-FU) and treated with glycine for the same period, the figures indicated to surviving cells in the basal half of the epithelium. This provided the regeneration cells formed multilayered form, mild vacuolated cells still observed, the lamina propria showed organized composed from fibers and fibrocytes separated the epithelium from the connective, normal capillaries and regeneration of collagenous fibers (fig 31).

The present study showed the lip sections from rats injected with (30mg/kg) of (5-FU) after (45) days several pathological changes were more developed than (21) days in both doses, represented by mild to moderate ulceration and destruction of external epithelial layer that was supported by connective tissue, granulation tissue observed mixed with neutrophils and cellular debris, the lamina propria separated from the underlying tissue which showed more inflammatory cells, vascular hyperemia , edema and hemorrhage (fig 32, 33). When high dose (60mg/kg) used the lip sections indicated to severe inflammation, the alterations became more developed, the lamina propria more vascularity with irregular rete ridges, connective tissue with numerous capillaries, edema and focal degeneration area with more hemorrhage (34). Resent findings on lips mucosa from rats injected with (30mg/kg) of (5-FU) and treated with glycine post (45) days indicated to variable histological improvement as restoration of normal stratified squamous epithelium with few epithelial cells vacuolization at basal layer, deposition of keratin on surface layer and separation of this layer from the mucosa in some regions, mild inflammatory cells at the rete ridges, vascularized lamina propria, the connective tissue with groups of striated muscle and still some of weavy muscle fibers and atrophied fibers observed (fig 35). Moreever the histological results revealed to the effect of glycine on lip mucosa sections from rats injected with (60mg/kg) of (5-FU) and showed moderate restoration of normal stratified squamous epithelium with still perinuclear vacuolated cells, mild mononuclear inflammatory cells infiltrate, but more fibroblast and collagen fibers bundles were observed and extended with perimysium of striated muscle (fig 36).

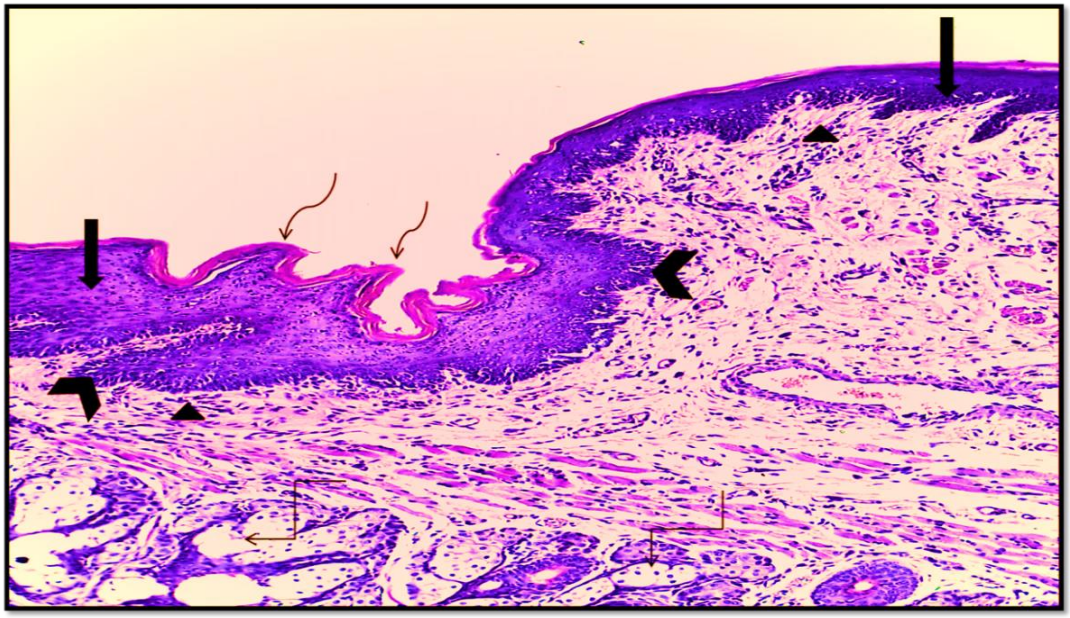


Figure (24): Section of lip in control rats showing the normal oral mucosa, normal stratified squamus epithelium ( ), mild keratin ( ), the mucosal layer penetrate the lamina propria ( ) with normal rete ridges ( ), and some of labial glands ( ) (H & E. 10 x).

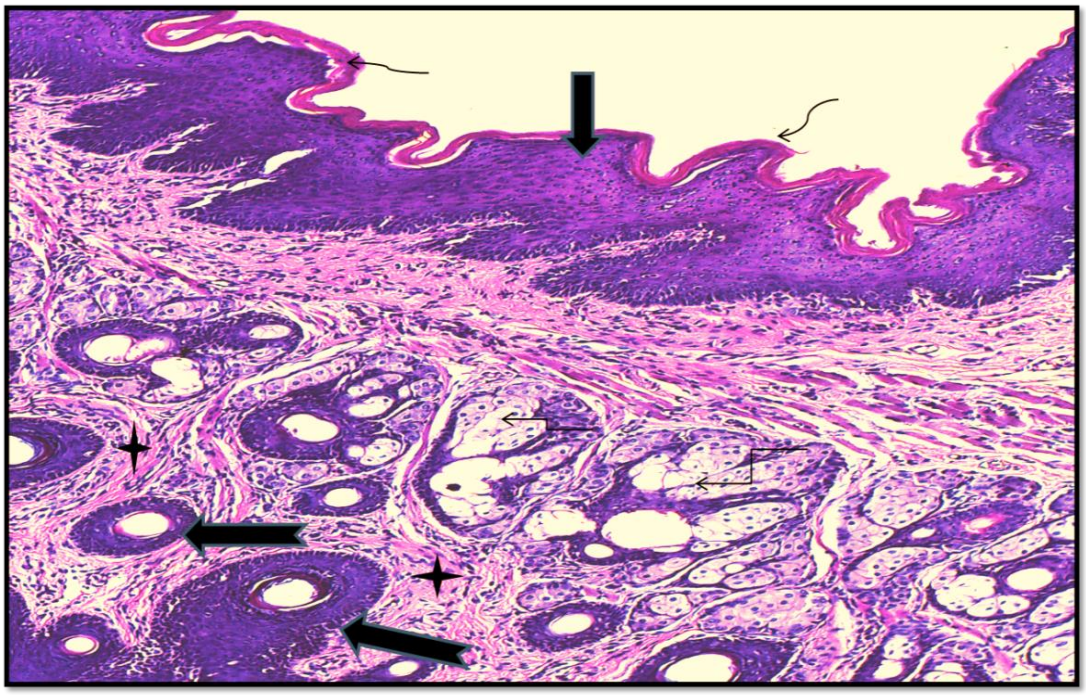


Figure (25) : Section of lip in control rats showing well developed oral mucosa ( ), mild keratin ( ), groups of labial glands ( ) and the lower side of lips (skin) showed hair follicles ( ) surrounded with collagen fibers ( ) . (H & E . 10 x).

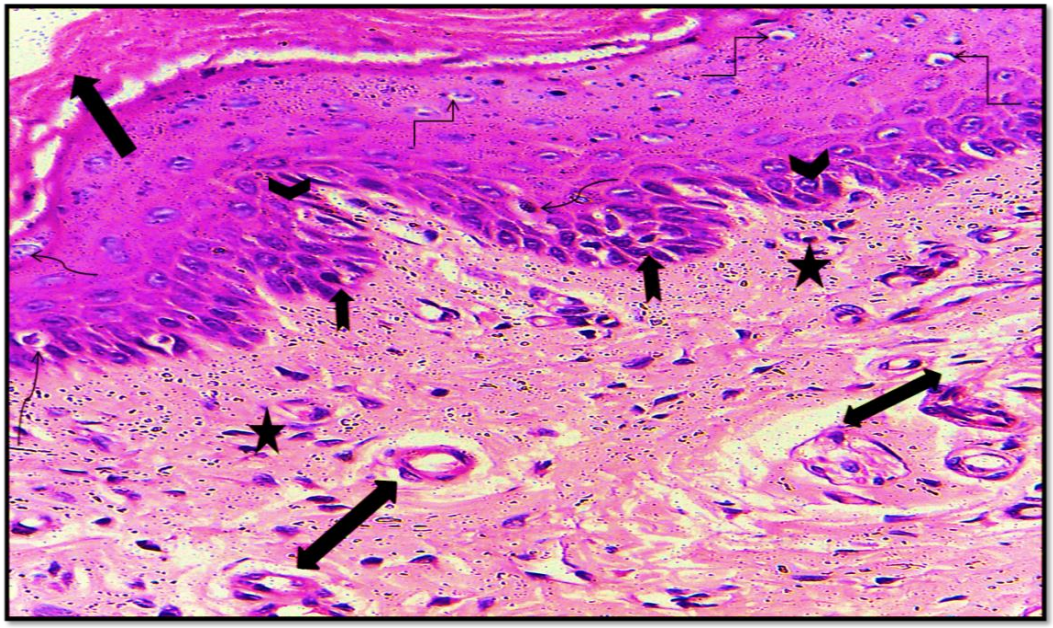


Figure (26) : Section in lip of rats treated with 30 mg\kg of (5-FU) after (21) days showing hyperkeratosis (➡), vacuolated keratinocytes (⤳), some cells with perinuclear and cytoplasm vacuolation (⤳), degeneration of basal (➡) and parabasal cells (➤) and inflammed lamina prpopria (★) with numerous capillaries (⟷) (H & E. 40 x).

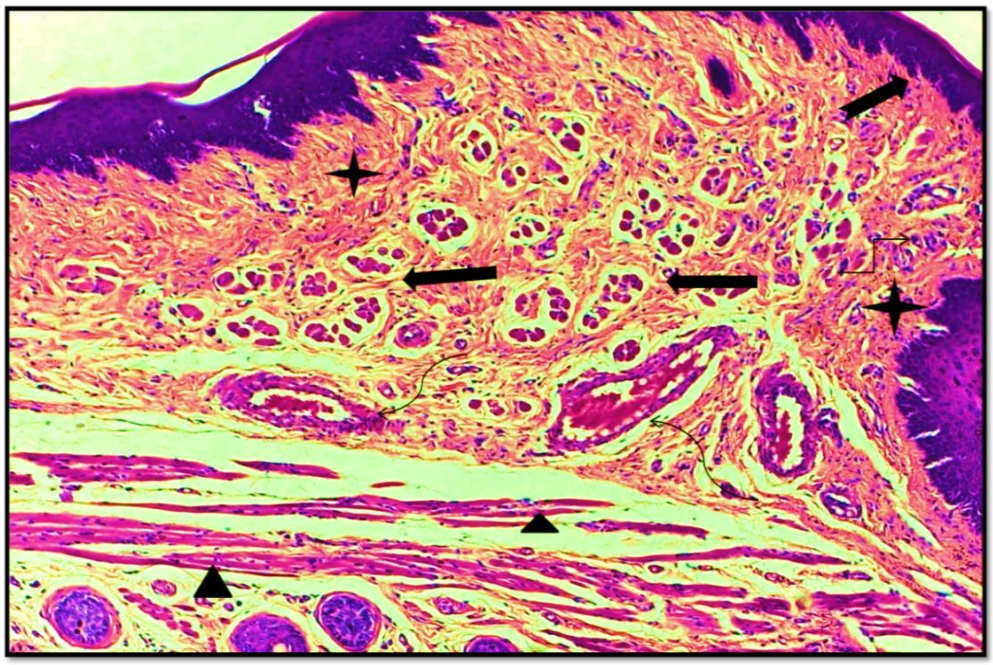


Figure (27) : Section in lip of rats treated with 30 mg\kg of (5-FU) after (21) days showing atrophied striated muscle fibers (➡) arranged in groups, separated by collagen fibers (✦), vascular hyperemia (⤳), inflammed lamina propria (➡) with highly vascularity (⤳) and smooth muscle fibers (▲). (H & E. 10 X).

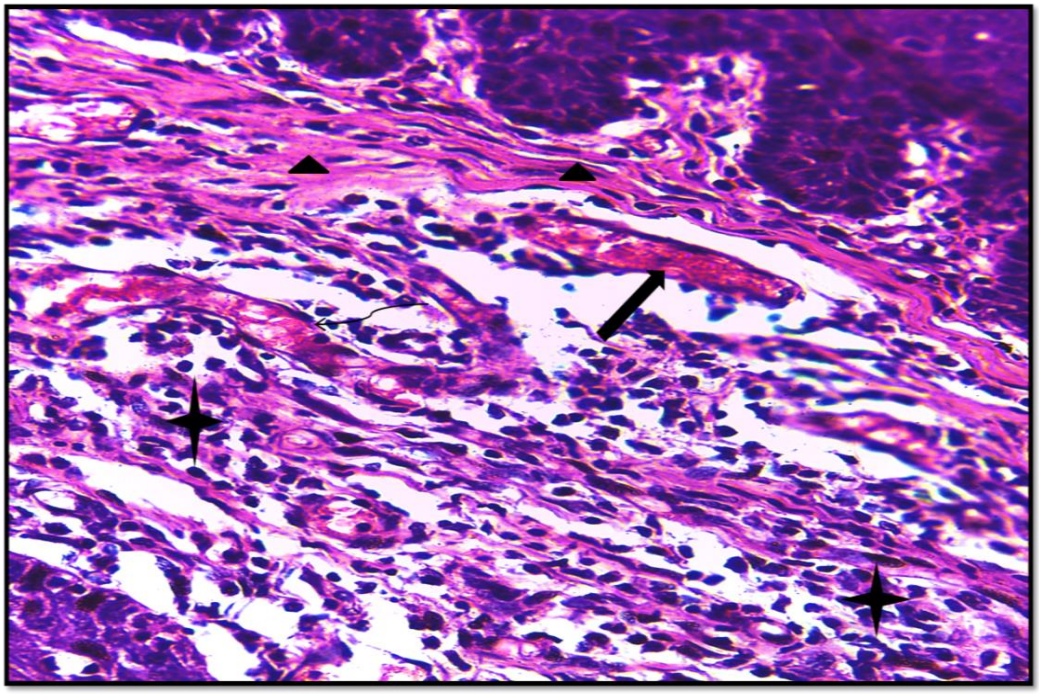


Figure (28) : Section in lip of rats treated with 60 mg\kg of (5-FU) after (21) days showing sever inflammation (✦) of lamina propria, congested blood vessels (➡) with collagen fibers (▲) and inflammatory cells . (H & E. 40 x).

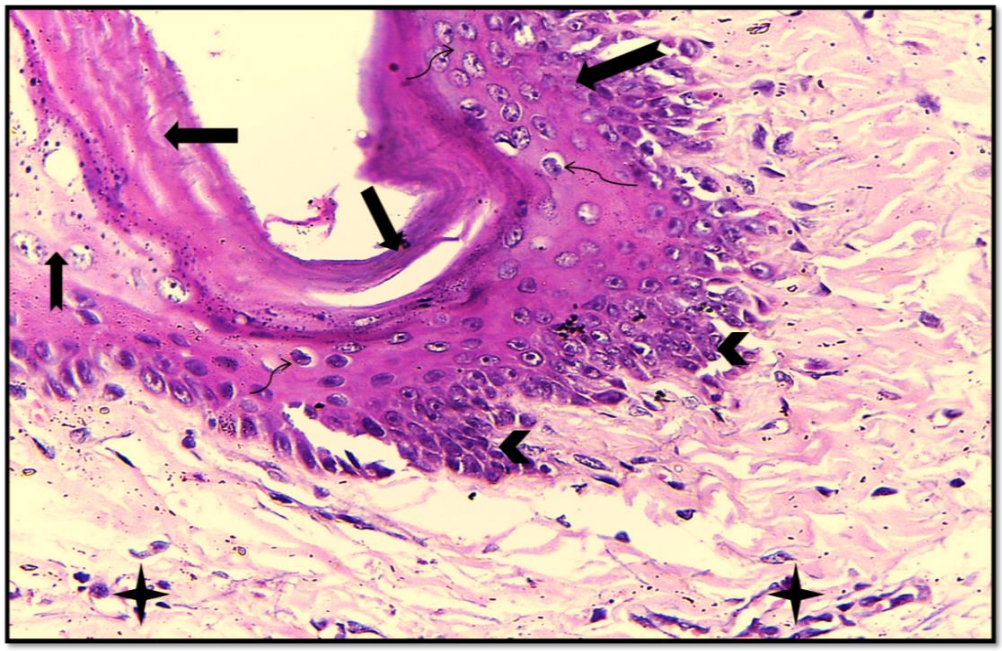


Figure (29): Section in lip of rats treated with 60 mg\kg of (5-FU) after (21) days showing hyperkeratosis (➡), destruction of epithelial layer (➡), degenerative vacuolation (⤳) proliferation of basal cells (◀) and inflammed lamina propria (✦). (H & E. 40 x).

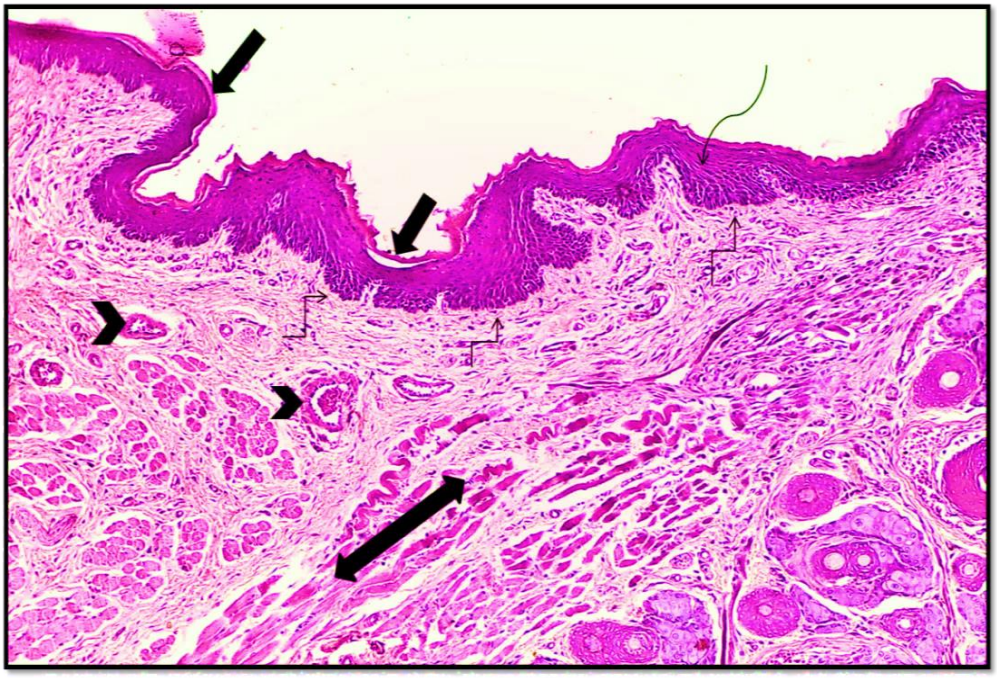


Figure (30): Section in lip of rats treated with (30 mg\ kg of 5-FU + glycin) showing deposition of keratin ( ), increase thickness of epithelium ( ), obvious and dilated rete ridges ( ), well develop collagen fibers ( ), and capillaries ( ). (H & E. 10X).

