

التأثير العلاجي للكويرسيتين لدى الفئران المصابة بقرحة المعدة التي يسببها الأسبرين

إعداد

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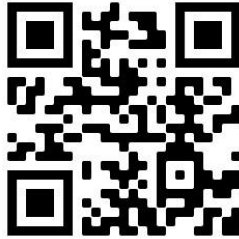
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Curative Effect of Quercetin in Rats Inflicted with Aspirin-Induced Gastric Ulcer

Abstract

Gastric ulcer disease affects a wide range of people worldwide, and is one of the most common diseases of the twenty-first century. This investigation was designed out to study therapeutic effects of quercetin in rats inflicted with acute gastric ulcers by aspirin. Twenty male albino rats ($200\pm 10g$ *b.wt.* each) were used in the study and divided into 4 groups ($n= 5$ rats), one of them left as control-ve group while the rest 3 groups were once orally administered by aspirin at a dose of $200mg/kg$ *b.wt.*, one of these three groups left as control +ve which fed on basal diet only and the rest twice groups orally administrated with quercetin at two doses 50 or 100 mg/kg *b.wt.*, each for seven days as an experiment period. Gastric ulcer length, gastric juice volume, pH value, percentage of total acidity were determined, and the histopathological changes were examined. The investigation results concluded that the quercetin twice doses 50 and 100 mg/kg *b.wt.*, had therapeutic effect for the stomachs by decreasing gastric ulcer length, gastric juice volume, percentage of total acidity, moreover, healing histopathological changes and increasing pH value of gastric juice. Furthermore, treated group with quercetin at a dose of 100 mg/kg *b.wt.*, reflected the highest significant decrease in gastric ulcer length, gastric juice volume, percentage of total acidity with highest increase in pH value as compared to a group orally administered with a dose of 50 mg/kg *b.wt.* Accordingly, quercetin has a curative effect in rats infected with aspirin-induced gastric ulcer.

Keywords: Gastric ulcer, quercetin, gastric ulcer length, histopathological changes.

Introduction

Gastric ulcer disease affects a wide range of people worldwide, and is one of the most common diseases of the twenty-first century. The exact pathogenesis of peptic ulcers is not clear, but diverse factors, including consumption of non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, stressful

lifestyle, alcohol consumption, *Helicobacter pylori* (*H. pylori*) infection, smoking, and family history are considered as risk factors in the pathogenesis of peptic ulcer (Mohammad *et al.*, 2015). The major goals of ulcer treatment are to relieve pain, promote healing, and prevent recurrence. Antisecretory drugs are also prescribed to relieve pain and allow healing; these may include proton-pump inhibitors, H₂-blockers, or antacids (as used in gastroesophageal refer disease (GERD) (Sharon *et al.*, 2009). Many synthetic anti-ulcer drugs (e.g., misoprostol) have been developed that can be specifically used for preventing or treating NSAID-induced gastric ulcers (Miyamoto and Haruma, 2013). However, these drugs have many side-effects such as diarrhea, itching, skin rash, and dizziness (Ertem, 2013).

Flavonoids are a group of naturally occurring compounds widely distributed as secondary metabolites in the plant kingdom found mainly in fruits, vegetables, leaves and grains (Goel and Bhattacharya, 1991). One of the most many natural flavonoids, found in a large number of fruits and vegetables, is quercetin (Kuo *et al.*, 1998). The defense of flavonoids against tissue oxidative stress is being proved in various animal models for a lot of pharmacological effects (Uma *et al.*, 2019).

Recent studies reported that quercetin (3,3',4',5,7-pentahydroxyflavone) has an antioxidant effects on injuries that caused by a lot of toxic agents in many different experimental models, depending upon the reduction of lipid peroxidation and increasing antioxidant enzymes activity (Ömer *et al.*, 2004). A lot of experimental researches studied the therapeutic benefits of quercetin and its derivatives on models of peptic ulcer disease. Quercetin antioxidant and anti-inflammatory properties are contributing factors to therapeutic efficacy for peptic ulcers (Manach *et al.*, 2005).

Therefore, this investigation aimed to study the therapeutic effects of quercetin doses in rats inflicted with acute gastric ulcers by aspirin.

Materials and Methods

1. Materials and rats

1.1. Experimental rats:

Twenty (20) Sprague – Dawley male albino rats weighting 200 ± 10 g b.wt., used in this study, obtained from the Animal House of Ophthalmology Hospital, Giza, Egypt.

