The potential protective effects of Aliskiren on diclofenac sodium induced gastric ulcer in a rat model

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Abstract

Introduction: Abrasion of stomach lining epithelium is caused by a defensive, protective mechanism, and aggressive factor imbalance. 20% of peptic ulcers begin as minor erosion of stomach lumen epithelia and extend to muscularis mucosa or submucosa 5 mm or larger. This research aims to minimise stomach mucosal lesions induced by NSAIDs by utilising Aliskiren. Method: 30 male albino rats were split into 4 groups. Negative control group A received 0.9% NaCl and tween 80 via oral gavage. Group B: Animals received ulcerogen (150 mg/kg Diclofenac sodium) orally and were not pre-treated. Group C: Diclofenac soduim and Omeprazole 20 mg/kg orally before ulcerogen. Group D received Diclofenac and Aliskierin via oral gavage before ulcerogen. Results: Diclofenac sodium at 150 mg/kg increases ($p>0.01$) stomach damage score, TNF-alpha expression, myeloperoxidase, malonaldehyde, and ulcer development % compared to healthy rats. Aliskierin 200 mg/kg pretreatment in Diclofenac-induced-ulcer in rats reduces ($p>0.01$) stomach damage score, TNF-alpha, myeloperoxidase, malonaldehyde, and ulcer formation percent, although less efficiently than Omeprazole and Aliskierin. Conclusion: Aliskierin inhibits Diclofenac. Aliskierin performed similarly to standard treatment (Omeprazole). Pirfenidone's protective effect was mostly due to its antioxidant and anti-inflammatory effects by reducing MPO, MDA, and TNF-alpha.

Keywords: protective effects, Aliskierin, diclofenac sodium, gastric ulcer, a rat model.

Introduction

Abrasion of gastric lining epithelium occurs due to imbalance between gastric defense, protective mechanism and aggressive factors represent about 20% of peptic ulcer start as mild erosion of epithelia lining of the stomach lumen and extend deeper to muscularis mucosa or submucosa in 5 mm in diameter or greater [1]. Gastric ulcer (GU) is the most widespread disease of the gastrointestinal tract approximately 5-10% of populations are affected [2]. A multifactorial gastric ulcer disease leads to prevalence differences between countries and appears to be more prevalent in developing countries and densely populated regions than in developed countries due to low socioeconomics and hygiene habits [3]. Gastric ulcer is highly linked to Helicobacter Pylori (H. pylori) infection and chronic non-steroidal anti-inflammatory drugs (NSAID) use, the incidence of gastric ulcer is about 80 percent in H. Pylori infected than non- infected patients and 10-30. percent of gastric ulcer is related to chronic NSAID use [4]. H. pylori prevalence increases with age (53.3%) and appear to be more in female 59.72 % than male 43.75 % [5]. Gastric ulcer is a multifactorial disease, endogenous and exogenous factors involved in GU development. Gastric ulcer is highly related to H. Pylori infection a common human pathogen responsible for ulcer formation usually found beneath the mucus layer, multi virulent factors involved in H. pylori toxic effect [6]. NSAIDs play an important role in gastric ulcer formation by reducing gastric defense layers through inhibition of prostaglandin synthesis by blocking cyclo-oxygenase (COX) enzyme isoforms [7]. Parietal cells secret acid and considered the first line of defense mechanism against bacterial overgrowth and colonization also enhance absorption of some important materials include iron, calcium and B12, over production of gastric acid cause mucosal damage by entering the gastric lumen through channels in mucus layer created by high glandular hydrostatic pressure during secretion thus lead to convert superficial erosion to a deeper lesion, disturb mucosal integrity and inactivate acid liable factors [8]. Analgesic, anti-inflammatory and antipyretic weak acid drugs with good gastrointestinal absorption, highly protein binding and bioavailability, it's most widely used worldwide especially by the elderly to relieve rheumatoid arthritis pain [9]. Aliskierin is a vel anti fibrotic agent with a minimal adverse effects [10]. Aliskiren is the first orally active renin inhibitor licenced as an antihypertensive drug. It is a non-peptide renin inhibitor, which blocks the first and rate-limiting stage of the renin-angiotensin system (RAS). aliskiren may treat hypertension alone or with additional drugs. Aliskiren is the first orally active renin inhibitor licenced as an antihypertensive drug. It is a non-peptide renin inhibitor, which blocks the first and rate-limiting stage of the renin-angiotensin system (RAS). Aliskiren may treat hypertension alone or with additional drugs [11]. Aliskiren reduces the level of plasma renin activity by high affinity binding to renin enzyme, preventing it from converting angiotensinogen to angiotensin I [12]. The objective of this study is to reduce gastric mucosal lesions caused by NSAIDs by using Aliskiren by investigating their effect on histological, gastric gross mucosal damage.