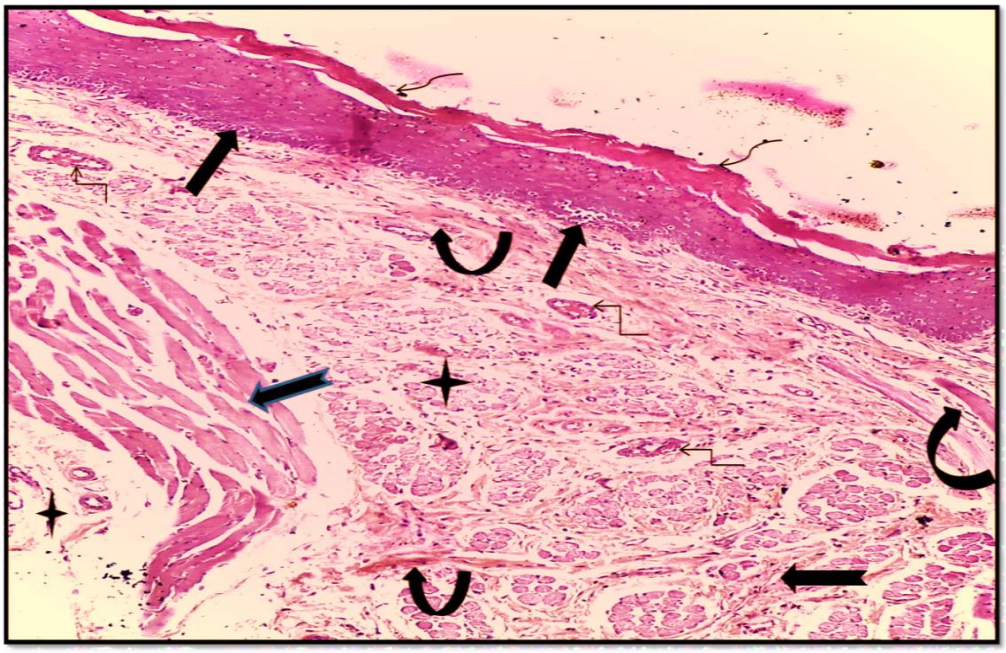


Figure (31): Section in lip of rats treated with (60 mg\ kg of 5-FU + glycin) showing proliferation of basal layer cells ( ), keratin deposition ( ), lamina propria with capillaries ( ), groups of striated muscle fibers ( ) separated by connective tissue ( ) and regenerated collage nous fibers ( ). (H & E. 10 X)

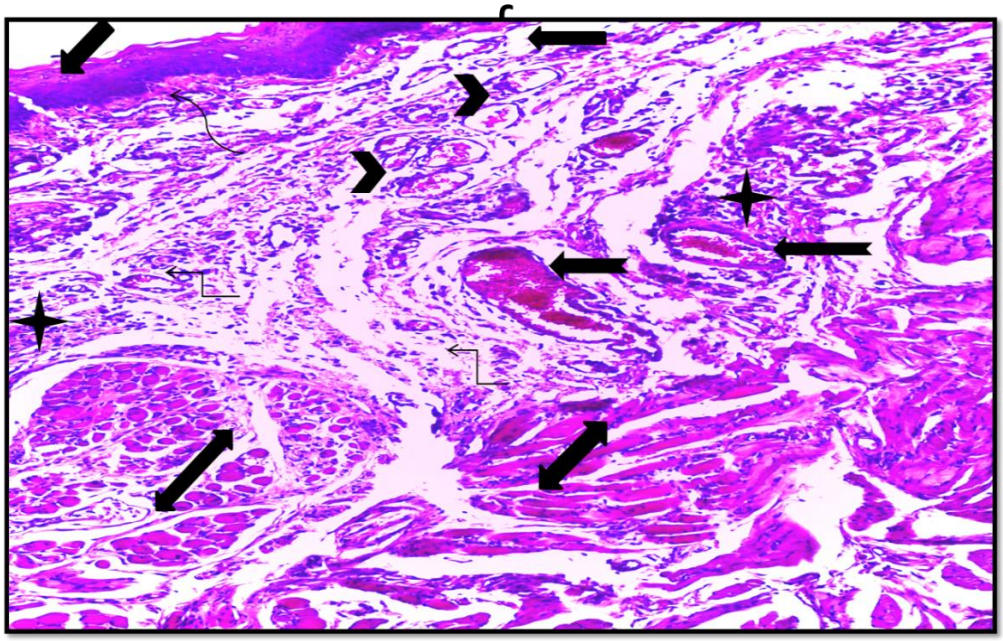


Figure (32): Section in lip of rats treated with 30 mg\ kg of (5-FU) post (45) days showing ulceration and destruction of mucosal layer (➡), inflamed lamina propria (⤳), vascularized connective tissue (⤳) with numerous capillaries (❯), inflammatory cells (✦) and congested blood vessels (➡), degenerated and atrophied bundles of muscle fibers (⟷). (H & E. 10 X).

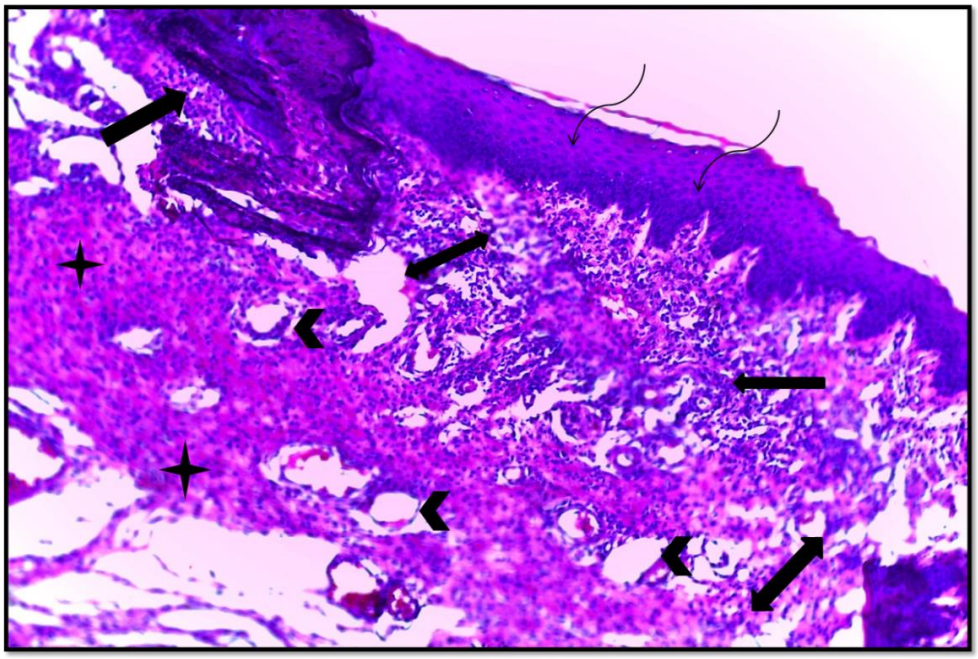


Figure (33): Section in lip of rats treated with 30 mg\ kg of (5-FU) post (45) days showing granulation tissue (➡) formed beneath the stratified epithelium (⤳), heavy infiltration of inflammatory cells (⟷), hemorrhage (✦) and degenerated capillaries (❯) within connective tissue . (H & E. 10X).

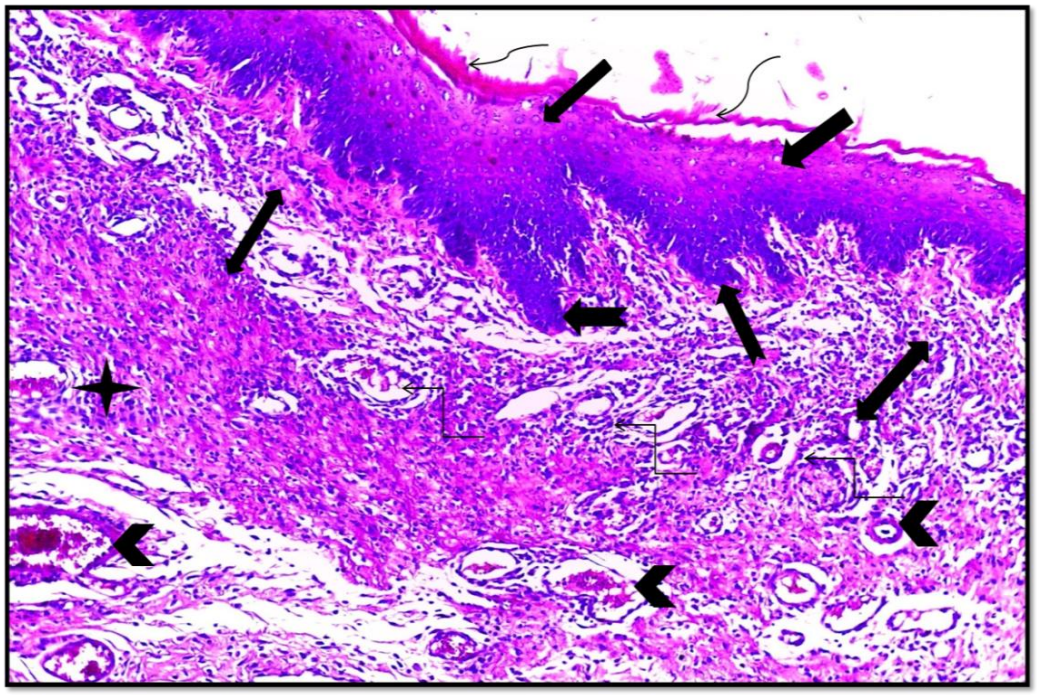


Figure (34): Section in lip of rats treated with 60 mg \kg of (5-FU) post (45) days showing separation of keratin (⤳), vacuolated epithelial cells (➡), irregular rete ridges (➡), sever inflammation of lamina propria (⟷), highly vascularized (⤳), hemorrhage (✦), and congested capillaries (❯). (H & E. 10X).

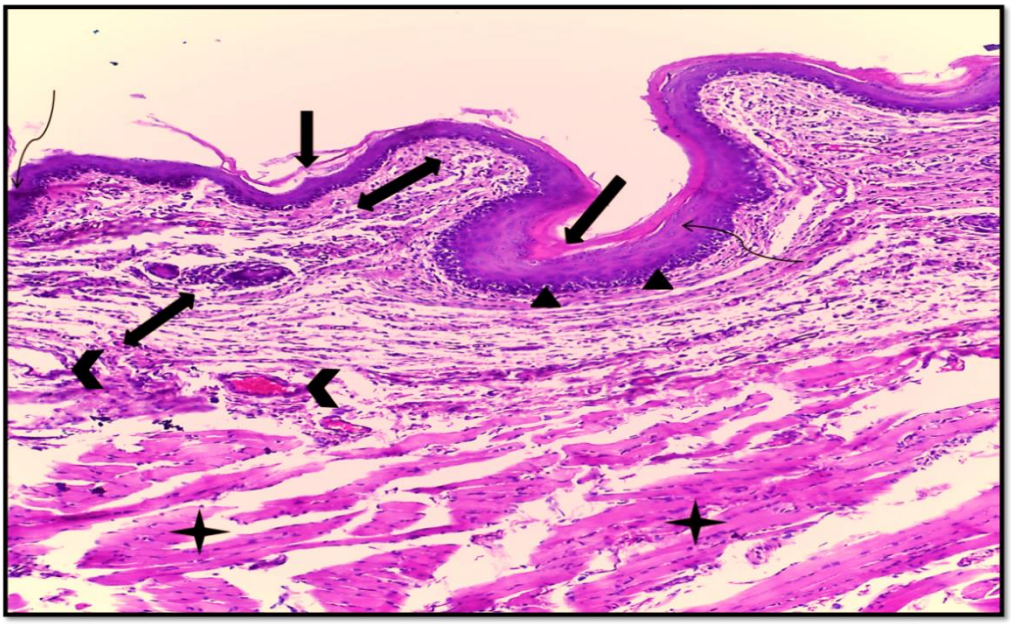


Figure (35): Section in lip of rats treated with (30 mg \kg of 5-FU + glycin) showing mild keratin deposition (➡), restoration of normal epithelial layer (⤳), vacuolated of basal cells (▲), mild sub-epithelial inflammation (⟷), vascularized connective tissue (❯) and still atrophied striated muscle fibers (✦). (H & E. 10 X).

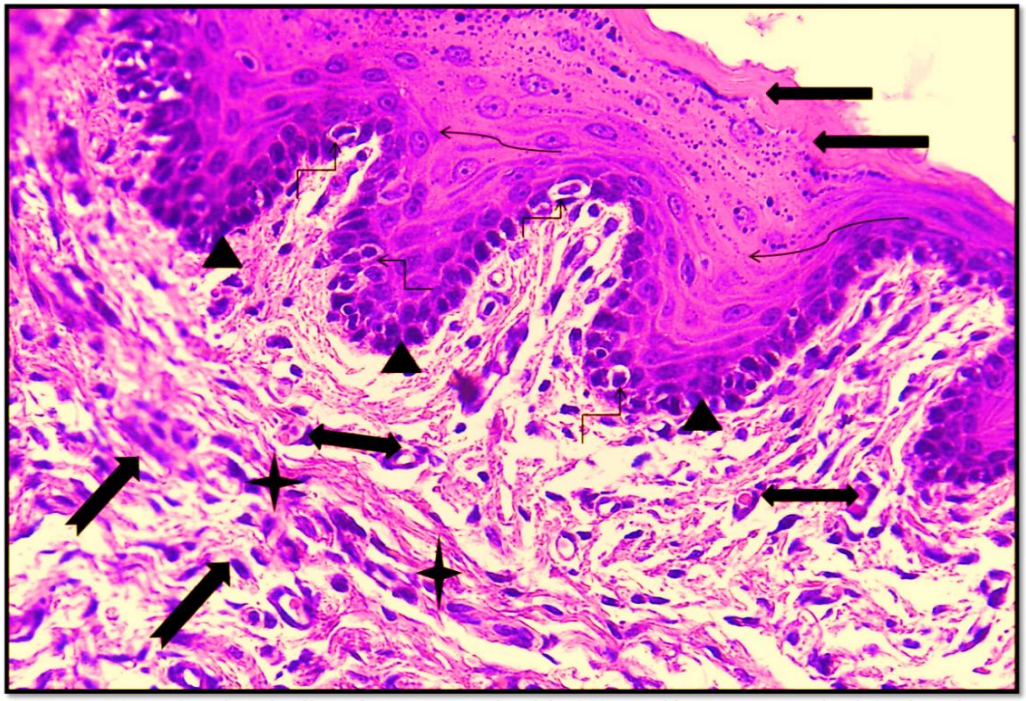


Figure (36): Section in lip of rats treated with (60 mg \kg 5-FU + glycin) showing keratin deposition (⟶), normal stratified squamus epithelium (⟶), organized basal cells (▲), still vacuolated keratinocytes (⟶), mild inflammatory cells with capillaries (⟷), collagen fibers (✦) and large number of fibroblast (⟶). (H & E. 40 X)

3.5.2.3. Cheek

Normal structure of rat cheek mucosa showed the normal stratified squamous epithelium, mild keratin deposition at the surface layer, deep rete ridges extend through the lamina propria, most cells of basal and para basal layer with dense, chromatic nuclei and the lamina propria as loose connective tissue with numerous small capillaries (fig 37).

Figures from cheek sections of rats injected with (30mg/kg) of (5-FU) after (21) days showed mild to moderate mucositis, mild keratin deposition, vacuolated epithelial cells and degeneration other cells, partially flattening of rete ridges, inflamed lamina propria, normal collagen fibers within connective

tissue beneath the mucosa and congested capillaries (fig 38). Moreover cheek mucositis from rats injected with (60mg/kg) of (5-FU) after same period showed inflammation process constituted by extended of inflammatory cells to the connective tissue with edema and numerous capillaries, the surface of mucosa showed stratified squamous epithelium with perinuclear vacuolated cells and mild keratin deposition, also dense collagen bundles with fibroblasts was obvious (fig 39, 40).

Microscopic examination of cheek sections from rats injected with (30mg/kg) of (5-FU) but treated with glycine for (21) days showed well epithelial layer, arranged cells with more proliferation of basal cells, mild vacuolation of keratinocytes, increased thickness of stratified squamous epithelium, the connective tissue with collagenous fibers and no signs of congested capillaries (fig 41). Compared with the cheek sections from rats group that injected with (60mg/kg) of (5-FU) and treated with glycine for the same period showed less healing signs as still perinuclear vacuolated cells and moderate inflammatory cells extend to the connective tissue beneath lamina propria, moreover irregular strands of collagen fibers and fibroblast, but on the other hand showed increased thickness of regenerated mucosal layer, restoration of most normal shape of rete ridges (fig 42).

When the treated period be longest, the changes on cheek mucosa from rats injected with (30mg/kg) of (5-FU) post (45) days were observed like ulceration of superficial layer, decrease thickness of epithelial layer, vacuolated keratinocytes, moderate inflammatory cells, dense connective tissue beneath lamina propria with randomly arrangement of atrophied muscle fibers and congested capillaries (fig 43). When the dose of (5-FU) was increased the cheek mucosa showed more severity changes as complete destruction and

ulceration of mucosa, separation of keratin layer, basal and para basal epithelium with perinuclear vacuolation cells, pyknotic nuclei cells and heavy infiltration of inflammatory cells, edema and degenerated area (fig 44).

Results revealed to the cheek mucosa sections of rats injected with (30mg/kg) of (5-FU) but treated with glycine for (45) days, well developed mucosa, re-epithelization of stratified squamous epithelium, proliferation of basal cells at rete pegs, more keratin deposition at the surface layer and the connective tissue beneath the mucosa with mild inflammatory cells, numerous capillaries and more fibroblast (fig 45). While changes of cheek sections in rats injected with (60mg/kg) of (5-FU) but treated with glycine for (45) days mentioned to restoration of oral mucosal, stratified squamous epithelium with normal cells and few perinuclear vacuolated cells, flat to well-organized of rete ridges in some regions, mild sub-epithelial inflammatory cells, the connective tissue exhibited normal collagen fibers with striated muscles and still congested large blood vessels (fig 46).