1.2. Basal diet:

The basal diet consisted of casein (12%), corn oil (10%), mineral mixture (4%), vitamin mixture (1%), cellulose (5%), chorine chloride (0.2%), methionine (0.3%) and the remained is corn starch (up to 100%) according to AIN (1993). The used vitamin mixture component was that recommended by Campbell (1963) while the salt mixture used was formulated according to Hegsted (1941).

1.3. Quercetin:

Quercetin which used as a treatment of aspirin induced acute gastric ulcer was used as a dry substance purchased from Al-Gomhoria Company for Chemicals then dissolved in water with the required concentration to make a dose of 50 and 100 mg/kg of rats b.wt..

2. Methods

2.1. Experimental design:

Twenty (20) male albino rats fed with basal diet for 7 days till the beginning of the experiment. Then, rats were divided to four equal groups (n=5 rats) as follows: Group 1 was fed during the whole experimental period on the basal diet only as a control negative group (C -ve). The rest three groups were once orally administered by aspirin at a dose of 200mg/kg b.wt., for acute gastric ulcer induction according to Agrawal *et al.*, (2000). All groups were divided according to these groups:

Group (1): Fed on basal diet only along the experimental period as (C -ve).

Group (2): Orally administered by aspirin at a dose of 200mg/kg b.wt., and fed on basal diet along the experimental period as (C +ve).

Group (3): Orally administered by aspirin at a dose of 200mg/kg b.wt., then orally injected with quercetin at a dose of 50 mg/kg b.wt., for 7 days.

Group (4): Orally administered by aspirin at a dose of 200mg/kg b.wt., then orally injected with (quercetin) at a dose of 100 mg/kg b.wt., for 7 days.

2.2. Measurement the gastric juice volume and the length of gastric ulcer:

At the end of the experiment (7 days), rats were fasted for 12 hrs, but only allowed for drinking water. All rats were sacrificed and their stomachs were tied around both openings (cardiac & pyloric sphincters) then injected with 3ml of distilled water. Gastric juice was collected in sterilized tubes and centrifuged at 500 r p m, for 5 minutes. The volume of gastric juice was measured by graduated cylinder and expressed as ml. After washing the stomachs with buffered saline, all stomachs opened longitudinally followed by examination under dissecting microscope for measuring gastric ulcer according to the method described by Akhtar and Ahmad (1995).

2.3. Determination the pH and total acidity of gastric juice:

The pH value of gastric juice was determined by pH meter and the total acidity was determined by titration of 1ml gastric juice in 10ml of distilled water with 0.01N NaOH using two drops of phenolphthalein as an indicator.

2.4. Histopathological study:

Stomachs from each group were fixed in 10% neutral buffered formalin (pH=7.0), dehydrated in ethyl alcohol, then cleared in xylol and embedded in paraffin; sections prepared and stained with hematoxylin and eosin for examining both fore and glandular parts of the stomach (Carleton, 1976).

2.5. Statistical analysis of data:

The obtained results were statistically analyzed using computerized SPSS (Ver. 22). Different treatments were analyzed by one way ANOVA test using Duncan's multiple range test at $p < 0.05$ indicating significance between different groups (Snedecor and Cochran, 1967).

Results and Discussion

1. Curative effects of quercetin at two doses on the length of gastric ulcer:

Data recorded in table (1) show the curative effects of quercetin at two doses on aspirin induced gastric ulcer in rats.

Table (1): Curative effect of quercetin at two doses on the length of gastric ulcer in rats

Groups		Aspirin and Quercetin	Doses (mg/kg B.Wt.)	Gastric Ulcer Length (mm.)
				Mean \pm SE*
Control -ve	1	-	-	0.00
Control +ve	2	Aspirin (Asp.)	200	6.08 \pm 0.05 ^a
Treated Groups	3	(Asp.) + Quercetin	50	4.11 \pm 0.06 ^b
	4	(Asp.) + Quercetin	100	1.99 \pm 0.03 ^c

- *SE means standard error of the mean.

- Values with different letters (a, b, c, etc.) differ significantly at $p \leq 0.05$.

It could be noticed from the table results that the mean \pm SE of gastric ulcer length in control positive group (C +ve) was 6.08 \pm 0.05 mm., compared to zero in control -ve normal group ($p \leq 0.05$). These results denote that there were significant increasing in gastric ulcer length for control positive compared to control negative group. All rats administered with aspirin at a dose of 200mg/kg b.wt., then orally injected with quercetin at two doses of 50 and 100 mg/kg b.wt., declared significant decrease in gastric ulcer length when compared to control positive group which were 4.11 \pm 0.06 and 1.99 \pm 0.03 mm, respectively. The group treated with quercetin at a dose of 100 mg/kg b.wt., reflected the highest significant decrease in gastric ulcer length.