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Method

The study was a prospective, comparative, pre-clinical study. After obtaining ethical approval from collage of medicine university of Baghdad, the study was conducted at the Iraqi center of cancer research and medical genetics, Baghdad-iraq.And lasted (5) months from March 2022 till August, and 2022. This study was conducted on 30 adult male's albino-Wister rats weighing (250- 280g), purchased from the Iraqi center of cancer research and medical genetics, Baghdad-Iraq. Animal were housed five per cage for one week prior to the experiment, they were maintained in normal laboratory conditions (25±2°C, 12-hour light-dark cycle) and had access to laboratory chow pellet with full access to tap water. The animals were divided in to four groups, ten rats in each group and as following: **Group A:** the animals did not receive any ulcerogen or test material except with the vehicle (0.9% NaCl and tween 80) via oral gavage rout, which was used to prepare the test material and this group served as negative control group. **Group B:** the animals received the ulcerogen (Diclofenac sodium 150 mg/kg) via oral gavage rout and were no pre-treated with any reference drug or test material and this group will serve as positive control group. **Group C:** the animals received the ulcerogen (Diclofenac soduim) and prior to the ulcerogen the animals were treated with a reference drug (Omeprazole 20 mg/kg) via oral gavage rout. **Group D:** the animals received the ulcerogen (Diclofenac sodium) and prior to the ulcerogen the animals were treated with Aliskierin 200 mg/kg via oral gavage rout. As the prior feeding of the animal has been shown to minimize the ulcerogenic action of some drugs, the animals were fasted for 24 hours before the administration of diclofenac sodium, during the fasted period the rats had full access to tap water which was held two hours before the procedure. All drugs were freshly prepared before administration on the day of the experiment. diclofenac sodium was used for the induction of gastric ulcer at a dose of (150 mg/kg) body weight in water, orally at a concentration (20mg/ml) dissolved in normal saline (NaCl 0.9%) to which tween 80 was added (2 drops of tween 80 (Tween 80 is a widely used non-ionic emulsifier that is added to cosmetics, pharmaceuticals, and foods (Alshahrani *et al.*, 2020)) in 10 ml 0.9% NaCl), tween 80 was used as surfactant and wetting agent. Aliskierin, and omeprazole were used as a protectant against gastric mucosal damage induced by diclofenac sodium, they were dissolved in the vehicle (0.9% NaCl and tween 80) at doses of (200), (300) and (20) mg/kg respectively. Tissue of rats of all groups was harvested at the end of experiment and histopathological changes of stomach of each rat were evaluated and scored as follows [11]. Quantification of protein expression was evaluated under light microscopy at 20X. The extent of the immunohistochemically reaction of proteins, such as MDA, MPO and TNFα was measured by percentage of positively stained cells according to the following scale [12]. The software used for data analysis was SPSS (Statistical Packages for Social Sciences) version 26. The data were represented by their mean, standard deviations as well as 95% confidence level in graphs. The analysis of variances (ANOVA) test was used to compare the variable mean according to study groups, then least the square differences test was used as a post- hoc test to find the comparable differences among groups.