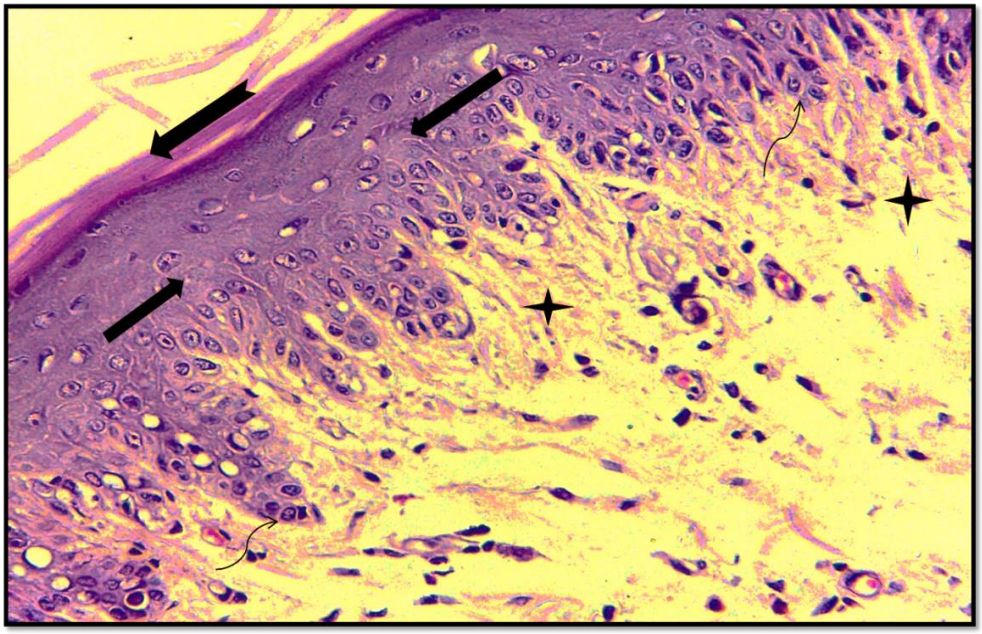


Figure (37): Section of cheek in control rats showing normal oral mucosa composed of stratified squamus epithelium ( ), mild deposition of keratin ( ) on surface layer and rete ridges ( ) well develop extend down to the lamina propria ( ). (H & E. 40 x)

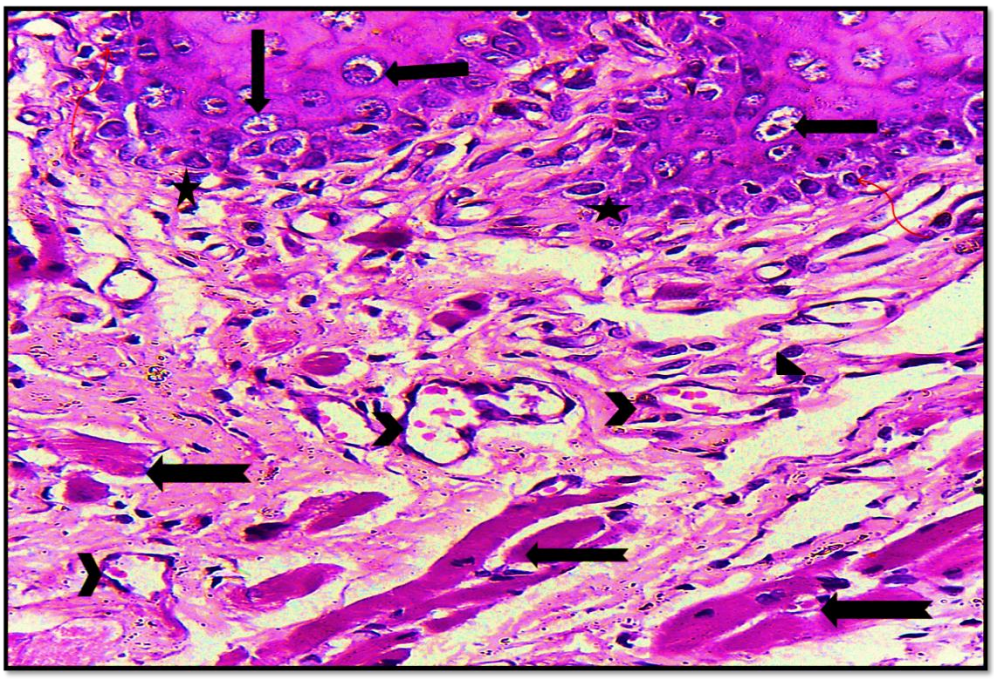


Figure (38): Section in cheek of rats treated with 30 mg \kg of (5-FU) post(21) days showing degeneration and cells vacuolation ( ), most cells of basal cells with perinuclear ( ), flattening rete ridges ( ), mild inflammation ( ), congested capillaries ( ), atrophied muscle fibers ( ) (H & E. 40 x).

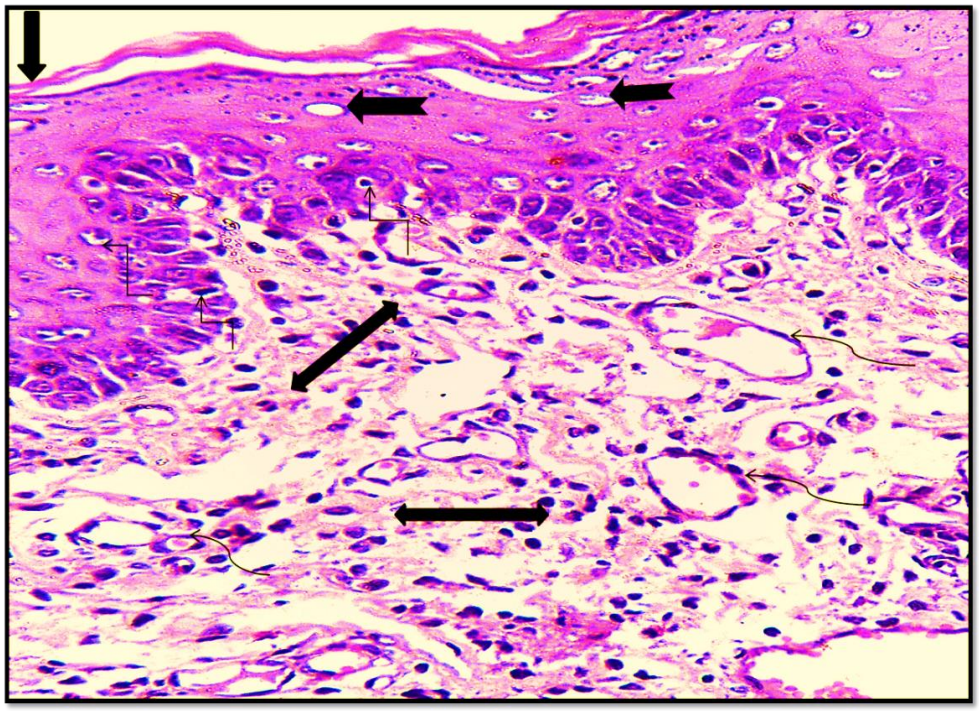


Figure (39): Section in cheek of rats treated with 60 mg \kg of (5-FU) post (21) days showing separation of keratin layer ( ), vacuolated cells ( ) of stratified squamous epithelium, most cells pyknotic nuclei ( ), lamina propria with lymphocytes ( ) and appeared vascularized ( ). (H & E. 40 x).

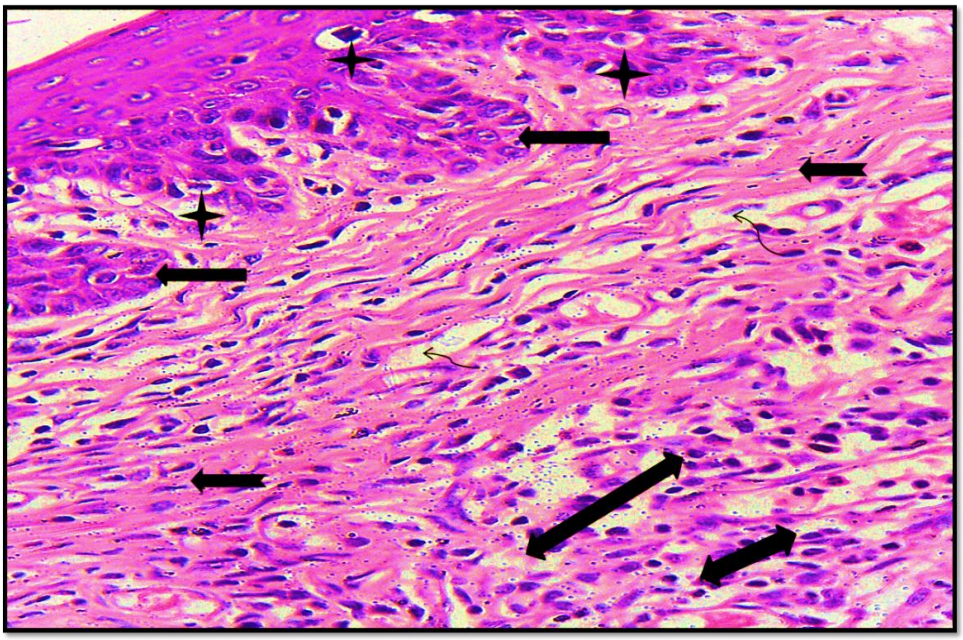


Figure (40) : Section in cheek of rats treated with 60 mg\ kg of (5-FU) post (21) days showing deep rete ridges ( ), vacuolated kerationcytes ( ), dense collagen fibers ( ), edema ( ) and large number of fibroblasts ( ). (H & E. 40 x)

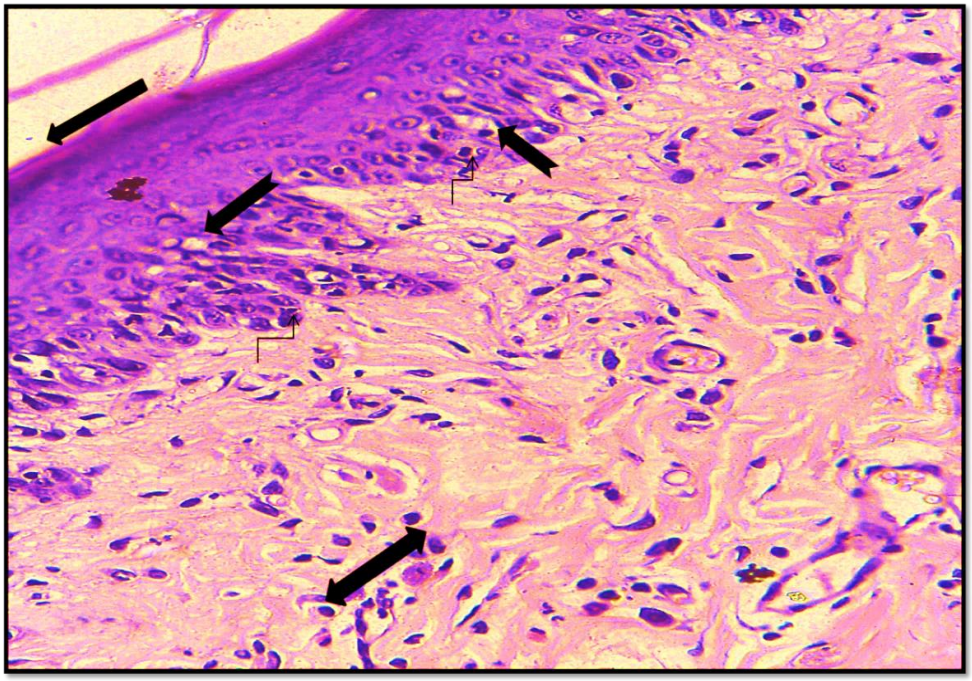


Figure (41): Section in cheek of rats treated with (30 mg\ kg of 5-FU + glycin) showing mild keratin (⟹),mild keratinocytes vacuolation (⟹), mild inflammatory cells (⟺) and more proliferation of basal cells (⤳). (H & E. 40 x).

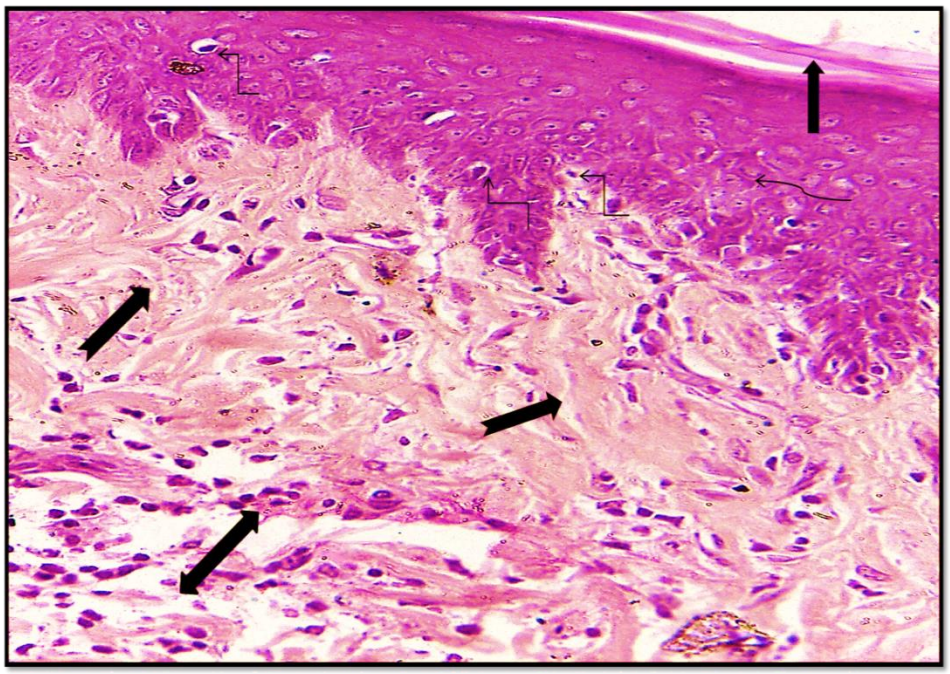


Figure (42): Section in cheek of rats treated with (60 mg \kg of 5-FU + glycin) showing separated of keratin layer(⟹), increase thickness of epithelial layer (⤳), still keratinocytes with perinuclear vacuolation (⤳) and inflammatory cells extend to deep layers (⟺), irregular collagenous fibers (⟹). (H & E. 40x)

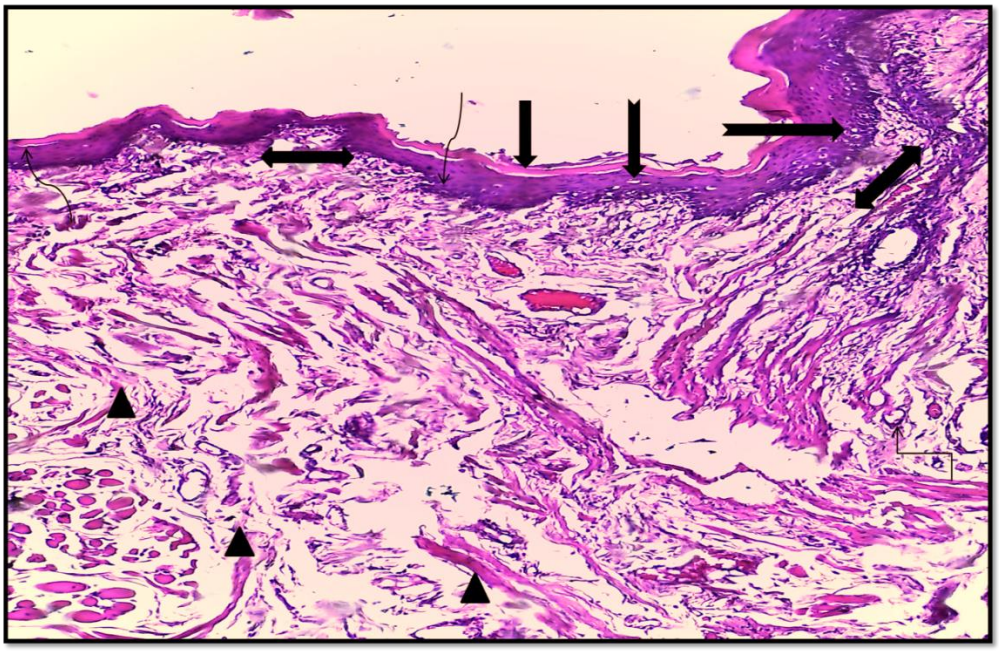


Figure (43): Section in cheek of rats treated with 30 mg \kg 5-FU post (45) days showing separation of keratin layer (⟶), reduced thickness of epithelium (⟶), vacuolated keratinocytes (⟶, moderated inflammatory cells ⟷) atrophied muscle fibers (▲) (H & E. 10 x).

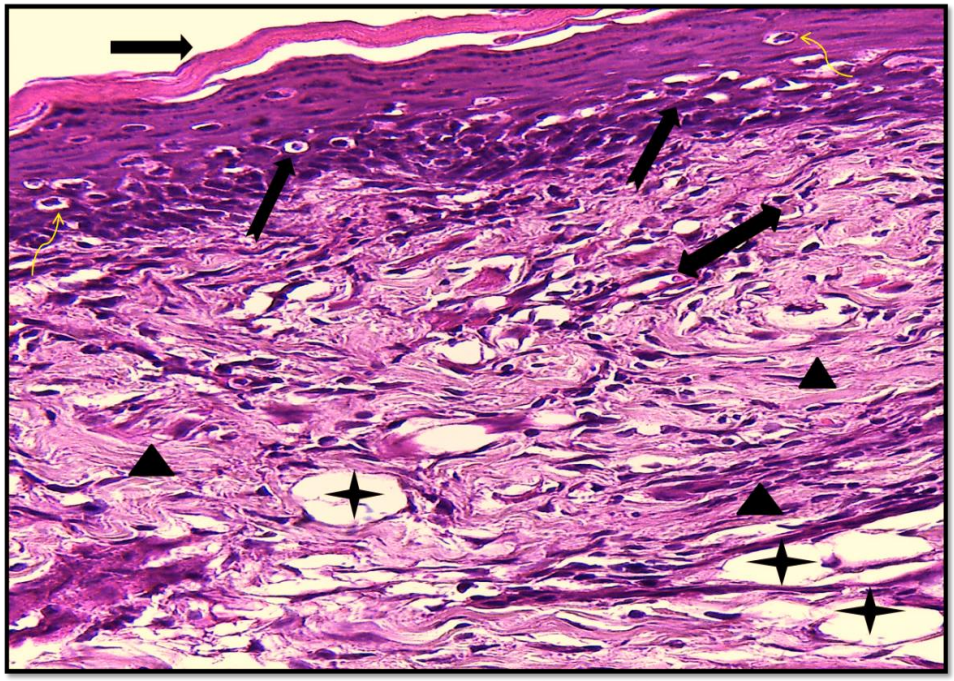


Figure (44): Section in cheek of rats treated with 60 mg \kg of (5-FU) post (45) days showing obvious separation of keratin layer (⟶), perinuclear vacuolation of cells (⟶) in basal and Para basal epithelium (⟶), irregular bundles of collagen fibers (▲), heavy infiltration cells (⟷), edema (✦) and degeneration (⟶). (H & E. 40 X).