These results were in agreement with Kelly *et al.*, (2009) who reported that flavonoids including quercetin have healing properties of gastric ulcers and these polyphenolic compounds considered as new alternatives for suppression peptic ulcers induced with *H. pylori*. In this respect Sumbul *et al.*, (2011) concluded that quercetin has been shown to have antiulcer and gastroprotective effects by anti-ulcer effect that it has been shown to inhibit growth of *H. pylori* in a dose-dependent manner in vitro. As soon as Ahmet *et al.*, (2003) demonstrated that

gastroprotective effect of quercetin is due to antiperoxidative, antioxidant and antihistaminic effects relating for decreasing the number of mast cells and reducing the gastric erosions area. Moreover, Mojzis *et al.*, (2001) showed that quercetin antioxidant properties and its mucus protective effect might be the main factors responsible for protective effect against induced gastric mucosal injury, recording that administration of low doses (25 and 50 mg/kg) of quercetin had slight effect on the healing of gastric mucosal lesion. Meanwhile, Lacasa *et al.*, (2000) reported that the protective curative effect of quercetin against absolute ethanol-associated gastric mucosal ulceration and necrosis is due to its anti-lipoperoxidant effect, as well as an improvement in enzymatic antioxidant performance.

2. Curative effects of quercetin at two doses on the gastric juice volume:

The curative effects of quercetin at two doses on the gastric juice volume are listed in table (2).

Table (2): Curative effect of quercetin at two doses on the volume of gastric juice in rats

Groups		Aspirin and Quercetin	Doses (mg/kg B.Wt.)	Volume of Gastric Juice (mL.)
				Mean \pm SE*
Control -ve	1	-	-	1.13 \pm 0.010 ^d
Control +ve	2	Aspirin (Asp.)	200	3.49 \pm 0.013 ^a
	3	(Asp.) + Quercetin	50	2.98 \pm 0.017 ^b
	4	(Asp.) + Quercetin	100	2.01 \pm 0.015 ^c

- *SE means standard error of the mean.

- Values with different letters (a, b, c, etc.) differ significantly at $p \leq 0.05$.

It could be demonstrated that rats administered with aspirin at a dose of 200mg/kg b.wt., without treatment (C +ve) showed significant increase ($p \leq 0.05$) in gastric juice volume which was 3.49 ± 0.013 mL., compared with (C -ve), normal rats, which was 1.13 ± 0.010 mL. All treated rats after oral administration with aspirin at a dose of 200mg/kg b.wt., reflected significant decrease in the volume of gastric juice as compared to rats orally

administered with aspirin at a dose of 200mg/kg b.wt., without treatment. Moreover, the highest significant decrease was recorded in the group of quercetin at a dose of 100 mg/kg b.wt., when compared to the group of 50 mg/kg b.wt., which were 2.01 ± 0.015 and 2.98 ± 0.017 mL., respectively.

These data agreed with that of María *et al.*, (2006) who reported that quercetin and naringenin caused significant decrease in ulcer length as compared to control group. Nevertheless, no decrease in either volume, acidity or pepsin observed; however, a significant difference in histamine secretion values was found. Also, Tandon *et al.*, (2004) mentioned that quercetin elevates nonenzymatic and enzymatic antioxidant agents (protein sulfhydryl, SOD, and CAT) and inhibits neutrophil infiltration. Furthermore, Bahare *et al.*, (2020) demonstrated that quercetin and its derivatives are naturally phytochemicals with promising bioactive effects.

3. Curative effects of quercetin at two doses on gastric juice pH:

Table (3) show the curative effects of quercetin at two doses (50 and 100 mg.kg B.Wt.) on the pH of gastric juice.

Table (3): Curative effect of quercetin at two doses on gastric juice pH in rats

Groups		Aspirin and Quercetin	Doses (mg/kg B.Wt.)	pH of gastric juice
				Mean \pm SE*
Control -ve	1	-	-	5.72 ± 0.009^a
Control +ve	2	Aspirin (Asp.)	200	2.11 ± 0.039^d
	3	(Asp.) + Quercetin	50	3.91 ± 0.033^c
	4	(Asp.) + Quercetin	100	4.79 ± 0.029^b

- *SE means standard error of the mean.

- Values with different letters (a, b, c, etc.) differ significantly at $p \leq 0.05$.

