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THE EFFECTS OF GLIBENCLAMID, AND SILIBININ ON INDOMETHACIN INDUCED GASTRIC ULCER IN RATS: HISTOPATHOLOGICAL STUDY

ABSTRACT

Gastric ulcer is the most prevalent gastrointestinal disorder and has been a major health problem. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used drugs for different indications with association of gastrointestinal (GI) adverse events. The current work was designed to evaluate the histopathological effects of, K+ATP channel blocker, glibenclamide, and silibinin on acute gastric injury induced by administration of indomethacin in the rat. The animals were allocated in six groups, Group 1: animals received normal saline, and served as negative control, Group 2: animals received single dose of 40mg/kg indomethacin, and this group served as positive control, Group 3: animals received single dose of 18mg/kg glibenclamid, Group 4: animals received single dose of 250mg/kg silibilin, Group 5: animals received 2.5 ml olive oil one hour before single dose of 40mg/kg indomethacin, and Group 6: animals received single dose of 250 mg/kg silibilin one hour before single dose of 40mg/kg indomethacin. Sections of stomachs were examined by light microscope at 100 and 400 × magnification. Glibenclamide show a mild degree of sloughing in mucosa, while normal architecture was seen after administration of silibinin alone, and a mild superficial gastric mucosal lesion was seen when silibinin was given one hour before indomethacin administration. As conclusion the results indicate that silibinin can to attenuate the gastric lesion induced by indomethacin.

KEY WORDS Gastric ulcer, silibinin, glibenclamide, K+ATP channel

1.INTRODUCTION

Severalfactors are known to influence the pathogenesis of gastric ulcerformation and an imbalance between 'aggressive' and 'defensive' forcesmay result in the loss of gastric mucosal integrity [1].

In physiological conditions, there is a balance between the aggressive factors (HCl, pepsin, bile and pancreatic enzymes) and the gastroprotective factors (mucus-bicarbonate, blood flow, prostaglandins (PGs) and glutathione). Corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs), smoking, alcohol, trauma, sepsis, shock, Helicobacter pylori, and stress have been shown to contribute to gastric ulcer formation [2].

NSAIDs are widely used for their analgesic, antipiretic and antiinflammatory effects [3], and gastrointestinal (GI) symptoms are the most common adverse events associated with NSAIDs therapy[4]. Moreover, drugs opening ATP-sensitive K+ (KATP) channels have been shown to protect against gastric injury caused by ethanol or indomethacin administration in rats[5].In addition to that KATPchannels, whose activity can be modulated by sulfonylureas, have also been identified in different excitable tissues such as heart, brain or smooth muscle[6].These channels are involved in several physiologic and pathologic mechanisms such as smooth muscle contraction, fluid secretion, ATP synthesis, cell apoptosis and reactive oxygen species (ROSs) production[7].

KATPchannel blocking bysulfonylureas, like glibenclamide, can exacerbateacetylsalycilicacid-induced ulcers in rat stomach[8], while, themechanisms involved in KATP channel-mediated protection of gastricmucosa seem linked to a vasodilation and to an inhibition of neutrophilactivity, which basically provide compensation for deleteriousprostaglandin deficiency [5].

Silibinin, a major bioactive flavanone in milk thistle seeds (Silybummarianum), has been accepted as a potent scavenger of most free radicals, such as hydroxyl and peroxyl radicals and hypochlorite ion[9].Silibinin has been also found to up-regulate the activities of

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superoxide dismutase and glutathione peroxidase GSH-pX in human red blood cells, protect blood vessels, and stabilize the membranes via the inhibition to lipid peroxidation[10].

The aim of the current study is the evaluation of thehistopathological effects of, KATPchannel blocker, glibenclamide, and silibininon gastric injury induced by acute administration of indomethacin in the rat.

2.MATERIALS AND METHODS

1. Animals

Wistar albino male rats,weighing170–180g with age of 10-12 weeks were obtained from the animal house of College of pharmacy at Baghdad University.

They were housed in cages in a temperature controlled room (20±5◦C) with 12h dark/light cycle and provided with standard diet pellets and water ad libitum.

2.Chemicals and drugs

Silibinin, as silibininhemisuccinates (SHS), was purchased from Tolbiac S.R.L. Buenos Aires – Argentina, and both indomethacin, glibenclamideareproduced byMedochemie –Cyprus. Bouin's fluid (picric acid, acetic acid and formaldehyde) is produced by BDH Inc. – Canada.