Results

Aliskiren caused a significant reduction ($p<0.01$) in gastric damage score mean, the ulcer's number score mean of Aliskiren was (1.4 ± 0.5) with (36.8% ± 13.6%) ulcer formation versus (3.8 ± 0.4) with (100%) ulcer formation for Diclofenac Sodium, while the mean scores of lesion severity for Aliskiren were found to be (1.5± 0.5) with (37.5% ± 13.2%) ulcer formation versus (4 ± 0) with (100%) ulcer formation for Diclofenac Sodium. As show in table 1 and 2.

Table 1: Mean scores of a number of lesions and damage percentage comparison, according to study groups, Values are expressed as Mean ± SD Different.

Groups	Mean ± Std. Deviation	Damage %
Healthy	0 ± 0 A	0% ± 0% A
Diclofenac sodium	3.8 ± 0.4 B	100% ± 11.1% B
Omeprazole + Diclofenac	0.4 ± 0.5 C	10.5% ± 13.6% C
Aliskiren + Diclofenac	1.4 ± 0.5 D	36.8% ± 13.6% D
P-value	<0.001**	<0.001**
ANOVA test, **significant at 0.01		

Table 2: Mean scores of lesion severity and damage percentage comparison, according to study groups, Values are expressed as Mean ± SD Different.

Groups	Mean ± Std. Deviation	Damage %
Healthy	0 ± 0 A	0% ± 0% A
Diclofenac sodium	4 ± 0 B	100% ± 0% B
Omeprazole + Diclofenac	0.3 ± 0.5 A	7.5% ± 12.1% A
Aliskiren + Diclofenac	1.5 ± 0.5 C	37.5% ± 13.2% C
P-value	<0.001**	<0.001**
ANOVA, **significant at 0.01.		

Microscopic investigation of this group showed a close to normal view, with some epithelial cells and lamina propri erosion, normal parietal cell distribution, normal gastric pit, and normal surface epithelium with a few detached superficial epithelial cells, The mean microscopic damage score in the Pirfenidone group was (1.3 ± 0.5) with (32.5% ± 12.1%) chance of ulcer formation which was significantly lower than the Diclofenac Sodium group which was (4 ± 0) with a (100% ± 0%) chance of ulcer formation, as show in table (3).

Table 3: Mean histopathological scores comparison, according to study groups, Values are expressed as Mean ± SD Different.		
Groups	Mean ± Std. Deviation	Damage %
Healthy	0 ± 0 A	0% ± 0% A
Diclofenac sodium	4 ± 0 B	100% ± 0% B
Omeprazole + Diclofenac	0.4 ± 0.5 C	10% ± 12.9% C
Aliskiren + Diclofenac	1.3 ± 0.5 D	32.5% ± 12.1% D
P-value	<0.001**	<0.001**
ANOVA test, **significant at 0.01		

The final group includes Aliskiren, showing a result falling in the middle of the above-explained results. Aliskiren exhibits an average level of MDA, the mean MDA score in this group was (2.3 ± 0.5) with a damage score percent of (60.5% ± 12.7%), showing significant differences from other groups, as show in table 4.

Table 4: Mean MDA scores comparison, according to study groups, Values are expressed as Mean ± SD Different		
Groups	Mean ± Std. Deviation	Damage %
Healthy	1 ± 0 A	26.3% ± 0% A
Diclofenac sodium	3.8 ± 0.4 B	100% ± 11.1% B
Omeprazole + Diclofenac	1 ± 0 A	26.3% ± 0% A
Aliskiren + Diclofenac	2.3 ± 0.5 C	60.5% ± 12.7% C
P-value	<0.001**	<0.001**
ANOVA test, **significant at 0.01.		

Finally, Aliskiren shows an average expression of MPO, with a mean score of MPO (2 ± 0), and a damage score percent of (54.1% ± 0%), showing significant differences from other groups, as show in table 5.