The data in the previous table showed that orally administration with aspirin at a dose of 200mg/kg B.Wt., without treatment (control +ve) caused significant decrease in the value of

gastric juice pH as compared to normal rats (C -ve) which were 2.11 ± 0.039 compared with 5.72 ± 0.009 respectively. Treated groups with the two doses (50 and 100 mg.kg B.Wt.) of quercetin, caused significant increase in the pH value of gastric juice compared to control positive group, and the highest significant increase was recorded for the group of quercetin at a dose of 100 mg.kg B.Wt.

These findings were in agreement with Shakeerabanu *et al.*, (2011), found increasing in pH and decreasing in total acidity, moreover, decreasing in acid output and pepsin concentration in ulcerated animals treated with quercetin, over and above protecting the mucosal layers from damage, acting thereby as antiulcerative agent. Moreover, Geraets *et al.*, (2007) said that quercetin is known to possess strong anti-inflammatory capacities. Furthermore, Rui *et al.*, (2020) demonstrated that quercetin could maintain relatively potent antitumor activities through its anti-inflammation effect.

4. Curative effects of quercetin at two doses on gastric juice total acidity percentage:

Data of the curative effects of quercetin at two doses on gastric juice total acidity percentage are recorded in table (4).

Table (4): Curative effect of Quercetin at two doses on gastric juice total acidity percentage in rats

Groups		Aspirin and Quercetin	Doses (mg/kg B.Wt.)	Total Acidity (%)
				Mean \pm SE*
Control -ve	1	-	-	0.021 ± 0.002^d
Control +ve	2	Aspirin (Asp.)	200	0.103 ± 0.007^a
	3	(Asp.) + Quercetin	50	0.098 ± 0.003^b
	4	(Asp.) + Quercetin	100	0.061 ± 0.002^c

- *SE means standard error of the mean.

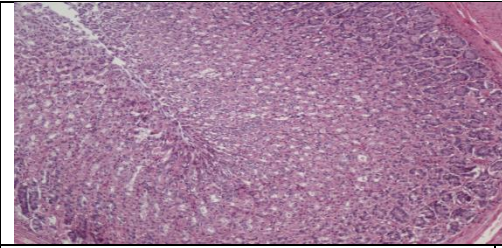
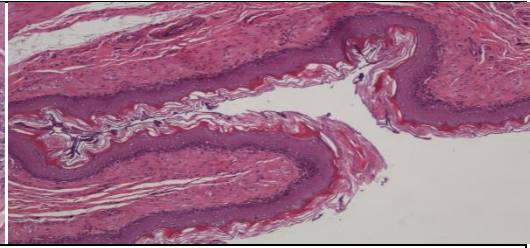
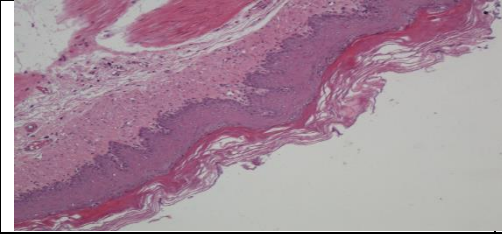
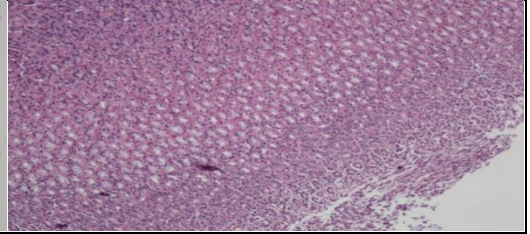
- Values with different letters (a, b, c, etc.) differ significantly at $p \leq 0.05$.

It could be observed from the table results that control positive group (ulcerated rats without treatment) showed significant increase of gastric juice total acidity percentage when

compared to control negative group (normal rats) which were 0.103 ± 0.007 and 0.021 ± 0.002 %, respectively. All rats orally administered with aspirin at a dose of 200mg/kg b.wt., then orally injected with doses of 50 and 100 mg/kg b.wt., of quercetin showed significant decrease in gastric juice total acidity as compared to control positive group which were 0.098 ± 0.003 , 0.061 ± 0.002 and 0.103 ± 0.007 %, respectively. Therefore, rats orally administered with aspirin at a dose of 200mg/kg b.wt., then orally injected with a dose of 100 mg/kg b.wt., quercetin showed the highest significant decrease in total acidity.