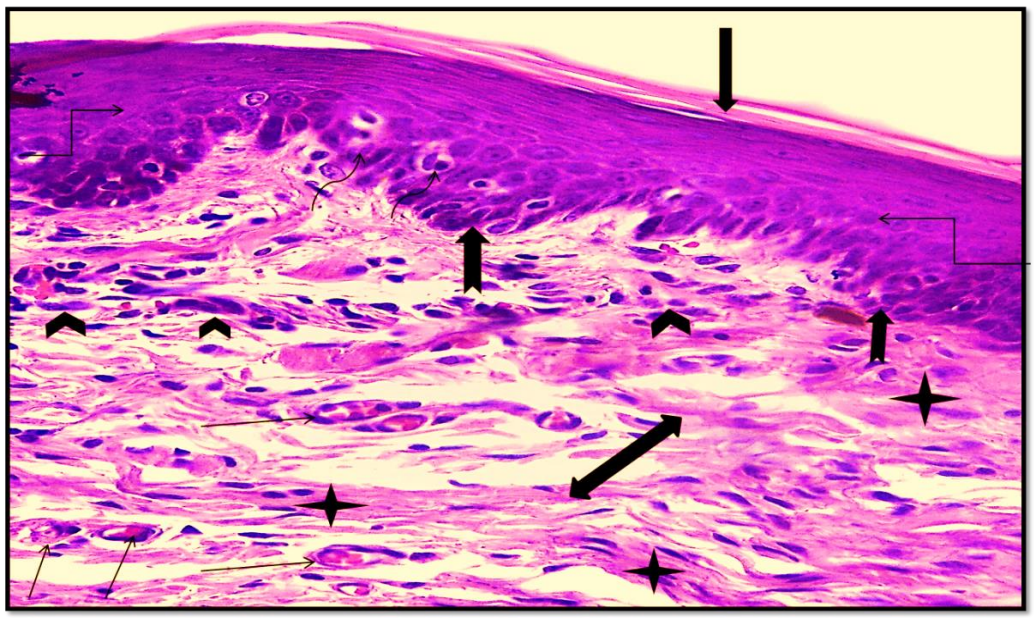


Figure (45): Section in cheek of rats treated with (30 mg \kg of 5-FU +glycin) showing mild keratin deposition (➡), re-epithelization of epithelial layer (⮕), proliferation of basal and Para basal cells (➡), few perinuclear cells (⮕), normal connective tissue (⬌) with collagen fibers (✦), fibroblasts (⮝) and capillaries (⟶). (H & E. 40 X)

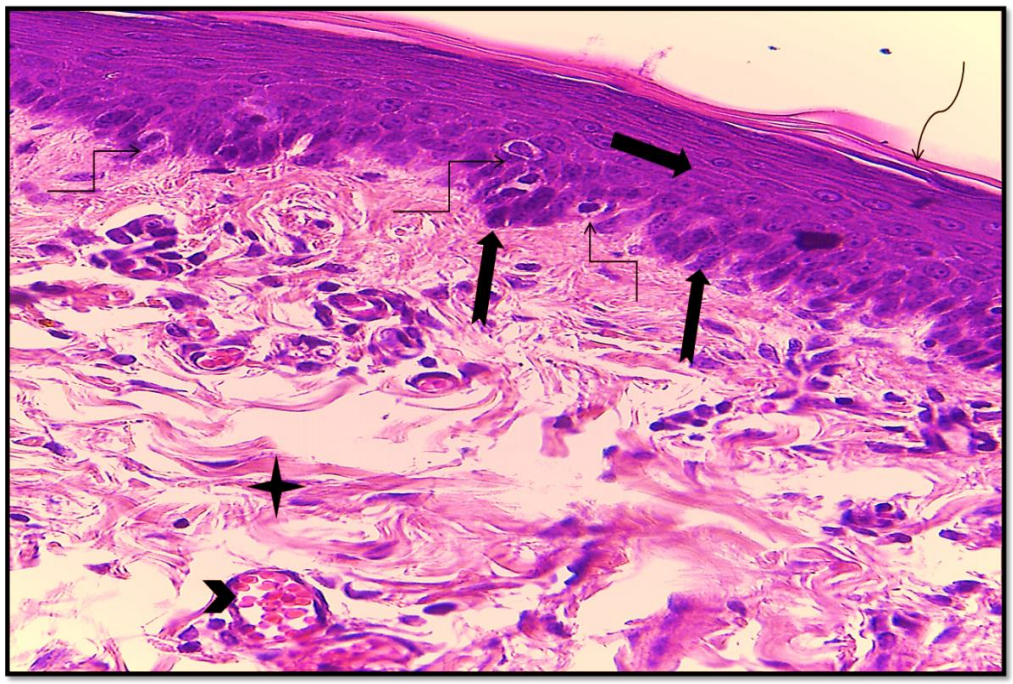


Figure (46): Section in cheek of rats treated with (60 mg \kg of 5-FU +glycin) showing restoration mucosal and stratified epithelial layer (➡), less keratin deposition (⮕), flattening to normal shape of rete ridges (➡), few perinuclear vacuolated cells (⮕), collagen fibers (✦) and large congested blood vessels (❯). (H & E. 40 X)

3.6. Immunohistochemistry examination of PCNA and BCL-2 expression:

Semi quantitative expression was performed by immunostaining of anti-PCNA and BCL-2. A positive control for PCNA confirmed the specific of the staining (fig 47, 48). Immunohistochemically results referred to strong and moderate immune expression to (PCNA) at basal and para basal cells within nucleus appeared as brownish in the tongue epithelial cells of control groups that treated with saline only and saline with glycine (fig 49, 50).

Figures from tongue sections related to rats treated with (30mg/kg) of (5-FU) post (21) days showed moderate positive staining of epithelial cells (fig 51), compared to the sections from rats treated with (60mg/kg) of (5-FU) for the same period which revealed mild immune reactivity confined at the basal cells within nucleus and some of keratinocytes at the surface layer with missing of the positive cells in some basal cells (fig 52). Moreover sections from rats tongue injected with (30mg/kg) of (5-FU) and treated with glycine for (21) days showed strong positive immunostaining of basal and supra basal cells of epithelial layer to (PCNA) protein expression (fig 53). Whereas sections from rats tongue injected with (60mg/kg) of (5-FU) and treated with glycine for the same period, showed moderate immune reactivity to (PCNA) expression and the basal cells stained with brownish to dark brown, and few positive cells of connective tissue were noticed (fig 54).

Also the study clarified the effect of (30mg/kg) of (5-FU) post (45) days on (PCNA) expression of rats tongue mucosa, the (IHC) staining referred to mild immune expression of basal cells and few keratinocytes at surface layer while most cells missing the stain with vacuolated fig (55). Compared with the

response to the immune expression of (PCNA) protein evaluated in rats tongue of group (60mg/kg) of (5-FU) post (45) days. The results showed few positive staining cells at basal cells and negative staining cells at whole thickness of epithelial layer, the cells missing of stain and noticed as blue stain cells (fig 56).

The study evaluated the role of glycine on rats tongue mucosa treated with (30mg/kg) of (5-FU) with glycine for (45) days, the (IHC) staining revealed to moderate positive staining to (PCNA) protein expression at basal cells, although some cells showed negative staining to (PCNA) (fig 57). While the sections in rats tongue treated with (60mg/kg) of (5-FU) and glycine showed moderate positive staining of basal cells appeared dark brown, mild staining to (PCNA) of supra basal cells with negative staining nucleus distributed through epithelial layer (fig 58).

Immunohistochemistry results of the positive control for BCL-2 expression of the tongue showed moderate to strong expression and obvious immune-reactivity in the cytoplasm of the basal and para basal layers of epithelium (fig 59, 60). Sections from the tongue mucosa treated with saline only and saline with glycine showed immunoreactivity of (BCL-2) in cytoplasm of most epithelial cells as brown deposition specially at surface of stratified squamous epithelial layer (fig 61, 62).

Results on tongue mucosa from rats treated with (30mg/kg) of (5-FU) post (21) days showed mild cytoplasmic reaction to BCL-2 agent and mild staining of epithelial cells at surface of mucosa as fine brown deposition (fig 63). While sections from rats tongue that related to (60mg/kg) of (5-FU) treated group

showed slightly immune reactivity and mild staining of the surface cells compared with control groups (fig 64).

Moreover, the immunohistochemistry of BCL-2 protein in tongue mucosa of rats treated with (30mg/kg of 5-FU + glycine) for (21) days showed moderate staining in whole thickness of epithelial layer and result expressed as positive immunoreactivity to BCL-2 in cytoplasm of epithelial cells and some of connective tissue cells (fig 65). Compared to the sections on tongue mucosa from rats group treated with (60mg/kg of 5-FU + glycine) which showed mild staining to BCL-2 in keratinocytes of surface layer, brown granules deposition within cytoplasm and also some cells of basal and para basal layer (fig 66).

Also the results revealed to mild immunoreactivity in tongue mucosa of rats treated with (30mg/kg) of (5-FU) post (45) days, the result was showed negative at basal and para basal layers accept positive staining to BCL-2 at epithelial cells cytoplasm of surface layer (fig 67). While the results clarified the immunoreactivity on tongue mucosa to BCL-2 protein in rats treated with (60mg/kg) of (5-FU) after (45) days and referred to negative results, the cells showed no staining either of cytoplasm or nucleus of epithelial layer cells (fig 68). As for sections in rats tongue that related to group treated with (30mg/kg of 5-FU + glycine) for (45) days showed increase in BCL-2 positive cells in cytoplasm of supra epithelial cells, compared to the sections of rats tongue related to the group treated with (60mg/kg of 5-FU + glycine) showed regeneration and positive cells to BCL-2 at the cells of epithelial layer but still negative immunoreactivity to BCL-2 on basal and para basal cells (fig 69, 70).

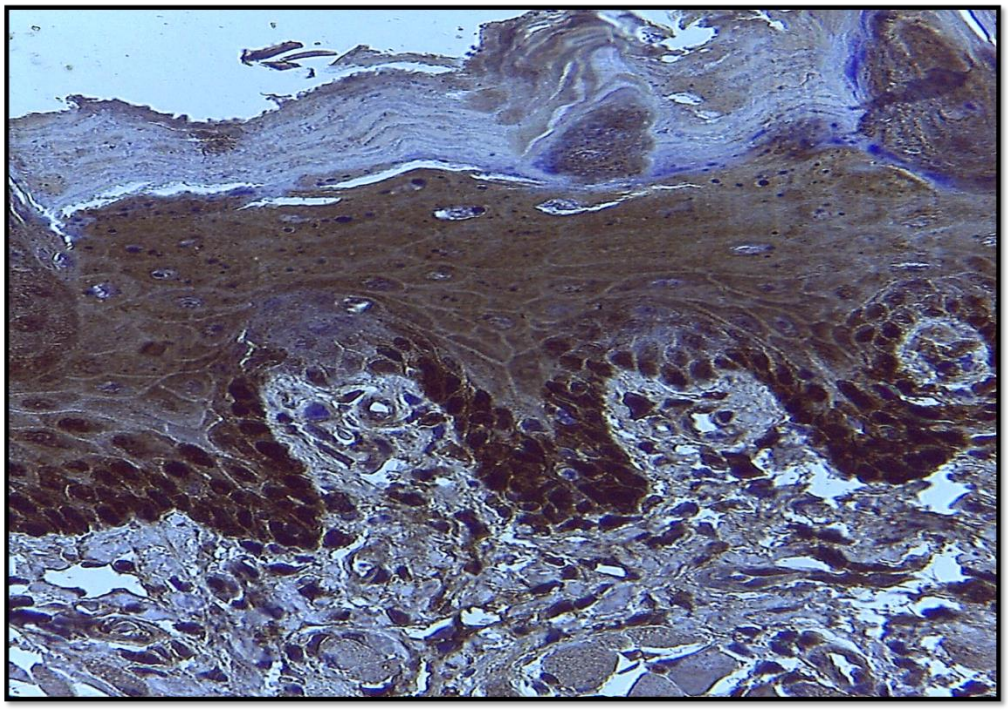


Figure (47): Positive control to PCNA protein expression in tongue of rats

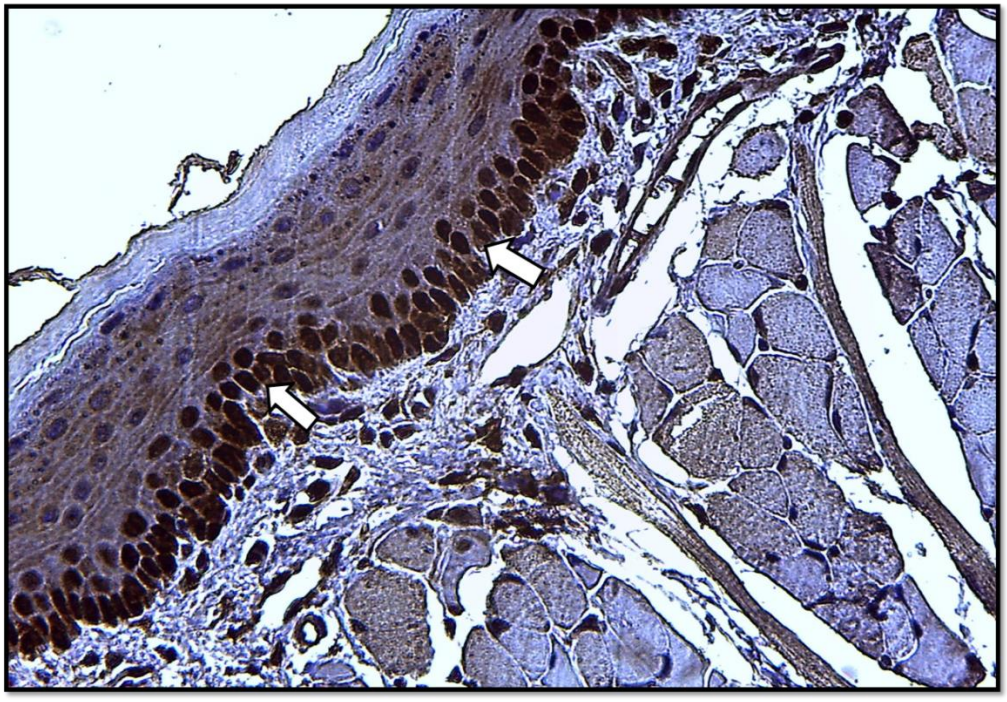


Figure (49): Section of tongue in control rats (treated with saline) showed moderate immune expression to (PCNA) in basal and supra basal cells (arrows) (IHC 40 X).

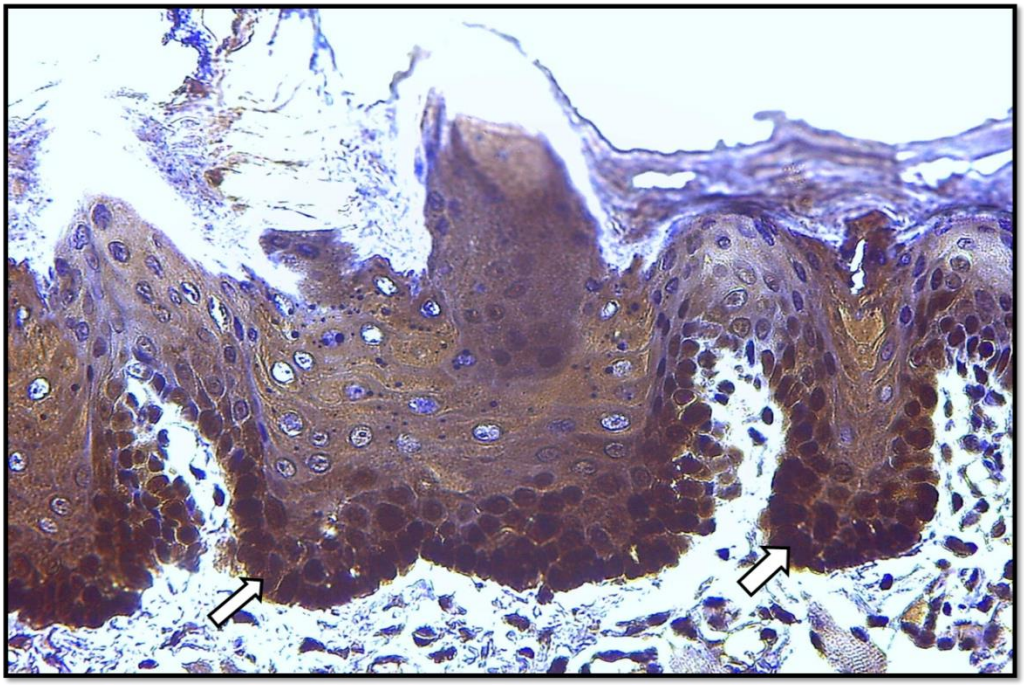


Figure (50): Section of tongue in control rats (treated with saline \glycine) showing strong positive immune expression to (PCNA) in basal and supra basal cells (arrows) (IHC 40 X) .

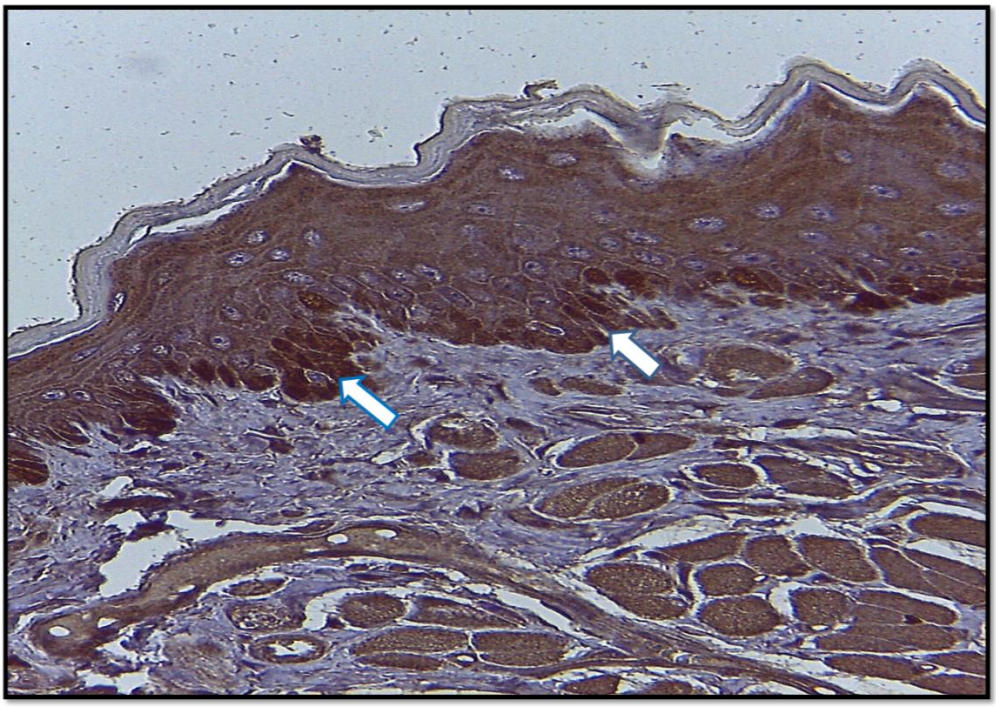


Figure (51): Section in tongue of rats treated with 30 mg \kg of (5-FU) post (21) days showed moderate staining to (PCNA) protein at basal cells (arrows) (IHC. 40 X).