Table 5: Mean MPO scores comparison, according to study groups, Values are expressed as Mean ± SD Different.		
Groups	Mean ± Std. Deviation	Damage %
Healthy	1 ± 0 A	27% ± 0% A
Diclofenac sodium	3.7 ± 0.5 B	100% ± 13.1% B
Omeprazole + Diclofenac	1 ± 0 A	27% ± 0% A
Aliskiren + Diclofenac	2 ± 0 C	54.1% ± 0% C
P-value	<0.001**	<0.001**
ANOVA test, **significant at 0.01		

Aliskiren shows a similar pattern in TNF-alpha like MDA and MPO, with an average expression of TNF-alpha, a mean score of TNF-alpha (2.4 ± 0.5), and a damage score percent of (60% ± 12.9%), showing significant differences from other groups, as show in table 6.

Table 6: Mean TNF scores comparison, according to study groups, Values are expressed as Mean ± SD Different.		
Groups	Mean ± Std. Deviation	Damage %
Healthy	1 ± 0 A	25% ± 0% A
Diclofenac sodium	4 ± 0 B	100% ± 0% B
Omeprazole + Diclofenac	1.2 ± 0.4 A	30% ± 10.6% A
Aliskiren + Diclofenac	2.4 ± 0.5 C	60% ± 12.9% C
P-value	<0.001**	<0.001**
ANOVA test, **significant at 0.01.		

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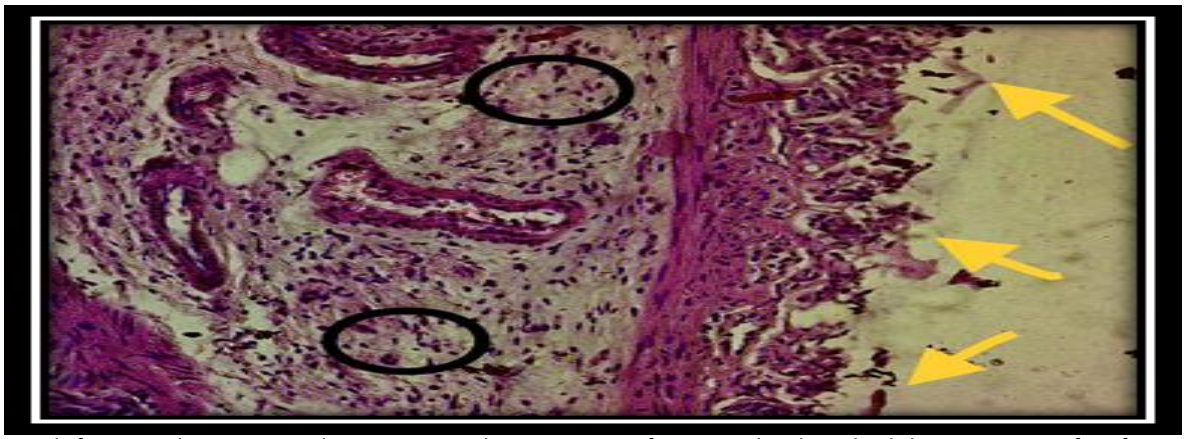

Fig 1: Diclofenac sodium 150 mg/kg (H&E, 20X degeneration of gastric gland marked degeneration of surface mucus epithelium with loss of histoarchitecture of gastric mucosa, submucosal layer ((yellow arrow)) with marked infiltration of inflammatory cells ((black circle)).