These results agreed with Brett and Ghica (2003) who reported that quercetin's anti-inflammatory activity appears to be due to its antioxidant and inhibitory effects on inflammation-producing enzymes (cyclooxygenase, and lipoxygenase), and the subsequent inhibition of inflammatory mediators, including leukotrienes and prostaglandins. Moreover, Kotob *et al.*, (2018) described the gastroprotective role of quercetin (Qu) and ellagic acid (EA) on aspirin-induced gastric ulcer (GU) in rats and concluded that both quercetin and ellagic acid caused gastroprotective effect on aspirin-induced gastric ulcer in rats. The mechanisms associated with stress-induced gastric lesion inhibition, oxidative stress attenuating, iron chelation and blunting ferritin level, modulating inflammatory cascade, and promoting the healing process, were studied by Vafadar *et al.*, (2020) who reported that quercetin can act as a cancer preventable through some mechanisms including anti-inflammation, anti-proliferation, pro-oxidation and cell cycle besides strengthening the impacts of other chemotherapeutic medications.

5. Histopathological results:

	
<p>Photo (1): Stomach of rat from control negative (C -ve) group showing normal mucosal gastric glands. (H and E \times 400).</p>	<p>Photo (2): Stomach of rat from (C +ve) group revealed gastro-esophageal wall with marked gastric mucosal gland necrosis and associated with lymphocyte cell infiltrate in the mucous. (H and E \times 400).</p>
	
<p>Photo (3): Stomach of rat from group 3 (Quercetin 50 mg/kg b.wt.) revealed gastric wall with focal superficial gastric mucosal gland necrosis and partial atrophy. (H and E \times 400).</p>	<p>Photo (4): Stomach of rat from group 4 (Quercetin 100 mg/kg b.wt.) revealed gastric wall mucosal showing partial atrophy of the mucosal glands. (H and E \times 400).</p>

These histopathological results agreed with that of **Alkushi and Elsayy (2017)** who demonstrated that no major damage of endothelial cells, pronounced reduction of necrosis and erosions with mild inflammatory cell infiltration recorded in quercetin-treated group.

Conclusion

Quercetin has curative effect in rats inflicted with aspirin-induced gastric ulcer, being more pronounced as the dose increased.

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د. السيد حامد علي بكر

المستخلص العربي

يصيب مرض القرحة المعدية مجموعة واسعة من الناس في جميع أنحاء العالم، وتعد أحد أكثر الأمراض شيوعاً في القرن الحادي والعشرين. تم تصميم هذا البحث لدراسة التأثيرات العلاجية للكويرسيتين في الفئران المصابة بقرحة المعدة الحادة بواسطة الأسبرين. تم استخدام 20 فأر ذكر من نوع الألبينو بمتوسط أوزان (10 ± 200 جرام) حيث تم تقسيمهم إلى 4 مجموعات (5 فئران لكل مجموعة)، تركت احدهم كمجموعة ضابطة سالبة أما الثلاث مجموعات المتبقية فقد تم حقنهم فمويًا بالأسبرين بجرعة 200 مليجرام/كجم من وزن الجسم، حيث تركت إحدى الثلاث مجموعات كمجموعة ضابطة موجبة وتم تغذيتها على العليقة الأساسية فقط أما المجموعتين المتبقيتين فقد تم حقنهم فمويًا بالكويرسيتين بجرعتين هما (50 و 100 مليجرام/كجم من وزن الجسم) لمدة سبعة أيام. تم قياس طول قرحة المعدة، وحجم العصير المعدى، ومستوى الـ pH، والنسبة المئوية للحموضة الكلية كما تم فحص التغيرات النسيجية للمعدة. وقد خلصت نتائج البحث إلى أن كلتا جرعتي الكويرسيتين 50 و 100 مليجرام/كجم من وزن الجسم كان لهما تأثير علاجي للمعدة عن طريق تقليل طول قرحة المعدة، وتقليل حجم العصير المعدى، وتقليل نسبة الحموضة الكلية، علاوة على ذلك، التئام التغيرات النسيجية للمعدة مع زيادة مستوى الـ pH للعصير المعدى. علاوة على ذلك، عكست المجموعة المعالجة بالكويرسيتين بجرعة 100 مليجرام/كجم من وزن الجسم أعلى انخفاض معنوي لطول قرحة المعدة وحجم العصير المعدى والنسبة المئوية للحموضة الكلية مع زيادة الـ pH مقارنة مع المجموعة التي حقنت فمويًا بجرعة 50 مليجرام/كجم من وزن الجسم. ووفقاً لذلك، فإن للكويرسيتين تأثير علاجي لدى الفئران المصابة بقرحة المعدة التي يسببها الأسبرين.

الكلمات المفتاحية: قرحة المعدة، الكويرسيتين، طول القرحة المعدية، التغيرات النسيجية.