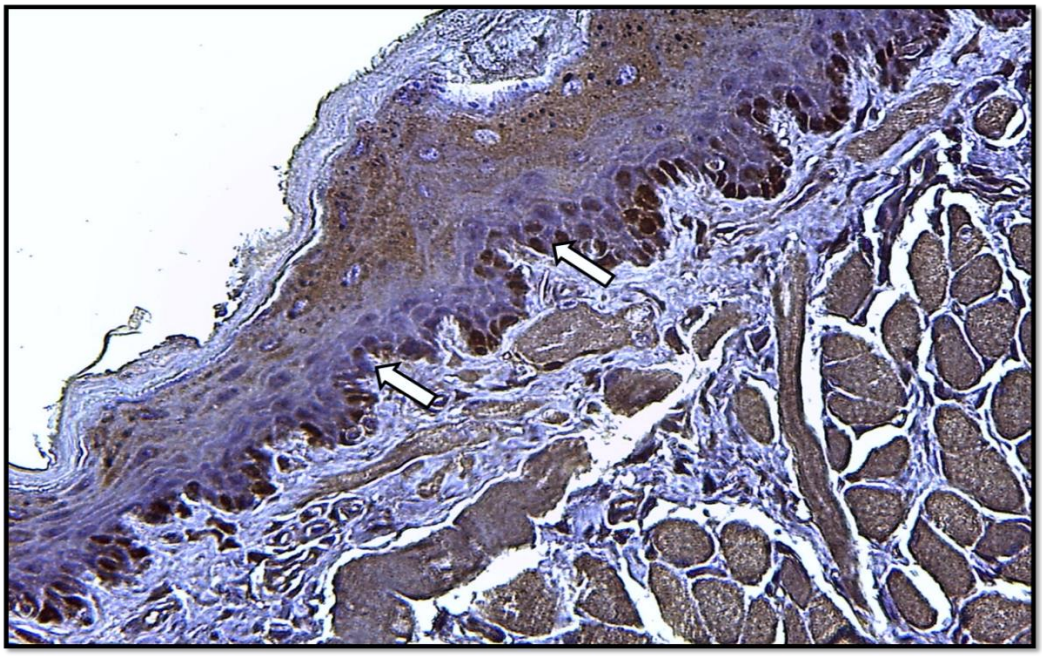


Figure (52): Section in tongue of rats treated with 60 mg \kg of (5-FU) post (21) days showed mild staining to (PCNA) protein at basal cells with missing of positive cells in some basal layer cells (arrow) (IHC. 40 X)

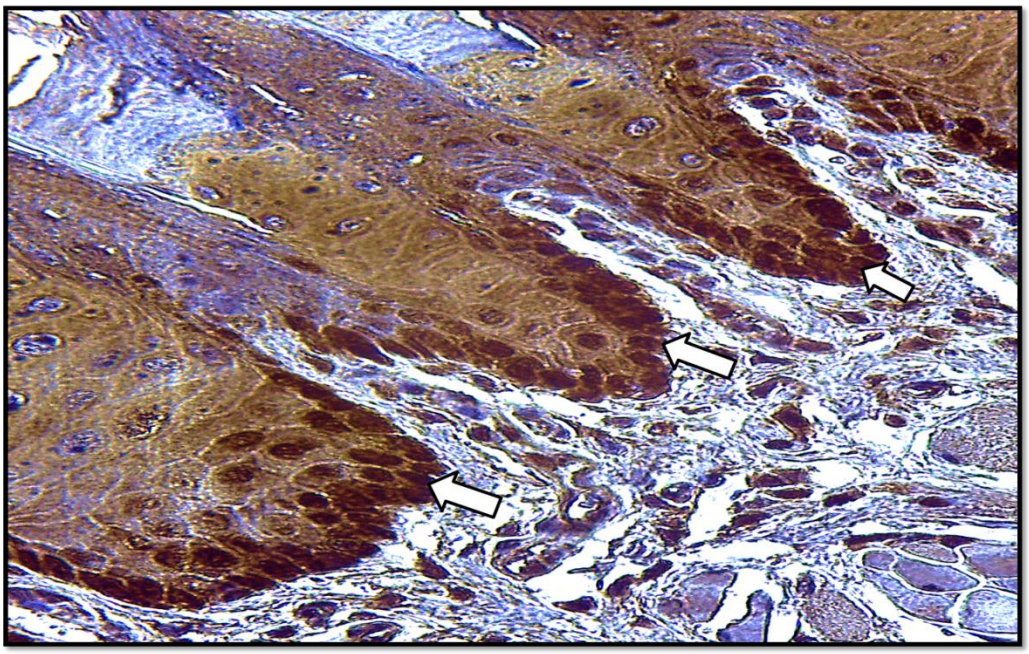


Figure (53): Section in tongue of rats treated with (30 mg \kg of 5-FU+ glycine) post (21) days showing strong immune expression in basal and supra basal cells to (PCNA) (arrows) (IHC. 40 X)

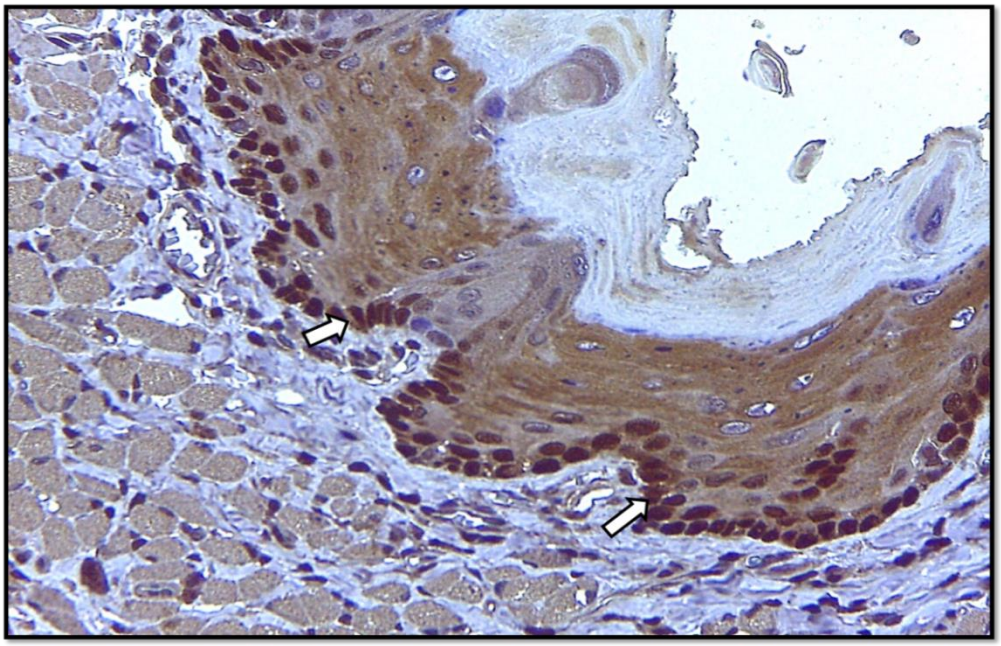


Figure (54): Section in tongue of rats treated with (60 mg \kg of 5-FU+ glycine) post (21) days showing moderate staining cells of basal layer to (PCNA) (arrows) (IHC. 40 X)

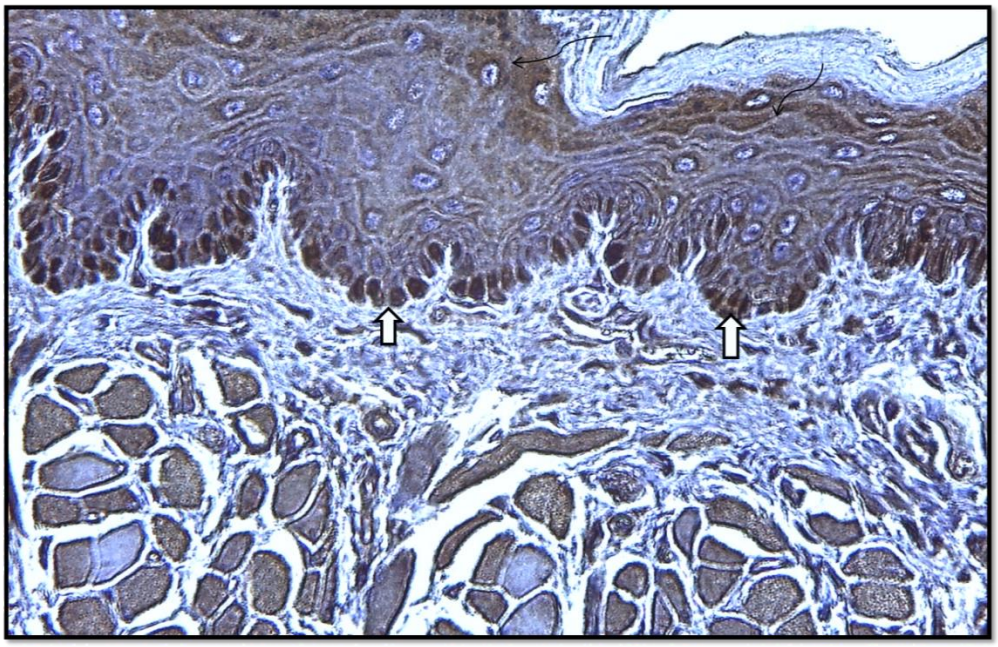


Figure (55): Section in tongue of rats treated with 30 mg \kg of (5-FU) post (45) days showing mild positive basal cells (arrows), few positive keratinocytes (↝) and most epithelial layer with vacuolated cells and missing staining (IHC. 40 X)

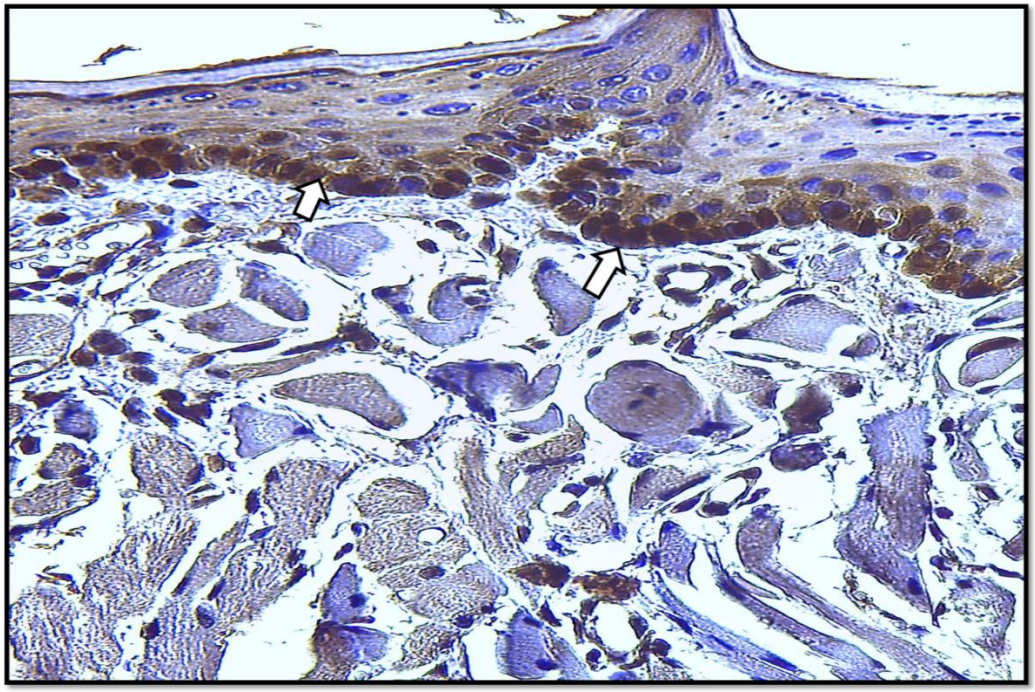


Figure (56): Section in tongue of rats treated with 60 mg \kg of (5-FU) post (45) days showing only few positive staining cells to (PCNA) at basal layer cells (arrows) while other cells were negative immuno reactivity (⟶) (IHC. 40 X)

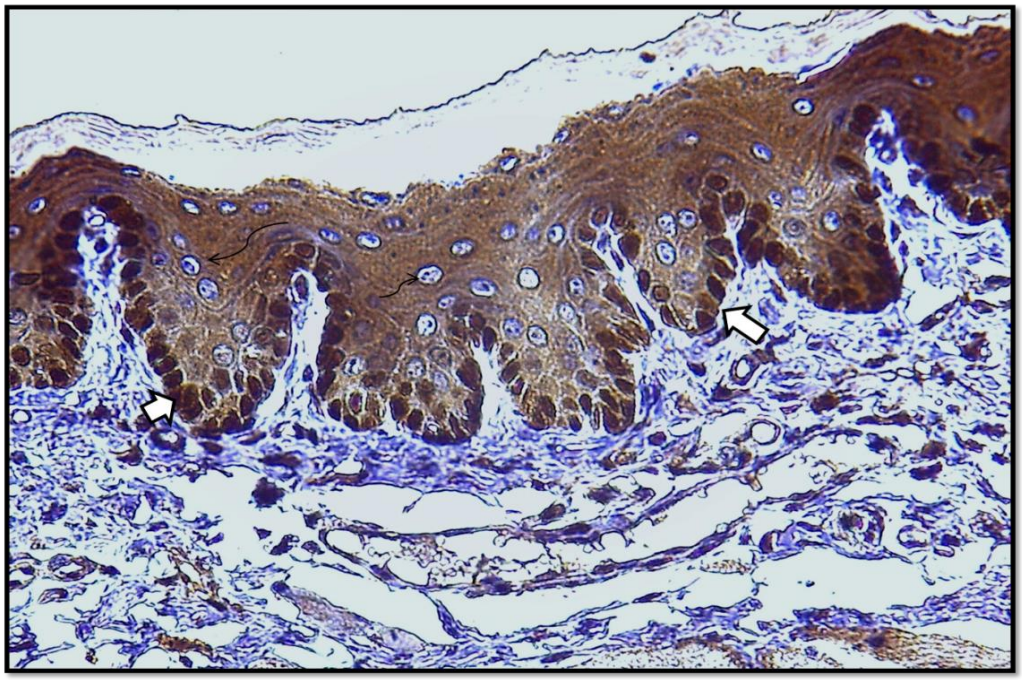


Figure (57): Section in tongue of rats treated with (30 mg\kg 5 of -FU + glycine) showed moderate staining to (PCNA) at basal cells (arrow) , some cells still showed negative staining (⟶) (IHC. 40 X)

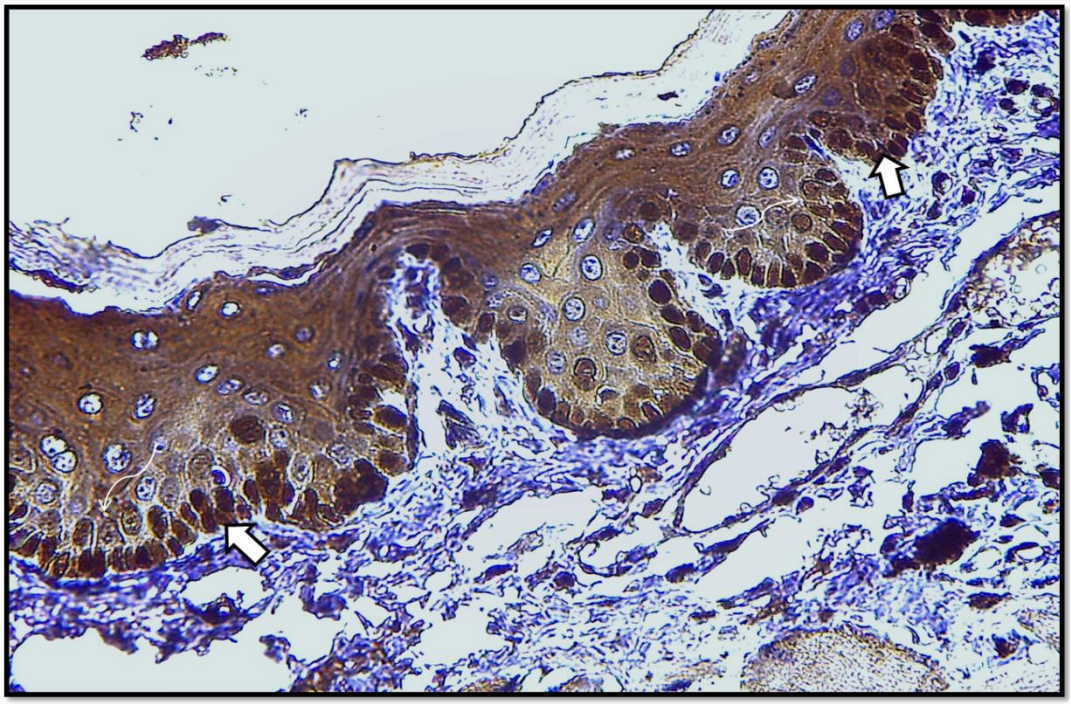


Figure (58): Section in tongue of rats treated with (60 mg \kg of 5-FU + glycine) showing positive staining to (PCNA) protein of basal cells (arrows), mild staining of supra basal cells (⟶) and negative staining nucleus also noticed (IHC. 40 X)

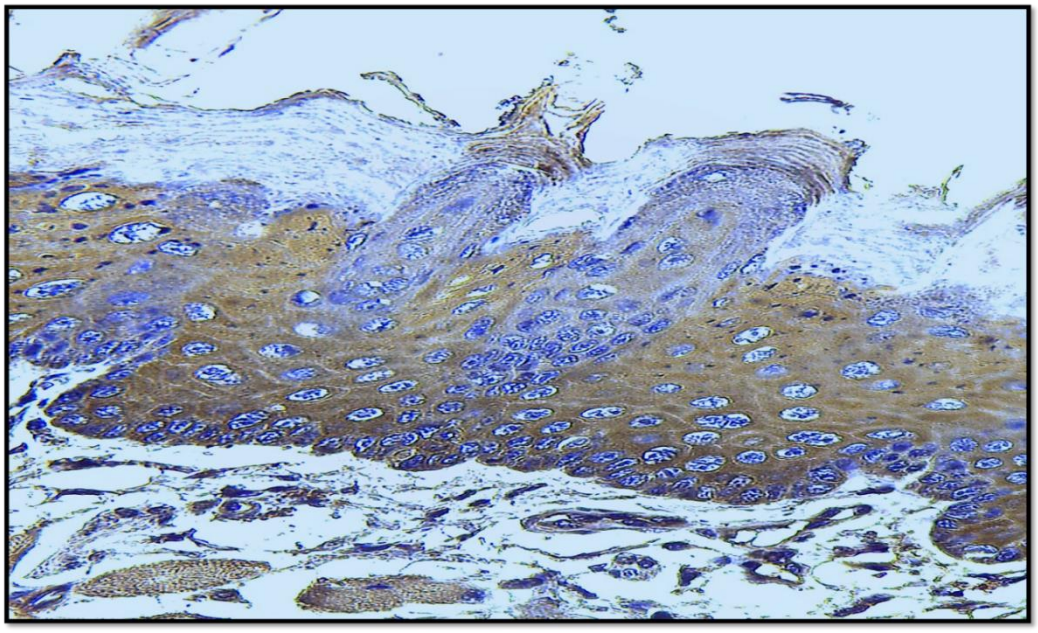


Figure (59): Positive control in cytoplasmic reaction to BCL-2 protein in tongue of rats.