3.Experimental Protocol and Groups

The animals were allocated in six groups, and each rat in the experiment received a specific treatment, as shown below:

- Group 1: animals received 2.5 ml normal saline, and served as negative control, to compare the normal architecture of the stomach with others that received different treatment.
- Group 2: animals received single dose of 40mg/kg indomethacin, and this group served as positive control, where an acute gastric ulcer can be induced [11].
- Group 3: animals received single dose of 18mg/kg glibenclamid.
- Group 4: animals received single dose of 250mg/kg silibilin.
- Group 5: animals received 2.5 ml olive oil one hour before single dose of 40mg/kg indomethacin.
- Group 6: animals received single dose of 250mg/kg silibilin one hour before single dose of 40mg/kg indomethacin.

Indomethacin, and glibenclamide were solubilized with saline, while silibinin was homogenized in olive oil, and the volume of oral doses were kept fixed with 2.5 ml in all groups.

The animals were fasted for 24 hour prior the receiving the treatment, with free access to water, then rats were sacrificed 4 hour after the last treatment by an overdose of ether.

4.Histological examinations of stomachs

Each stomach was removed and opened along the greater curvature. The stomachs were washed with cold saline, and then the perfuse-fixed stomachs placed in Bouin's fluid overnight, and processed for routine paraffin embedding. The stomachs were cut by rotary microtome into 5-µm sections. Three serial sections per stomach were mounted on slides, deparaffinized, rehydrated, and stained with

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haematoxyline - eosin stain[12.] Sections of stomachs were examined by light microscope at 100 and 400× magnification.

3.RESULTS AND DISCUSSION

A histiopathological study of mucosal lesions was done by an observer unaware of the treatment protocol. Figure 1 show the normal histology of rat gastric wall. In group 2, administration of indomethacin resulted in increasing in inflammation markers in submucosa layer (vascular congestion, and infiltration of inflammatory cells mostly neutrophils (Fig.2) and marked epithelial sloughing in mucosa layer as a sign of gastric mucosal ulcer(Fig.3)). While in group 3, glibenclamide show a mild degree of sloughing in mucosa(Fig.4). Normal architecture was seen after administration of silibinin alone (group 4)(Fig.5), and results from group 6 showed a mild superficial gastric mucosal lesion was seen when silibinin was given 1 hour before indomethacin administration (Fig.6).In group 5, picture of gastric mucosal ulcer was seen when indomethacin administered 1 hour after giving olive oil(Fig.7) with marked vascular congestion and increase in inflammatory cells (Fig.8).

The results of present work confirmed the method that proposed by Rainsford and Whitehouse [11] in producing acute gastric ulcer model in rat by oral administration of indomethacin, moreover this method has many advantages, as it is: simple, time and cost effective, and give a clear results.

It was reported by others that gastric lesions induced by indomethacin are characterized by significant oxidative injury, reduced mucosal blood flow and reduced secretion of mucus/bicarbonate, mainly due to inhibition of PGs secretion [13].
In the group of rats treated with KATP channel blocker, glibenclamide, the histological changes were represented by a mild degree of sloughing in mucosa and these results were consistent with results of Rahgozar et al. [14], where the last found that glibenclamide is able to worsen the gastric lesions.

KATP channels are involved in several physiologic and pathologic mechanisms such as smooth muscle contraction, fluid secretion, ATP synthesis, cell apoptosis and reactive oxygen species production [7].

On another hand, the results from group 4 showed that using of silibinin alone has no adverse effects on gastric architecture, moreover in group 6, results showed that silibinin has the ability to attenuate the deleterious effects of indomethacin when it used one hour before administration of indomethacin. The cyto-protective effect of silibinin may be due to several mechanisms operating at various cell levels. As, silibinin acts as an effective antioxidant[15]; its radical-scavenging activity could also be partly involved in cell regulatory pathways based on ROSs[16]. To exclude the effects of the vehicle that used to homogenize silibinin, olive oil was given alone to a rat before administration of indomethacin (group 5), and a signs of ulcer with marked vascular congestion and increase in inflammatory cells were observed and it were similar to that have been seen after indomethacin administered alone (group 2).

D-FIGURES

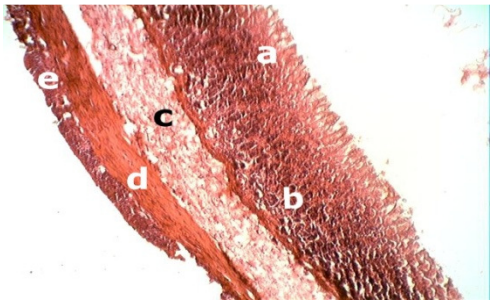

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Fig.1 (H&Ex100) normal histology of rat gastric wall composed of a. mucosa, b. muscularismucosa, c. submucosa, d. muscularispropiria, e. adventitia.