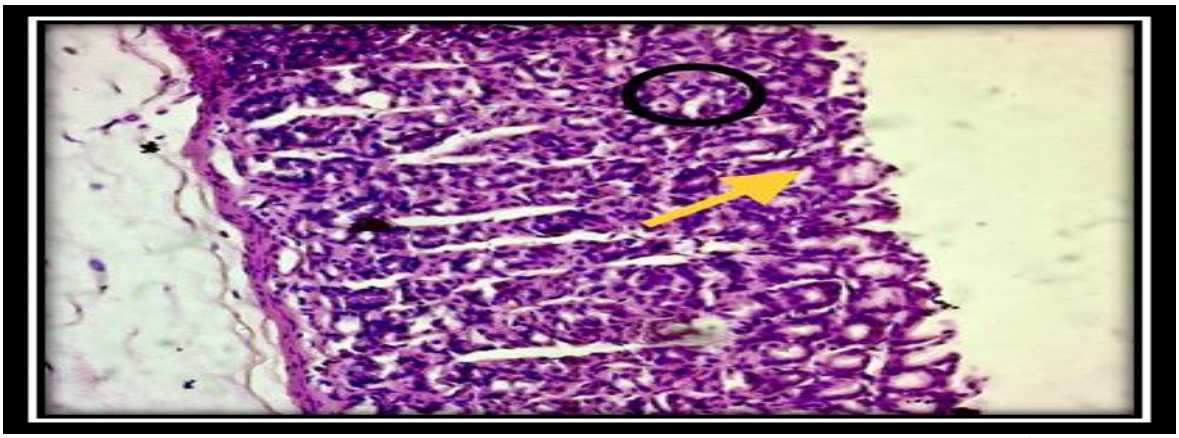

Fig 2: Omeprazole 20 mg/kg (H&E, 20X) show histopathological appearance similar to those of normal ((yellow arrow).

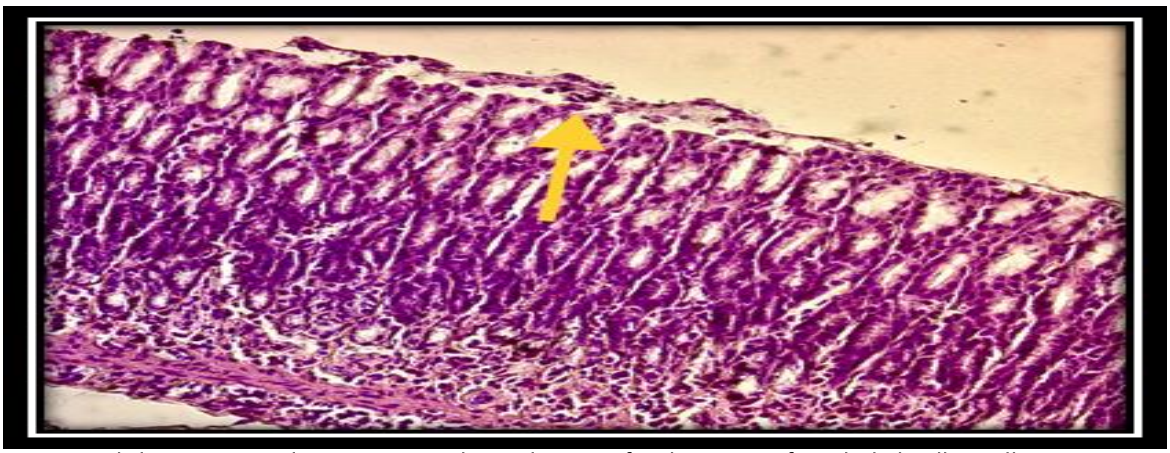

Fig 3: Aliskiren 200 mg/kg (H&E, 20X) showed a superficial erosion of epithelial cells ((yellow arrows)).

Discussion

Gastric ulcer is one of the most common chronic gastrointestinal diseases characterized by a significant defect in the mucosal barrier. The frequent long-term use of non-steroidal anti-inflammatory drugs and Helicobacter pylori (H. pylori) infection are major factors involved in gastric ulcer development. Acid inhibitors and antibiotics are commonly used to treat the gastric ulcer. However, in the last few decades, the accumulating evidence for resistance to antibiotics and the side effects of antibiotics and acid inhibitors have drawn attention to the possible use of new medications in the prevention and treatment of gastric ulcers [14]. Diclofenac, one of the most effective NSAIDs, has several adverse effects on the gastrointestinal tract by the overproduction of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-8) is considered to be an important inducer of these lesions [15]. Because of its inhibitory effect on prostaglandin synthesis, it can harm stomach tissue by increasing gastric acid and pepsin activity, as well as increasing lipid peroxidation and oxidative stress by generating free radicals like (MDA, and MPO) [16]. In this study the sites where Diclofenac sodium-induced gastric mucosal lesions were the glandular portion of the rat's stomach. The macroscopic appearance of these mucosal lesions is in accordance with the finding, in which they expressed a very similar gastric mucosal