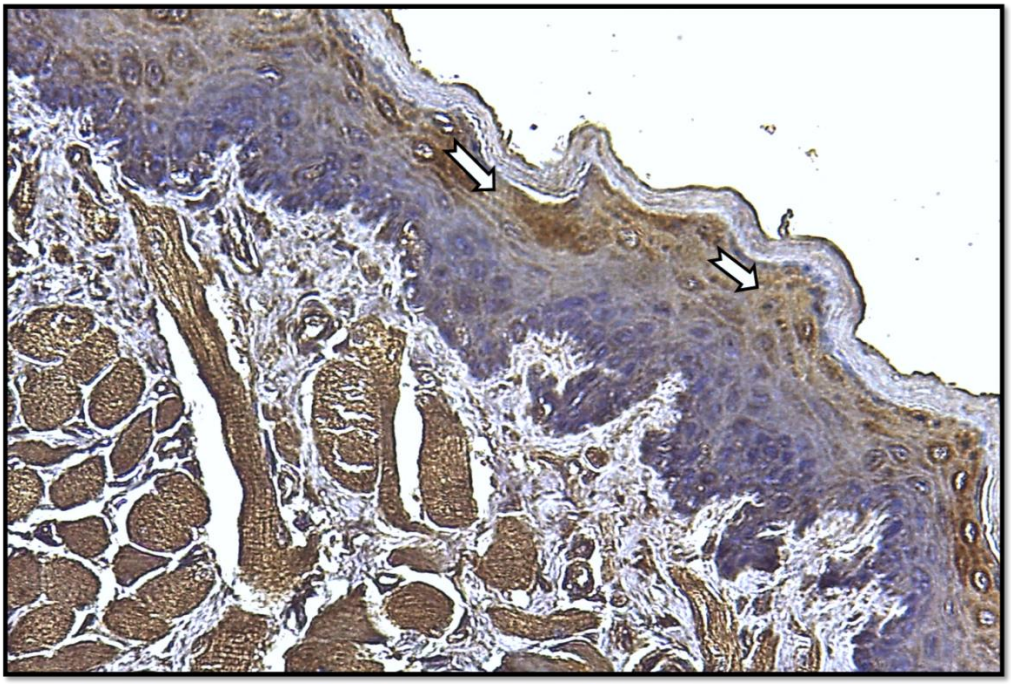


Figure (61) : Section in tongue of rats treated with normal saline showing immune reactivity (arrows) in cytoplasmic reaction to BCL-2 of epithelial cells (IHC. 40 X)

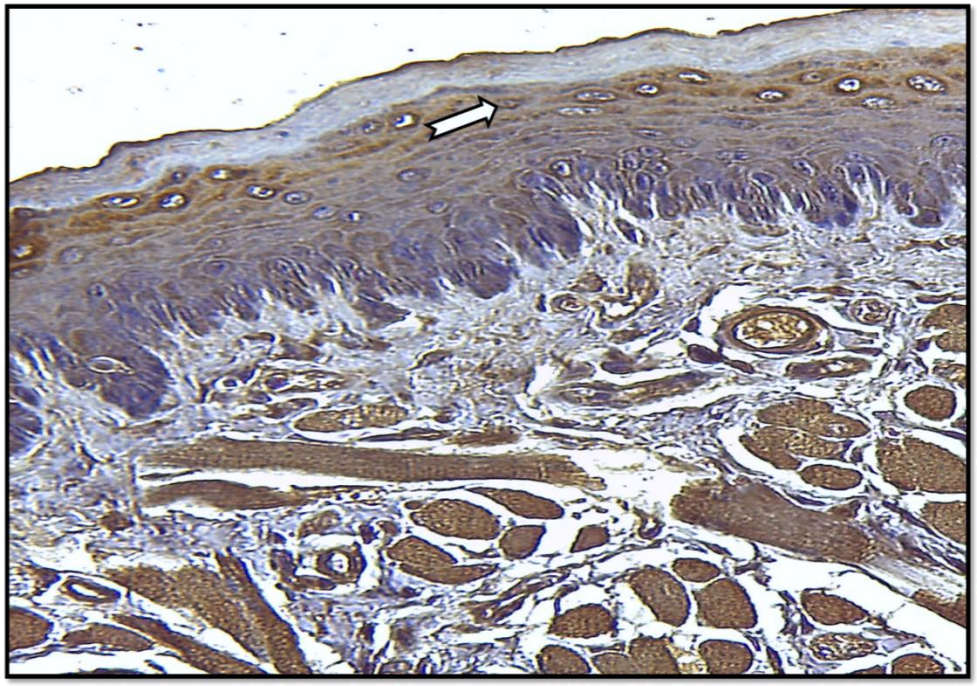


Figure (62): Section in tongue of rats treated with saline \glycine showing moderate immune reactivity to BCL-2 of epithelial cells cytoplasm (arrow) (IHC. 40 x)

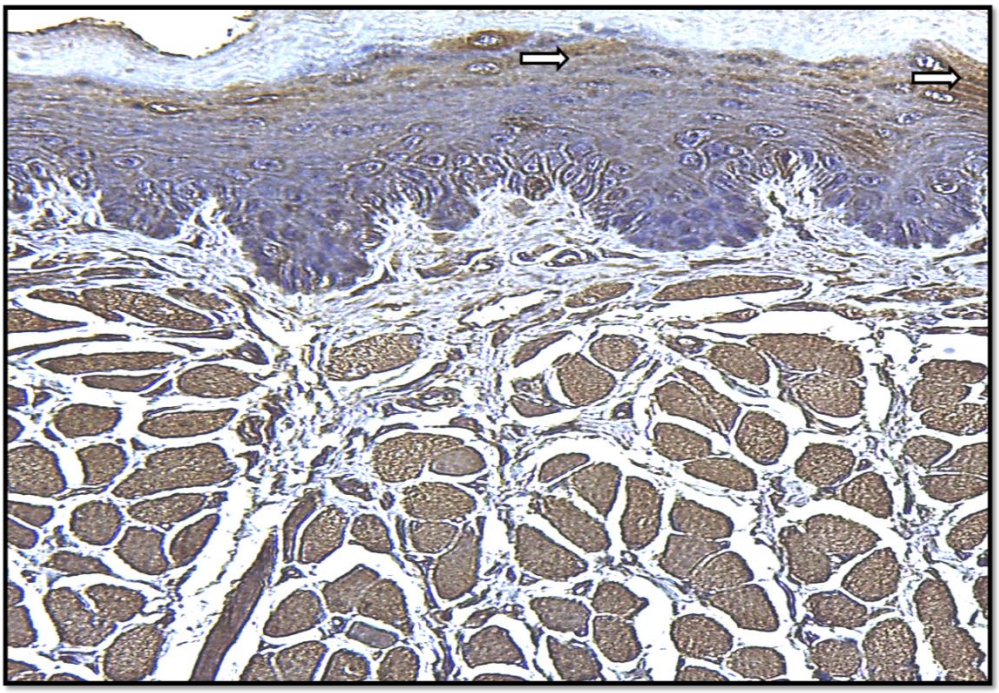


Figure (63): Section in tongue of rats treated with 30 mg \kg of (5-FU) post (21) days showed mild cytoplasmic reaction to BCL-2 activity (arrows) (IHC. 40 X)

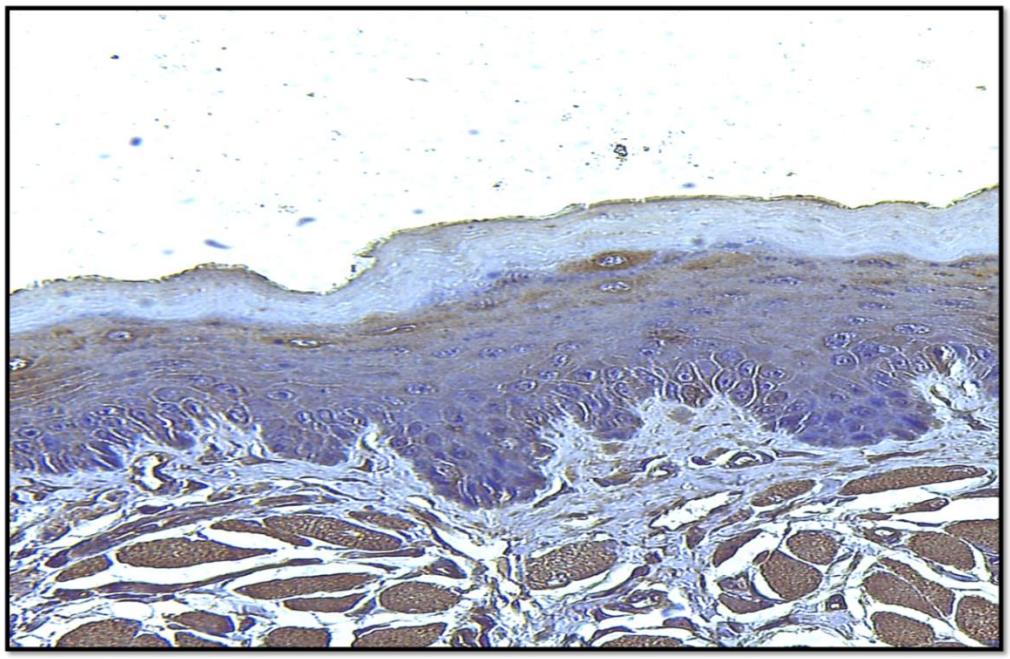


Figure (64): Section in tongue of rats treated with 60 mg\kg of (5-FU) post (21) days showed slightly immunoreactivity to BCL-2 in some of epithelial cells cytoplasm at surface layer (IHC. 40 X)

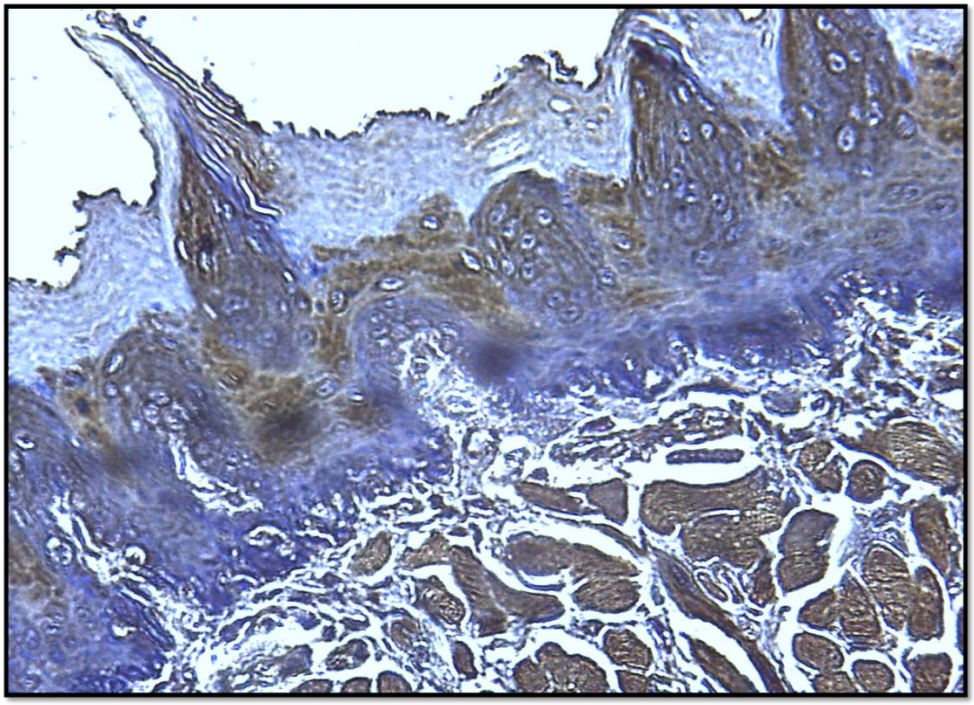


Figure (65): Section in tongue of rats treated with (30 mg/kg of 5-FU + glycine) for (21) days showed positive immune staining to BCl-2 in whole thickness epithelial layer, basal and supra basal cells (IHC. 40 X)

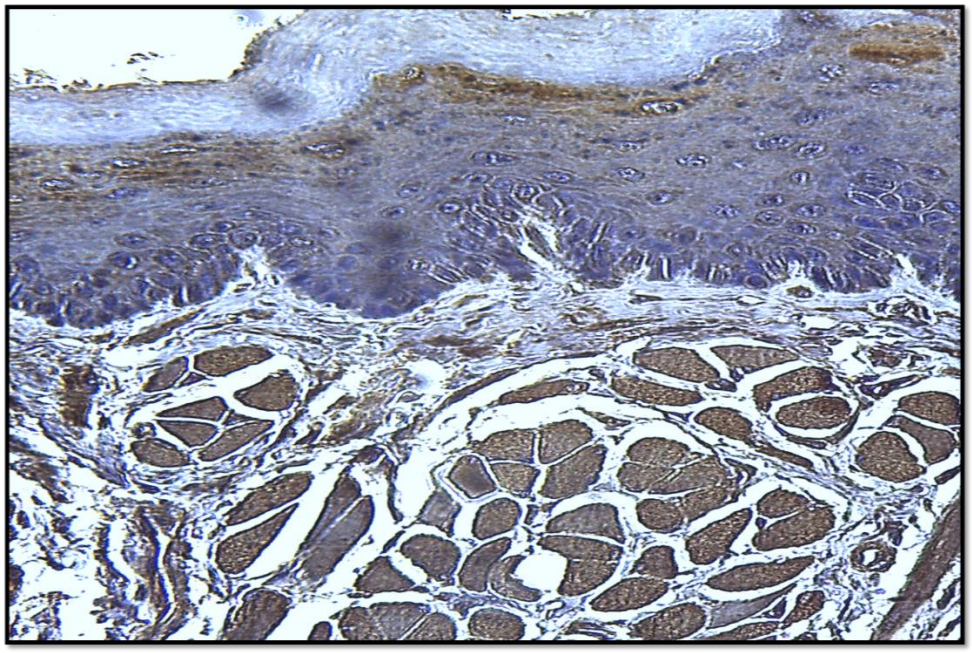


Figure (66): Section in tongue of rats treated with (60 mg\kg of 5-FU + glycine) for (21) days showed positive staining at surface layer, brown granules deposition in epithelial cells cytoplasm and few cells at connective tissue (IHC. 40)

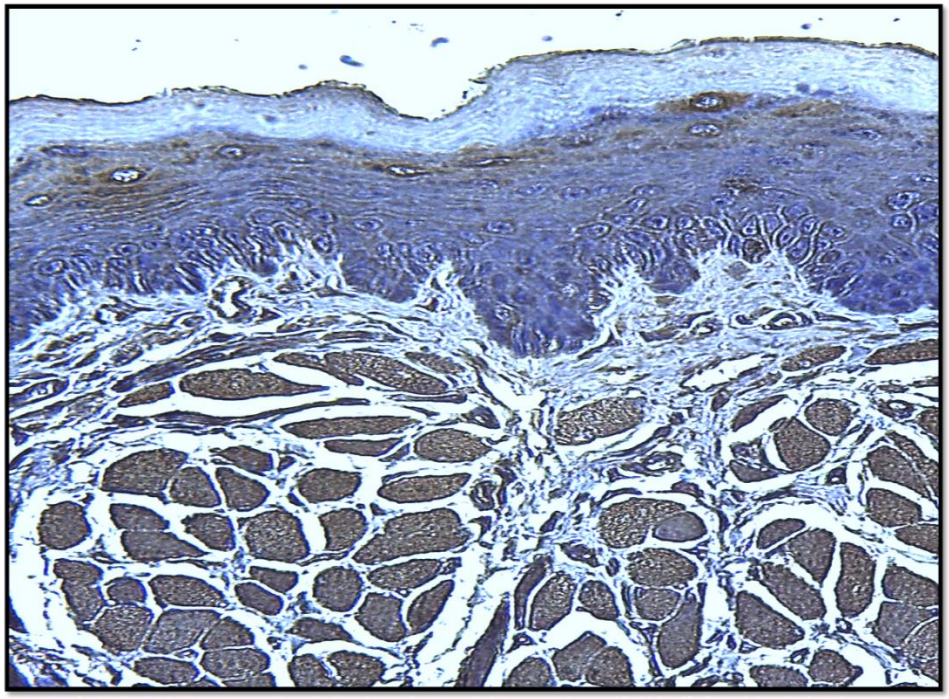


Figure (67): Section in tongue of rats treated with 30 mg\ kg of (5-FU) post (45) days showed mild expression to BCL-2 at surface of epithelial layer (IHC. 40 X)

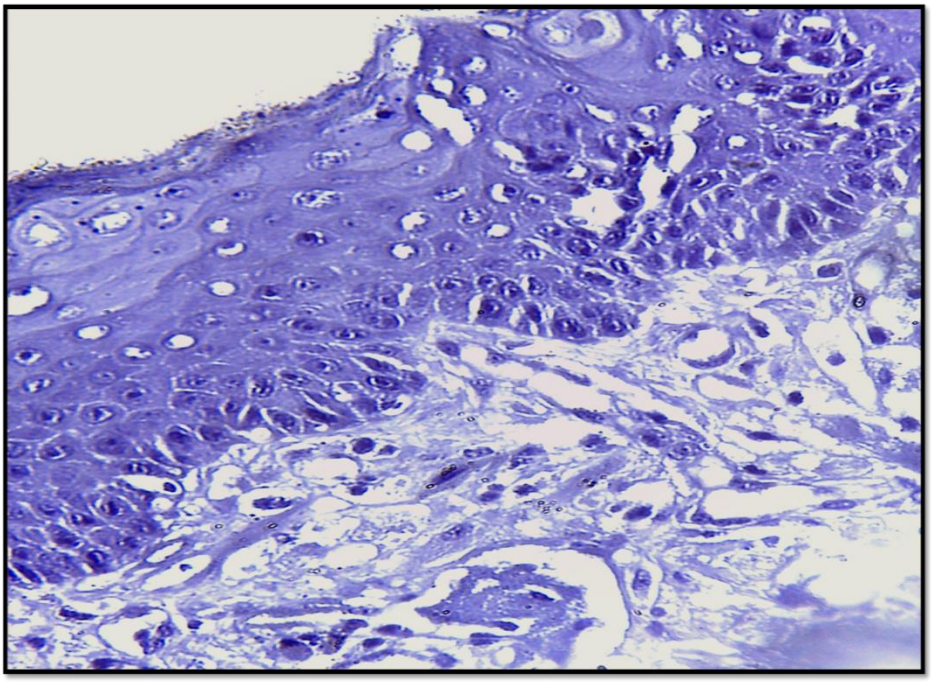


Figure (68): Section in tongue of rats treated with 60 mg \kg of (5-FU) post (45) days shows negative immune reactivity in cytoplasm reaction to BCL-2 of epithelial cells (IHC. 40 X)

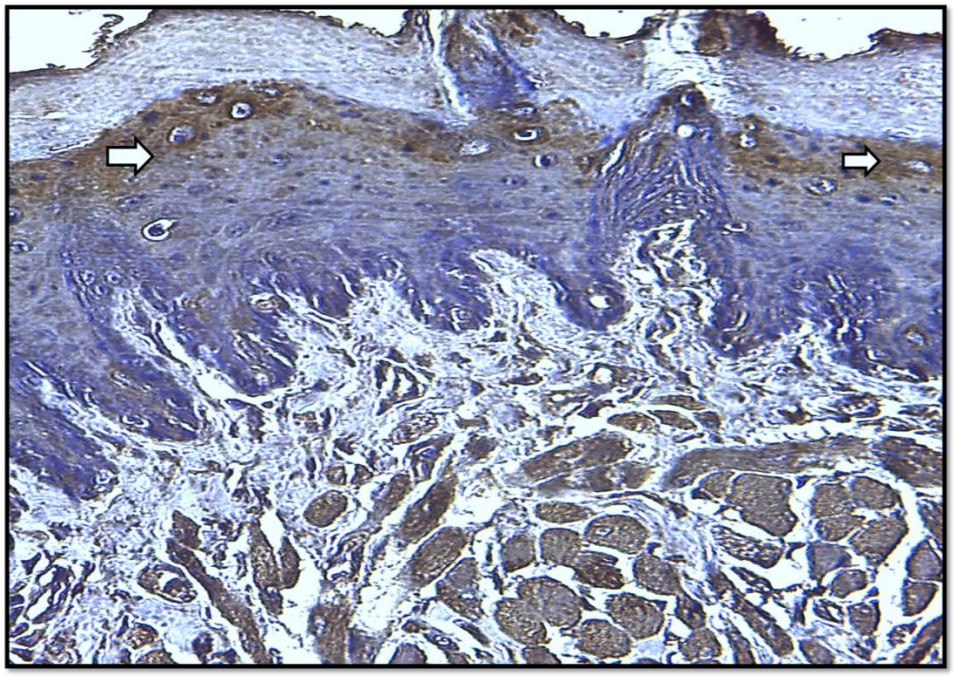


Figure (69): Section in tongue of rats treated with (30 mg \kg of 5-FU + glycine) for (45) days showing positive immunoreactivity to BCL-2 in cytoplasm of keratinocytes cells at surface layer (arrows) (IHC. 40 X)

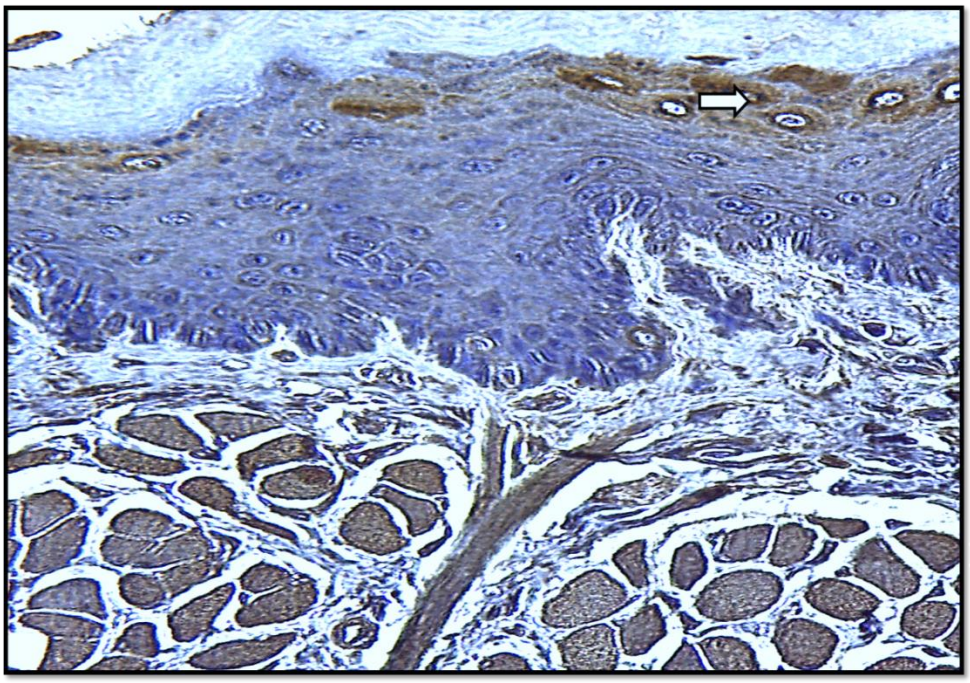


Figure (70): Section in tongue of rats treated with (60mg/kg of 5-FU + glycine) for (45) days showed mild immunoreactivity to BCL-2 protein expression at surface of epithelial layer cells (arrow) (IHC. 40 X)

Chapter Four

Discussion

Chapter Four

Discussion

1.4. Chemotherapy induced-oral mucositis toxicity

Oral mucositis (OM) was popular problem of the oral mucosa inflammation either induced through irradiation and chemotherapy treatment in most cancerous afflicted patients (Sonis and Fey, 2002). The prevalence of (OM) differs depending on the treatment managed and individual patient features, this adverse reaction is recurrently started by damage of cells in the basal epithelium layer and underlying connective tissue of oral mucosa and so may be mild shape by erythema or moderate to severe like erosions and ulcerative lesions of oral cavity (Freitas *et al*., 2012; Watanabe *et al*., 2013).