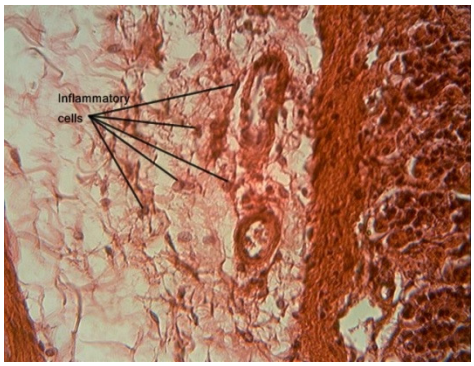

Fig.2 (H&Ex400) infiltration of inflammatory cells in submucosa after indomethacin administration

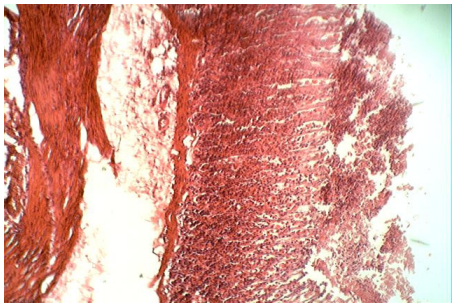

Fig.3 (H&Ex100) Inflammatory cells in submucosa with marked epithelial sloughing after administration of indomethacin

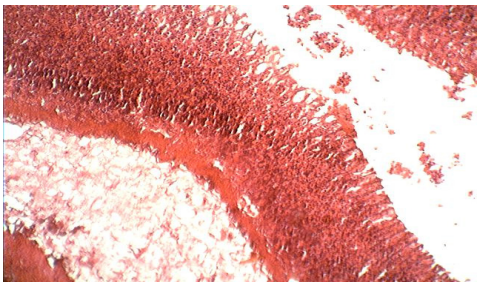

Fig.4 (H&Ex100) mild degree of sloughing in mucosa after receiving glibenclamide

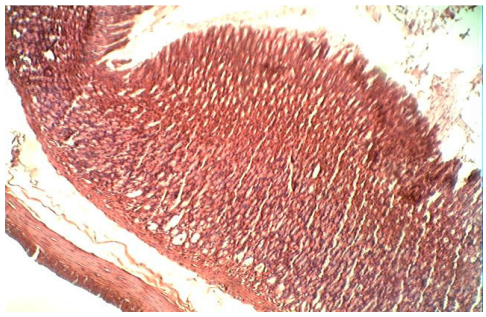

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Fig.5 (H&Ex100) normal architecture after administration of silibinin

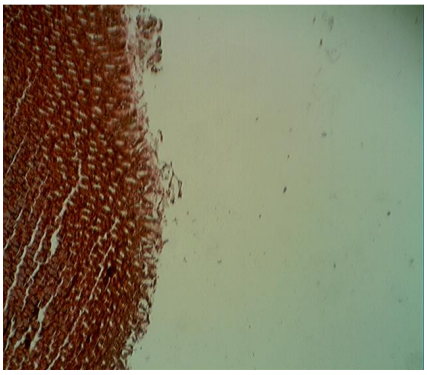

Fig.6 (H&Ex100) mild superficial gastric mucosal lesions resulting from indomethacin administrationpreceded by silibinin treatment.

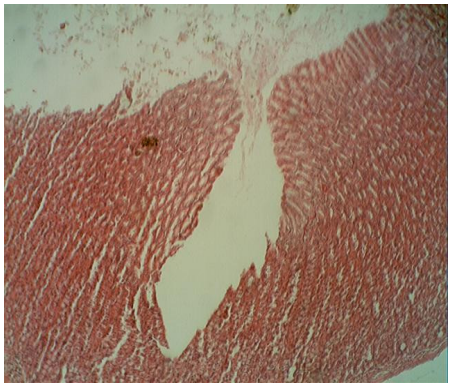

Fig.7 (H&Ex100) deep gastric mucosal ulceration resulting from indomethacin administration after receiving olive oil

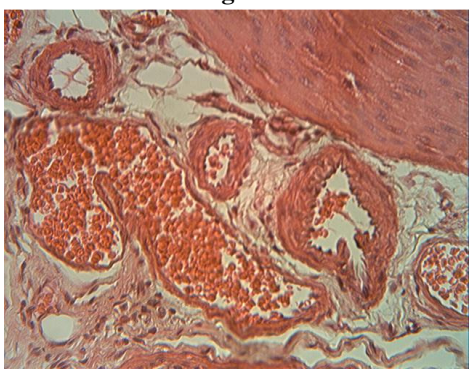

Fig.8 (H&Ex400) marked vascular congestion and increase in inflammatory cells resulting from indomethacin administration after receiving olive oil

5.CONCLUSION

In conclusion, glibenclamide was able to induce a mild gastric lesion, while silibinin alone has no such effect and didn't show any histological changes as normal architecture of gastric wall layers was observed after treatment; moreover silibinin was capable to attenuate the gastric lesion induced by indomethacin.

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