damage with close features for this current study [17, 18]. The present study revealed that there is a significant increase in the lesion number and lesion severity (which is represented by the total linear lengths of lesions). in ulcerated rats following Diclofenac sodium oral administration (150 mg/kg). These findings agree with the findings of (Mostafa, R.E. *et al.*, 2020); and (Tandoh, A. *et al.*, 2021), these studies show that Diclofenac does increases lesions number and severity significantly due to the mechanisms mentioned above [17, 18]. The increased levels of MPO and MDA in the stomach of Diclofenac-ulcerated rats is a symptom of accelerated lipid peroxidation and free radical overproduction, resulting in mucosal injury, according to the current study which came in agree with (Akinrinde, A.S. and Hameed, H.O., 2022) [19] in which they expressed elevation of serum MPO activity (83.30%), when compared with control, and also in agreement with (Fornai, M. *et al.*, 2020) [20] in which they expressed increments of MPO and MDA, overexpression. One of these drugs is Aliskierin, not a large number of studies have focused on examining the antioxidant properties of Aliskierin in *vitro*. It has been reported that Aliskierin has the ability to reduce reactive oxygen species generation by activated neutrophils and macrophages and also by TGF-β-stimulated murine mesangial cells [21, 22]. Aliskierin also has been found to decrease the level of lipid peroxidation, Different studies have been done on the biochemical level in order to define the mechanism of the antioxidant activity of Aliskierin, and all have reached the same results. Two studies suggested that Aliskierin turns off the hydroxyl radical generated by Fenton chemistry (the reaction of hydrogen peroxide with ferrous iron). however, there is still controversial information regarding the ability of Aliskierin to complex iron and eventually stop the generation of hydroxyl radical instead of acting as a radical quencher [23]. Regarding the ability of Aliskierin to turn off superoxide anion, three reports claimed that this effect is either not related to Aliskierin, it is not a prominent effect [23, 24]. The current study shows a significant degree of prevention using Aliskierin (200 mg/kg) as a gastro-protective medication due to the above-explained mechanism. Macroscopically, Aliskierin showed a normal-like stomach with a significant decrease of ulcer number and severity, compared with the Diclofenac group and very similar to the results of the Omeprazole group. Histopathologically, Aliskierin showed a very close to normal view, with some epithelial cells erosion, normal parietal cell distribution, normal gastric pit, and normal surface epithelium with a few detached superficial epithelial cells, compared to the Diclofenac group. Due to the lack of any pre-clinical or clinical study using Aliskierin in gastric ulcer, the above obtained results of Aliskierin use as a protective medication against gastric ulcer came in agreement with, in which they found a similar results using Aliskierin in lung ischemia and due to the same mechanism [25]. The significant decrease in MPO and MDA activity in rats pre-treated with Aliskierin, on the other hand, omeprazole is an apparent indicator of anti-peroxidative and hence antioxidative potential. In comparison to the Diclofenacl group, Aliskierin is Similar to omeprazole, significantly reduced TNFα expression this may be due to anti-inflammatory action of Aliskierin via reducing pro- inflammatory cytokines. This is in agreement with (Bozkurt, I. *et al.*, 2022), this experimental study evaluated the effect of Aliskierin, for its anti-fibrotic, anti- inflammatory, and anti-oxidative properties [26].

Conclusion

This study indicates that Diclofenac sodium is toxic to the stomach mucosa of rats. Aliskiren shown a less protective effect against Diclofenac-induced stomach ulcer than Omeprazole or Pirfenidone. Aliskiren protect by lowering oxidation markers like MPO and MDA and inflammatory cytokines like TNF-alpha.

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