In this study (5-FU) drug was used to induce oral mucositis in albino rats (*Rattus norvegicous)*, 5-FU was the most drug employed as anticancer therapeutic agents caused oral mucositis with multiple side effects so it was selected in the current study, also using of rat model was justified by Sonis *et al*., (1990) who reported that administration of (5-FU) to rodents produce histological alterations in oral mucosa almost mimic to those found in human oral mucositis.

Several animal models of (OM) have been developed to investigate potential remedies for (OM), because that OM is importantly debilitating and aching in patients. The results were supported by previous study that (5-FU)-induced oral ulceration possesses traditional inflammatory form and is the most common model

for (OM) in rodents (rats, hamsters, mice and rabbits) (Bowen *et al*., 2011; Stringer, 2013).

The mechanism action of this drug cytotoxicity has been attributed to the disincorporation of Fluor nucleotides into RNA and DNA and to the suppression of thymidylate synthase enzyme (a critical enzyme in nucleotide metabolism), both of which lead to cell death (Toyodart *et al*., 2000; Kuilenburg, 2004). Furthermore, it is though that the progress of oxidative stress and an overproduction of reactive oxygen species (ROS) caused by chemotherapeutic agents are major actions in most ways leading to mucositis, additionally, ulcerative mucositis outcomes in the damage of the oral mucosa for example an anatomical barrier, so the mouth becomes easy infected with enteric microbes, fungi and viruses and lead to elevate infection risk, especially through immune suppression (Zegarelli, 1993; Watanabe *et al*., 2009).

Hence, the disease characterized one of the public health problems; therefore, it was comprised in this study to evaluate the efficacy of glycine supplementation which can be used for new beneficial options for the remedy of oral mucositis which showed potential protective effects. Because of simple to execute as well as, has a low cost and does not need invasive way of remedy

Results showed that the 5-FU caused a significant decrease in the body weight ($P \leq 0.05$) as compared to the control groups. This may be related to the reduced amount of food that consumed by rat because of inflammation and tongue ulceration, Similar observation was conveyed in the previous studies by several authors (Clarke *et al*., 1999; Mitsuhashi *et al*., 2006; Huang *et al*., 2009; Han *et al*., 2011) who proposed a relation between little immune ability and

mucositis acuity with weight reduction, decrease of the body weight may result from reduced food intake because the presence of oral ulcerations.

The improvement in the body weight of rats by treating them with glycine may be attributed to the effect of this agent on oral cavity ulcers and healing of these ulcers. Other studies explained that glycine having antioxidant properties and prevent the lipid peroxidation greatly and these might be regarded as demonstration for the more bettering role of amino acids as glycine supplementation against 5-FU toxicity (Wu, 2013; Wu *et al.*, 2013). So, the body weight gain of animals was significantly greater in treated groups with glycine compared to the groups treated with 5-FU only. This may be elucidated that the curative of (OM) was quicker in these treated groups, due to allow the animals to consume more food and water.

4.2. Biochemical study

According to the results of the present study serum MDA level was measured as indicator of the extent of lipid peroxidation products in all experimental rats, 5-FU caused a significant increase in mean value of serum MDA oxidative enzyme concentration in (OM) rats at all periods compared to the treated group and control. These results may be due to the side effects and the cytotoxicity of 5-FUand its metabolism within cells and tissue in addition to tissue inflammatory response. Our results are in agreement with other studies which indicate that chemotherapy triggered the progress of oxidative stress , alterations in structure and function of the cell membranes, disorder of the peroxidation antioxidant and over production of reactive oxygen species (ROS), inactivation of detoxification system or extreme consuming of antioxidants, which were causal elements in the oxidative injure of cellular structures and molecules includes lipid, protein and nucleic acid (Davies *et*

al., 2008; Kisaoglu *et al*., 2013). This result was discussed by several researchers who reported that the lipid peroxidation products raised with chemotherapy (Weijl *et al*., 1997; Simone *et al*., 2007; Alirezaei *et al*., 2012). Also Cell membranes were rich in unsaturated fatty acids so the cellular structures liable to free radical attack (Miyazono *et al*., 2004).

The data showed that treated groups with glycine supplementation plus 5-FU to the rats may support the antioxidant defense, have role in protein synthesis and protect cell membrane structure all these significantly reduced serum MDA level in comparison to the groups (OM) group treated with 5-FU alone. Furthermore, anti-neoplastic therapy triggered oxidative stress plays main role in the progress of most of the adverse impacts including (OM) that induced significant injurious changes in antioxidant. These results were similar with the results of (Wagner *et al*., 1992; Rubbo *et al*., 1994; Froh *et al*., 2002) who clarified that the glycine reducing the formation of harmful free radicals and additional toxic mediators. The free radical nitric oxide or other derivatives can give rise to lipid peroxidation .So, the supplementation of glycine had protected from the toxicity of 5-FU as indicated by the significant restoration of serum MDA level.

Recent study revealed that a significant decrease in (SOD and GPx) oxidative enzymes level in all rats treated with 5-FU in comparison with control. These findings were related to 5FU toxicity, reduced anti-oxidative activity and effect of free radicles. Our data discussed in previous studies which were recorded by (Yamashita *et al*., 1999; Yoshino *et al*., 2012; Rashid *et al*., 2013; Sajid *et al*., 2016) concluded that the reducing in antioxidant enzymes action (SOD and GPx) in 5-FU treated groups proposes that excess ROS production may have triggered redox imbalance. Also Al-Asmari *et al*., (2016) reported that the decrease in SOD and GPx is possibly attribute to inactivation via the rise in ROS or lipid

peroxidation when oxidative damage is extreme and that oxidative stress intervenes in the pathophysiology of 5-FU toxicity.

Moreover results showed that the glycine in combination with 5-FU was significantly (P≤0.05) increased GPx and SOD activities compared to (OM) group treated with 5-FU alone. Our results are similar to other studies which indicated that glycine significantly attenuated the 5-FU-induced decrease of SOD and GPx levels in blood serum suggested that the preventive effect of glycine supplementation increased SOD and GPx activity due to its free radical scavenging, by eliminating some of ROS includes peroxy- radicals, superoxide radicals and oxygen (Senthilkumar *et al*., 2004; Zeb and Rahman, 2017).

4.3. Hematological study

Our study showed that WBCs count was significant decreased (P≤ 0.05) in mucositis groups treated with two doses of 5-FU and for each periods in comparison with normal control, this may be regarded to the cytotoxicity caused by this drug and to suppression of immune response. These results consisted with these of (Rofe *et al*., 1994; Medeiros *et al*., 2011) who observed leukopenia in chemotherapy treatment animals and the danger of myelosuppression is raised with chemotherapeutic agents are offered, leukopenia already happened because of the toxic effect of 5-FU on myeloid in the bone marrow, hence fluorouracil remedy was considered as immune-suppressive drug.

Also the study recorded obvious variations in differential WBCs count of neutrophils, lymphocytes and monocytes in mucositis groups as compared to the control. Neutrophils significantly increased at (P≤0.05) post (21) days while the

lymphocytes significantly decreased at the same periods, whereas neutrophil significantly decreased at ($P \leq 0.05$) after (45) days, in return significantly increased the lymphocytes. These observations were similar to that recorded by Vaghasiya *et al*. (2009) who noticed that the differential leucocyte counts altered from 66% neutrophils and lymphocytes before remedy administration to 90% after two weeks of treatment in experimental guinea pig.

In addition, RBCs count and Hb concentration was also decreased significantly at ($P \leq 0.05$) in mucositis group. This can be referred to great inflammation in oral mucosa especially after (45) days from treatment with 5-FU and few eating with drinking of rats leads to dehydration and loss of weight. Also the chemotherapeutic agents and inflammatory infections caused suppression of bone marrow can cause to anemia (Singh *et al*., 2008).

Administration of glycine in combination with 5-FU in rats attenuated significantly the effect of 5-FU on blood parameters in comparison with mucositis group. These effects displayed restoring most blood parameters especially WBCs counts post (21) days from treatment with glycine and also improvement of other differential leukocyte counts, Glycine and 5-FU together were improved significantly the decline RBCs count and Hb concentration in comparison with 5-FU group alone .This could be related to beneficial glycine supplementations and it role in reducing the impact of fluorouracil toxicity on myeloid and erythroid in bone marrow.

4.4. Histomorphmetrical study

Morphometric analysis revealed to significant decrease in the mean of epithelial layer thickness in mucositis group for all regions of oral mucosa (tongue, cheek and lower lip) in both doses of 5-FU and for both periods at ($P \leq 0.05$) compared with normal control, this result related to the direct effect of 5-FU on surface epithelial layer resulted in damage and ulcers formation and sluggish the oral mucosa, our results was consistent with other study which showed that the direct restrained impacts by chemotherapy on DNA replication and mucosal cellular propagation consequence in reduction in the regeneration ability of the basal epithelium, which lead to reduce epithelial layer thickness (Naidu *et al*., 2004; Zhao *et al*., 2009; Munaretto *et al*., 2011). Moreover, reduced basal epithelial layer thickness is ascribed to loss of basal cells in mucositis group which require replicative and regeneration such as in colon genic death of basal epithelial stem cells lead to decrease restoration capability of epithelium layer under high dose of 5-FU chemotherapy producing experimentally ulceration (Sonis, 2009).

Morphometric criteria of oral mucosa was determined in tongue, cheek and lip sections ,the results showed that glycine supplementation caused significant increase in the mean thickness of epithelial layer in rats treated with (glycine + 5-FU), this revealed to positive impacts of glycine supplementation on changes in the oral mucosal tissues in rats by increased healing of the surface layer , less inflammation or by preserve the basal layer and increase the regeneration in most regions, this result discussed by other researches clarified that the amino acid can alleviate pathological ways as oxidative stress and as outcome motivates of tissues to antioxidant properties of glycine (Alvarado *et al*., 2006; Bahmani *et al*., 2012).

4.5. Pathological study

4.5.1. Macroscopically examination

Current macroscopically findings showed mild to moderate changes in oral mucosal tissues lining (tongue, cheek and lower lip) post (OM) induction with two doses of (5-FU) drug at each two periods (21, 45) days followed by scratching of oral cavity in all rats as compared with normal control, the result explained that scratching and treated with 5-FU establish the ulcers formation in experimental model with inflammatory signs as redness, hyperemia and hemorrhage after (14) days from administration of (5-FU) drug followed by irritation of the mucosa in all rats at both periods. These signs caused clear epithelial ulceration of the surface layer at end of experimental periods approximately in all rats treated with dose (60 mg\kg) of (5-FU) post 21 days and both doses post 45 days, our observations were similar with previous researches (Lima *et al*., 2005; Leitão *et al*., 2007) which suggested that the greatly keratinized nature of rat oral mucosa. It is difficult to trigger ulceration, so the current animal model comprised the combination of chemotherapy with mechanical irritation as superficial scratching to create mucosal injure in animal mimic to mucosal damage in human.

The role of glycine supplementation was very obvious morphologically on of oral mucosa tissues of the treated male rats post (21 and 45) days of treatment through alleviating the signs related with oral mucositis as mild redness without epithelial ulceration as compared to rats of mucositis group.

4.5.2. Histopathological changes

Histological results were also confirmed the injurious effects of 5-FU on oral mucosa of (tongue, cheek and lip), whereby, 5-FU-treated group animals (mucositis group) showed variable histopathological changes after (21) days of treatment with dose (30mg\kg. B. W) in tongue sections including mild loss of normal appearance shape of papillae, secession of keratin layer, mild flattening of rete ridges, most epithelial layer with perinuclear cells and other cells with vacuolated cytoplasm, mild inflammatory cells in lamina propria, numerous congested blood vessels, while the administration with (60mg\kg. B. W) of 5-FU post (21) days was more effect than the dose (30mg\kg) of 5-FU and showed hyperkeratosis, keratin layer acanthosis , sever atrophied papillae, vacuolated cells at basal and supra basal layers, heavy infiltration of inflammatory cells in lamina propria with edema , congested large blood vessels and atrophied muscle fibers. These changes may be due to the cytotoxic impact of 5-FU caused suppression of epithelial cells proliferation and inflammatory response, keratin deposition and effect of inflammatory cells. These results agreed with (Barasch and Peterson, 2003; Taiwo *et al*., 2009) who reported that 5-FU induced oral mucositis initiated after short period of remedy, and reached intensity peak during two weeks. Furthermore 5-FU caused heavy infiltration of inflammatory cells and induction of mucositis with subsequent formation of oral ulceration in (OM) model (Lionel *et al*., 2006; Chen *et al*., 2007).

After (45) days of 5-FU administration the results clarified that the impact of (30 and 60mg / kg. B.W) of 5-FU revealed to sever atrophied papillae, reduced thickness of epithelium, degeneration regions with hemorrhage and vacuolated epithelial cells, heavily lymphocytic infiltration and congested blood vessels were observed. This indicated to the direct effect of chemotherapeutic agent led to

inflammatory phase, which triggers the early liberation of chemical-mediators via neutrophils, macrophages and lymphocytes. These findings were also suggested in previous studies reported fast inflammatory response was induced by pro-inflammatory cytokines and mediators released from inflammatory cells leading to an elevated vascular permeability and tissue response (Shih *et al*., 2003; Molan, 2006).

The cytokines that secreted from endothelial cells, keratinocytes as well as the cells of lamina propria promote cells injuries, at the same time, and chemotherapeutic drugs stimulate enzymes that rise apoptosis. Also ROS that generate by chemotherapy which is harmful to the DNA of epithelial cells and cells of connective tissue led to trigger a cascade of biological actions, which in role outcome in the production of different pro-inflammatory cytokines , so producing tissue damage and raised sub-epithelial vascularity (Cawley and Benson, 2005; Motallebnejad *et al.*, 2008).

The recent study evaluates the role of glycine on (OM) post (21 & 45) days with both doses of 5-FU, it is clearly ameliorated the structure of the tongue which was damaged by 5-FU alone, when co-administrated in dose (30mg/kg. B. W) of 5-FU with (2mg/g B. W) of glycine supplementation especially post 21 days were more amelioration. The results showed several observations like increase thickness of epithelium seemingly degree of re- epithelization with restoration of normal rete ridges, keratin layer formation, restoring most normal shape of papillae and mild or absence of inflammatory cells infiltrate , vacuolated keratinocytes and large congested blood vessels especially with dose (60mg\kg. B. W) of 5-FU were observed at the same period, but sill moderate inflammatory cells within lamina propria, dilated blood vessels with neoform of capillaries post (45) days of treatment with glycine and 5-FU dose (60mg/kg. B. W). This may be due to cyto-

protective and anti-inflammatory impact of glycine in oral mucosa to repair the damage tissues, increase epithelial layer thickness, synthesis of proteins and the important role of glycine in fibroblasts proliferation. These results were clarified and supported the findings of some investigators (Corzo *et al*., 2005), who reported that nutritional supplementation with 5% glycine supplementation has accelerated the process of wands healing; recover the weight gain, antioxidant ability and immunity.

The anti-inflammatory impacts of glycine are supposed to be mediated, because of its method of action in the cell membrane where it stimulates the chloride channel that stabilizes the membrane possible (Hartog *et al*., 2007). Furthermore, glycine prevents the raise of intracellular calcium which stimulates the forming of the cytokines waterfall by suppressing cells that trigger the inflammatory method through preventing stimulation of TNF-α and NF- κB and therefore reducing the production of free radicals and additional toxic mediators also decrease TNF-α, infiltrated of inflammatory cells and edema (Radi *et al*., 1991; Li *et al*., 2001; Mauriz *et al*., 2001). In addition glycine contributes in the creation of collagen so glycine supplementation may improve basement membrane constancy (Pöschl *et al*., 2004).

Microscopic figures on oral mucosa lining cheek and lip of (OM) rats' post 21 and 45 days of treatment revealed mild to moderate mucositis with dose (30mg/kg. B. W) after 21 days in both of cheek and lip compared with control, like decrease thickness of epithelial layer, separation of keratin layer from mucosa, cells of epithelial layer with vacuolated cytoplasm, less inflammatory cells in lamina propria with vascular hyperemia, while the effect of dose (60mg/kg. B. W) more injurious in the same period, particularly in cheek sections. This may be related to the effect of 5-FU metabolism and the toxic effect of the drug on cells

activity and their proliferation. These results were in agreement with some researchers (Chang *et al*., 2012; Patel *et al*., 2014; El-Bermawy, 2015) who demonstrated that 5-FU was powerful chemotherapeutical agent triggering damage of oral mucosa which was a structural barrier. Furthermore, the most potential mechanism of 5-FU was triggered (OM) by producing numerous inflammatory cytokines, progress of oxidative stress and increase of (ROS) with obvious expression of nuclear factor kappa-(NF- κB), other mechanism included the delay of cell propagation and inhibition of DNA and RNA synthesis (Chang *et al*., 2015; Generoso *et al*., 2015).

Histopathological changes on cheek and lip mucosa from rats injected with both doses of (5-FU) post (45) days were revealed to sever ulceration with destruction of surface epithelial layer, granulation tissue formed, inflammatory cells infiltration , edema and hemorrhage. This result explained that the direct impact of chemotherapy agent on DNA recurrence and mucosal cellular propagation consequence in a decrease the regeneration ability of the basal epithelium, so the immediate stomato-toxicity of the chemotherapy take place. These events are approved to outcome in atrophy of mucosal layer, collagen interruption and ultimately ulceration (Bronchud *et al*., 1994; Gallagher *et al*., 1995).

Further, with double dose of 5-FU severity changes noticed like complete destruction and ulceration of mucosa, severe inflammation with more vascularity in lamina propria, congested blood vessels, heavily infiltration of inflammatory cells most of lymphocytes , edema with degenerated area. These results may be regarded to the tissue response caused by epithelial layer destruction, the region exposed easy to infection and that led to more complications such as delay healing and more sever inflammation. Our

observations were similar to other studies clarified that 5-FU agent triggered expression of numerous cytokines like tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6), which induced inflammatory tissue response result in sever infiltration of inflammatory cells (Mostafa, 2014; Atalay *et al*., 2015; Quarteiro *et al*., 2015). Moreover, the disorder of oral mucosal fence might give rise bacterial infestation, these microbes were as well chemotactic influence for mononuclear cells resulting to mucositis and eventually mucosal ulceration. While the excessive of blood vessels in lamina propria of lip and cheek sections may indicate the presence of chronic inflammation (Abbas *et al*., 2010). This result is consistent with previous study which referred that the propagation of blood vessels is motivated via vascular endothelial growth factor (VEGF), expression of VEGF is stimulated through angiogenesis and ischemia .Also, the presence of edema in these tissues happened because the chemotherapy drug and might be a consequence of vascular alterations (Dai *et al*., 2009).

On the other hand, the present results clarified that administration of glycine with 5-flourouracil drug treated group for different periods showed variable improved re-epithelization nearly similar to control especially with dose (30mg\kg. B. W) with renewal of normal stratified squamous epithelium, keratin layer deposite, and well-organized lamina propria compared to mucositis group. These results were demonstrated by some previous investigators (Pan *et al*., 2005; Howard *et al*., 2010), who stated that the glycine supplementation promotes curing in a diverse shape of lesions because it has antioxidant effect which may impact the amount of injuring ROS, are produced though mucositis stage and which are ability to renewal of epithelium layer. So, the glycine triggered a fast remodeling of tissues as compared with rats group without glycine treatment.

From our data, treatment of glycine in combination with 5-FU attenuated the cellular inflammatory actions within oral mucosa that occurred after induction of OM for both doses and at both periods. These results related to the effect of glycine which may be act as protective factor, has role in collagen synthesis and increase the immune response so glycine has anti-inflammatory potential in the present study; but did not estimate the impact of glycine on expression of cytokines. Our results in agreement with Zhong *et al*., (2003) who proved that anti-inflammatory possible of glycine has been demonstrated to be because of its ability to decrease the expression of important pro-inflammatory cytokines as (IL-1β and TNF- α), which have revealed to play significant role in the pathophysiology of OM in progressing the lesions and arbitrating damage.

Moreover, it has been proposed that glycine is capable to perform on inflammatory cells include macrophages to inhibit motivation of inflammatory transcription elements and the creation of free radicals and inflammatory cytokines (Spittler *et al*., 1999).

Also, the present studies showed restore the balance in the number of blood vessels and smooth muscles. These issues had perversely been discussed by (Yin *et al*., 2000; Yamashina *et al*., 2001), who clarified that the glycine suppress propagation and immigration of smooth muscle cells, relation among endothelial cells, smooth muscle cells and fibroblast cells play a crucial part in the arranging of angiogenesis.

4.6. Immunohistochemistry study

Immunohistochemistry was used to investigate specific cellular localization of the anti-proliferating cell nuclear antigen (PCNA) and anti-apoptosis (BCL-2) in rat tongue mucosa and the toxicity impacts of 5-FU drug on the distribution of

(PCNA) and (BCL-2) expression, the positive immunoreactivity PCNA antigen was visible strongly in the nuclei of epithelial layer while BCL-2 was visible in the cells cytoplasm of the oral mucosa lining tongue.

PCNA is a greatly preserved 36 kDa nuclear polypeptide recognized as the helpful protein of DNA polymerase delta, which is expressed through the cell phase and its concentration is raised more in S-phase (Essers *et al*., 2005). The results of the study showed there was a marked weak stain after (21) days especially with dose (60mg/kg. B. W) while minimal to negative stain after (45) days in expression of PCNA in the tongue tissues of 5- FU drug treated rats as compared to normal rats. This result is in coincide with (Lofty and Zayed, 2009; Aboushady *et al*., 2012; Won *et al*., 2013), who found that the poisonousness of chemotherapeutic factors like 5- FU and other chemotherapy agents reduces propagation of mucosa cells and causes deterioration of their cellular restoration, so lead to significant reduction of PCNA immune-staining expression of mucosa cells.

BCL-2 is a gene encodes a protein situated in the nuclear membrane on the inner surface of mitochondria and inner surface of endoplasmic reticulum. It is the maximum significant gene of bcl-2 family and has been described to extend the existence of cells via particularly suppressing apoptosis, the equilibrium between mitotic action and apoptosis is thought to adjust ordinary development (Ribeiro *et al*., 2005). Results from current study revealed that BCL-2 protein was minimal to slightly immune-staining expression post 21 days for both doses, whereas, slightly to negative post 45 days for both doses in expression of BCL-2 of 5-FU treated rats compared to control. This result may be related to the effect of 5-FU on nucleus, alterations in DNA, nuclei pyknosis and degeneration of epithelial cells. These result discussed by other studies that illustrated the administration of 5-FU

was escorted by a significant decrease in BCL-2 positive cells, chemotherapy causes suppression of DNA synthesis with decrease in cell propagation and the generation of ROS, also harm in the metabolism in ancestor cells which cause suppression of mitosis and raise of apoptosis via activation of apoptotic way (Kitajima *et al*., 1993; Zamzami *et al*., 1998).

Tiberio *et al*., (2002) were described that BCL-2 protein plays a part in preserving stem cells populace proposing that BCL-2 proto oncogene represents significant role in contrasting the committing of keratinocytes cells to differential and protective stem cells.

Tongue sections from rats treated with glycine post (OM) induction, showed there was increased both PCNA and BCL-2 immuno-reactivity expression in most epithelial layer cells when compared to 5-FU treated group alone. By this means indicating that glycine supplementation increased epithelial propagation and anti-apoptosis, it is possible that this preventive impact of glycine may demonstrate beneficial in clinical practice to reduce (OM) subsequent from 5-FU remedy.These results were supported by other studies mentioned to glycine supplementation has antioxidant and cytoprotective characteristic, or its anti-inflammatory action via decreasing TNF- and IL-6 output (Nafarzadeh *et al*., 2013; Razak *et al*., 2017). Glycine causes a simple regulation of anti-apoptotic particles that can terminate the decease cascade via inhibiting mitochondrial injure to preserve the integrity of the electron passage chain and stimulate oxidative phosphorylation (Yang *et al*., 1997). Also, the anti-apoptotic impact of glycine is a unique feature which might be modified by the glycine receptor (GIyR). Furthermore, there is a study revealed to the role of glycine in protects endothelial cells and the impact was not mediated via glycine receptor type (GIyR) (Holmes and Assimos, 1998; Duenschede *et al*., 2006).

Chapter Five

Conclusions & Recommendations

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5.1. Conclusions

- 5-Flourouracil chemotherapy associated with irritation has a deleterious effect on the rats' oral mucosa leading to obvious pathological and biochemical alteration of rat male *Rattus norvegieus*.
- Long duration and high dose (60 mg\kg for 45 days) of taking 5-FU can cause more cytotoxic and damaging impact to the rats oral mucosa.
- Preventive effects of glycine supplementation has been established and it protective role against 5-flourouracil toxicity has been proved.
- The anti-oxidant activity of glycine observed to their ability in ameliorating of disorders of oxidative stress indicators in all experimental animals.
- Effective role of glycine supplementation by improving mucosal damage, increased Malonedialdehyde (MDA) level, and increased with recover expression of PCNA and BCL-2 in most epithelial layer of tongue mucosa of male rats.
- According to the study results a simple amino acid of glycine supplementation formed has anti-oxidant with anti-inflammatory beneficial power in restore the tissues and can accelerate the curing process by decreasing the damage caused by 5-FU toxicity.

5.2. Recommendations

The following points are recommended:

- Additional studies should measure the expression of TNF- α and (IL-1) as expression of inflammatory mediators that play vital role in the pathogenesis of 5-FU-triggered mucositis.
- Further studies to clarify the toxicity impact of 5-flourouracil on the other organs as stomach, liver, intestine and reproductive system.
- Study the role of glycine supplementation clinically to determine their efficacy in OM individuals undergoing cancer therapy.
- Further studies are necessary to demonstrate the fundamental molecular mechanism of glycine supplementation.

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الخلاصة

صممت الدراسة الحالية لتقييم التأثيرات السمية لعقار الفلورويوراسيل ومضاعفاته المتمثلة بالتهاب الغشاء المخاطي المبطن للفم والمستحث في الجرذان المختبرية نوع (*Rattus norvegicus*) مع تقييم الدور الوقائي للحامض الاميني الكلايسين كعامل وقائي.

استعملت في هذه الدراسة ذكور الجرذان البالغة بعد التأكد من سلامتها الصحية وبعدد(96) جرذ ومعدل وزن(200-250) غم وبعمر (10-12) اسبوع بعد تربيتها في البيت الحيواني لكلية العلوم/ جامعة البصرة, قسمت الحيوانات الى مجموعتين اساسيتين ولفترتين زمنيتين (21 و 45) يوم وبمعدل (48) جرذ لكل فترة زمنية, ثم قسمت كل مجموعة الى ثلاثة مجاميع ثانوية (16) جرذ لكل منها: المجموعة (1) : مجموعة السيطرة ,المجموعة (2): مجموعة حث الالتهاب المخاطي, المجموعة (3): هي مجموعة المعالجة (الحث + الكلايسين).

في نهاية الفترة الزمنية للتجربتين, خدرت جميع الحيوانات باستخدام الكلوروفورم وتم جمع الدم وعزل المصل وحفظ لغرض اجراء الاختبارات البايوكيميائية المتضمنة تقدير مستوى انزيم الاكسدة MDA وبعض الانزيمات المضادة للاكسدة مثل (GPx & SOD) مع تقييم بعض المعايير في الدم, و شملت الدراسة قياس اوزان اجسام الجرذان في مجاميع التجربة فضلا عن الدراسة القياسية النسجية للطبقات الطلائية ضمن الطبقة المخاطية المبطنة للفم كما تم اخذ عينات من كل من اللسان, الشفتين والخدين للدراسة النسجية وايضا تقنية التصبيغ المناعي النسجي Immunohistochemistry لتقدير التعبيرالجيني لكل من البروتينات(PCNA & BCL-2) في جميع المجاميع وخلال الفترات المحددة في الغشاء المخاطي للسان.

اظهرت الدراسة ان لعقار الفلوريوراسيل تاثيرا على وزن الجسم للمجاميع المعاملة حيث سجل انخفاضا معنويا في وزن الجسم لجرذان مجموعة الحث مقارنة مع مجموعة السيطرة وعاد الوزن الى الطبيعي تقريبا في المجموعة التي حث فيها الالتهاب المخاطي بعد استخدام الكلايسين للمعالجة .

بينت الدراسة تباين في تراكيز المعايير الكيموحيوية خلال الفترات الزمنية للدراسة, حيث يزداد تركيزانزيم MDA وبفارق معنوي ($P\leq 0.05$) في مجموعة الحث بعد فترة 21 و 45 يوم في مصل الجرذان في حين هناك انخفاض معنوي احصائي لتركيز الانزيم في مصل جرذان المجموعة التي عولجت مع الكلايسين , كما اظهر الفحص انخفاض معنوي في تراكيزالانزيمين المضادين للاكسدة GPx & SOD

) في المجاميع المستحث فيها الالتهاب المخاطي الفموي مقارنة مع مجموعة السيطرة في حين هناك ارتفاع معنوي في المجاميع المعالجة بالحامض الاميني الكلايسين وخلال نفس الفترتين (45,21) يوم .

اوضحت نتائج فحص بعض المعايير الدموية انخفاض معنوي احصائي عند ($P\leq 0.05$) في عدد كريات الدم البيض في مجموعة الحث خلال الفترتين الزمنيتين وخاصة بعد (45) يوم مقارنة مع مجموعة السيطرة. وايضا لوحظ هناك اختلافات واضحة في العدد التفريقي لكريات الدم البيض وتشمل الخلايا اللمفية, والعدلة و الخلايا الاحادية في جرذان مجموعة الحث مقارنة مع جرذان السيطرة, بينما المجاميع المعاملة بالفلورويوراسيل والتي عولجت بالحامض الاميني الكلايسين سجلت ارتفاع ملحوظ في اعداد كيات الدم البيض حيث تبدو النسب والبيانات قريبة من الحد الطبيعي في مجموعة السيطرة خاصة بعد (21) يوم من الحث والعلاج. كما كانت نتائج فحص اعداد كريات الدم الحمراء ومستوى الهيموكلوبين تشير الى انخفاض معنوي في معدل الكريات الحمراء ونسب هيموكلوبين الدم في مصل جرذان مجموعة الحث وعودة تدريجية للمعدلات الطبيعية في المجموعة التي عولجت بالحامض الكلايسين بعد استحثاث الالتهاب المخاطي الفموي فيها. اظهرت نتائج الدراسة القياسية النسجية وجود انخفاض معنوي في سمك الطبقة الظهارية في مقاطع اللسان, الشفة والخدين في المجاميع المعاملة بلفلوريوراسيل بعد (21) يوم من المعاملة لكن لوحظ انخفاض اكثر بعد (45) يوم من المعاملة مقارنة مع مجموعة السيطرة. بينما سمك طبقة الظهارة يزداد معنويا ($P\leq 0.05$) في مجاميع الحث والمعالجة بالكلايسين خلال الفترتين الزمنيتين 21 و 45 يوم مع وجود فرق مقارنة مع مجموعة السيطرة في المقاطع النسجية من جرذان المجاميع المختبرية المعاملة بعد الفترة الزمنية (45) يوم .

اشارت نتائج الدراسة الى التغيرات العيانية لمنطقة التجويف الفموي المتمثلة بظهور الدلائل الالتهابية مثل الاحمرار, الاحتقان ,وجود مناطق بيضاء والنزف بعد 14 يوم من حث الالتهاب المخاطي الفموي وخلال الفترتين الزمنيتين (45,21) يوم بعد الحث مقارنة مع مجموعة السيطرة حيث يظهر التجويف الفموي خالي من اي علامات غير طبيعية, كما تسبب الفلورويوراسيل المستخدم في مجموعة الحث بتقرحات واضحة في المنطقة السطحية للطبقة الظهارية مع وجود قرح بيضاء اللون محددة واحمرار شديد في مناطق متفرقة من التجويف الفموي واللسان بعد (45) يوم من استخدام عقار الفلورويوراسيل . بينما مجاميع الحث والتي عولجت باستعمال الكلايسين كانت مناطق اللسان والتجويف الفموي مظهريا خالية من التقرح ما عدا بعض الاحمرار واختفاء القرح خاصة بعد 21 من المعالجة مقارنة مع مجموعة الالتهاب الغشاء المخاطي المستحث.

اظهرت نتائج الفحص النسجي لمقاطع من اللسان, الشفة و الخدين للمجاميع المعاملة بالفلوريوراسيل مقارنة مع مجموعة السيطرة, حدوث العديد من التغيرات النسجية في نسيج الغشاء المخاطي للسان في المجاميع المعاملة بعد (21) يوم وبالجرعة (30ملغم/كغم) من العقار, حدوث تغيرات مظهرية في شكل الحليمات للسطح الظهري للسان, انفصال جزء من طبقة الكيراتين عن الطبقات السطحية, ومعظم خلايا الطبقة الظهارية ذات انوية محيطية مع ارتشاح طفيف للخلايا الالتهابية, مقارنة مع مقاطع اللسان المعاملة بتركيز (60ملغم/كغم) لنفس الفترة الزمنية لوحظ فرط ترسيب الكيراتين , ضمور الحليمات, ارتشاح كثيف للخلايا الالتهابية في الصفيحة الاصيلة , تنكس , تكوين وذمة وعائية وايضا احتقان الاوعية الدموية الكبيرة. كما اشارت الدراسة النسجية الى تغيرات اكثر تطورا وشدة مع زيادة الفترة الزمنية للحث ولنفس الجرعة (30ملغم/كلغم) بعد (45) يوم من المعاملة تمثلت بضمور شديد جدا في حليمات السطح الظهاري للسان, نقصان سمك الطبقة الظهارية مع ارتشاح كثيف للخلايا الالتهابية وحيدة النواة , مقارنة مع مقاطع اللسان المعاملة بتركيز (60ملغم/كلغم) لنفس الفترة الزمنية اظهرت النتائج تغيرات اكثر شدة وتطور من الجرعة المعتمدة السابقة في السطح الظهري والبطني للسان متمثلة بفقدان المظهر الطبيعي تلف الحليمات وايضا ارتشاح كثيف للخلايا الالتهابية . اما بعد استخدام مزيج الفلوريوراسيل والكلايسين في المعالجة لوحظ استعادة معظم التركيب الطبيعي للغشاء المخاطي للسان كزيادة سمك الطبقة الظهارية, اعادة تكوين الحليمات مع ارتشاح طفيف للخلايا الالتهابية وزيادة في تضاعف خلايا الطبقة القاعدية خاصة بعد (21) يوم من المعالجة الذي اظهر النسيج فيها قدرة للشفاء واعادة الالتئام اكثر من تلك التغيرات بعد الفترة الزمنية (45) يوم من الحث. كما سجلت التغيرات النسجية لمقاطع من الشفتين والخدين للجرذان في مجموعة الحث وباعتماد الجرعة (30ملغم/كلغم) من العقار تغيرات مثل اختزال سمك الطبقة الظهارية وتفجي السايتوبلازم لخلايا هذه الطبقة, احتقان الاوعية الدموية مقارنة مع المقاطع النسجية للجرذان المعاملة بالجرعة (60ملغم/كلغم) من العقار حيث كان التاثير اكثر وضوحا خاصة في مقاطع الخدين .اما التغيرات بعد (45) يوم من المعاملة تمثلت بتقرحات شديدة مع تلف المنطقة السطحية للبطانة, ارتشاح كثيف للخلايا الالتهابية, وذمة مع نزف, بينما اشارت الدراسة الى مجموعة الحث والمعالجة بالكلايسين تحسن واضح في اعادة تشكيل الطبقة الظهارية مشابه للتركيب النسجي الطبيعي , تجدد طبقة الخلايا الطلائية الحرشفية الطبقية, ترسيب في طبقة الكيراتين بشكل منتظم وانتظام طبقة الصفيحة الاصيلة.

بينما اوضحت نتائج الدراسة المناعية النسجية Immunohistochemistry تاثير الفلوريوراسيل على التعبير الجيني لبروتينات (PCNA & BCL-2), حيث كان هناك انخفاض معنوي احصائي في

التعبير الجيني لهذه البيروتينات في المجاميع المعاملة وللفترتين (21و 45) يوما مقارنة مع مجموعة السيطرة. بينما اشارت النتائج الى زيادة في التعبير الجيني لهذه البروتينات لكلا الفترتين الزمنيتين وبكلا التركيزين بعد المعالجة بالحامض الاميني الكلايسين. اشارت البيانات من الدراسة الحالية ان الكلايسين عامل وقائي وفعال بايولوجيا ضد المضاعفات الجانبية لعقار الفيورويوراسيل المستخدم في علاج السرطان وانه عمل على الاصلاح والتجديد للطبقة المخاطية الفموية.

الوصف النسجي للتاثير الوقائي لمكمل الكلايسين على التهاب الغشاء المخاطي للفم المستحدث بواسطة الفلوريوراسيل في ذكور الجرذان

اطروحة

مقدمة الى مجلس كلية العلوم- جامعة البصرة

كجزء من متطلبات نيل درجة الدكتوراه في فلسفة

علوم الحياة في علم الانسجة

من قبل

فاتن لطيف خفي

ماجستير انسجة 2013

باشراف

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2021