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Protective Effect of Quercetin Against Indomethacin Induced Gastric Ulcer In Rats.

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Abstract

Aim: This study was designed to study the protective effects of Quercetin against indomethacin induced gastric damage in rats.

Materials and Methods: Adult male albino rats weighing 180 – 200 gm were divided into 3 groups: group 1 (control group), group 2 received an ulcerogenic dose of indomethacin (200 mg/kg body weight) in drinking water. Group 3: receives quercetin orally at a dose of 50mg/kg body weight for one week before induction of gastric ulcer with indomethacin.

Results: A significant reduction in number and the mean area of gastric ulcer, gastric tissue malondialdehyde (MDA) and the plasma levels of tumor necrotic factor alpha (TNF-α) and interleukin 1-B (IL-1B) and a significant increases in the PH, gastric tissue superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were observed in quercetin treated rats compared to indomethacin ulcerated rats.

Conclusion: Quercetin exerts a potent anti-inflammatory gastroprotective effect besides its clear antioxidant effect against indomethacin induced gastric ulcer.

Introduction

Peptic ulcers are a common disorder of the entire gastrointestinal tract that occurs mainly in the stomach and the proximal duodenum. This disease is multifactorial and its

treatment faces great difficulties due to the limited effectiveness and severe side effects of the currently available drugs. The use of natural products for the prevention and treatment of different pathologies is continuously expanding throughout the world [1]. It is caused by many factors like stress, drugs, alcohol, etc. and is reported to be due to an imbalance between offensive acid-pepsin secretion and defensive mucosal factors like mucin secretion and cell shedding [2].

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most frequently prescribed drugs worldwide which are useful as analgesic and anti-inflammatory agents. Indomethacin (IND) is one of the most popular NSAIDs which prescribed extensively for to treatment of rheumatoid arthritis, osteoarthritis, cervical spondylitis, ankylosing spondylitis and acute musculoskeletal disorders and infective inflammation. A long-term use of NSAIDs among patients is associated with a range of oesophagho-gastro-duodenal changes with a very high morbidity and mortality rates [3]. It accounts for gastro duodenal mucosal erosions in approximately 35 – 60% of patients, gastric or duodenal ulceration in 10 – 25% of patients and severe complications, such as gastrointestinal hemorrhage or perforation in 1% of patients [4].

The toxicity of NSAIDs is mainly attributed to inhibition of prostaglandin synthase activity that inhibits prostaglandin production in the GI tract resulting in accumulation of intracellular arachidonic acid [5], induction of mitochondrial injury [6] and production of reactive metabolites that covalently bind to critical cellular Proteins [7].

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. Quercetin (3,5,7,3',4'-pentahydroxyflavone) is one of these plant derived flavonoids which is found in black and green tea, apples, onion, red grapes, citrus fruits, tomato and leafy green vegetables [8]. Flavonoids have been recognized for having interesting clinical properties, such as anti-inflammatory, antiallergic, antiviral, antibacterial, and antitumoral activities [9]. They can inhibit a series of enzymes which are activated in inflammatory process. Many studies support the idea that reactive oxygen

species (ROS) generating in a situation of oxidative stress plays an important role in inflammation [10].

Based on the previous data, the present study was directed towards assessment of the gastroprotective efficacy of quercetin against NSAID (indomethacin) induced gastric ulcer and to clarify the possible mechanisms underlying this effect.

Materials and Methods:

I-Chemicals used:

1- Indomethacin provided in tablets, each one containing 25mg and manufactured by Misr CO., Egypt. It was dissolved in drinking water.
2- Quercetin provided as powder manufactured by Sigma CO., USA.
3- Tissue MDA, SOD GSH-Px kits (Ransod and Ransel and Randox Laboratories GmbH, Netherland).
4- IL-1 and TNF- were determined by ELISA according to the manufacturer's instructions (Assay Designs, Ann Arbor, MI; Bender MedSystems, SanDiego, CA).
5- Diethyl ether: available in the form of solvent ether from laboratory Rasayan (1 L. M.W. 74.12).

II- Animals used:

Experimental protocol for the study was approved by the ethics committee on animal experiments in Benha University.

Thirty healthy adult male albino rats weighting 180 – 200 g. averaging 16 weeks old were brought from Experimental Animal Breeding Farm, Helwan - Cairo to be utilized in this study. They were housed in cages (5 rats/cage) under standard laboratory conditions (12h light/dark cycle, 20 – 25 °C, relative humidity 55%).The animals were given commercial standard caloric diet (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and tap water ad libitum. All animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences. After acclimatization for 1 week, the rats were randomly classified into 3 equal

experimental groups: **Group I (Control group):** received no medication and given free access to food and water. **Group II (Indomethacin ulcerated group):** received an ulcerogenic dose of indomethacin (200 mg/kg body weight) in drinking water. **Group III (Quercetin and indomethacin):** receives quercetin orally at a dose of 50mg/kg body weight for one week before induction of gastric ulcer with indomethacin.

IV-Procedure of the experiment:

At the end of the experiment, the rats were anaesthized by ether and both chest and abdominal wall were opened. Intracardiac blood samples were collected then put in the incubator till it is clotted and the plasma was taken and kept at -20ºC till the time of measurement of plasma TNF-α and IL-1B. The stomach of each rat was removed after the lower oesophageal [11] and the pyloric ends have been ligated [12].

1-Measurement of the number and area of gastric ulcer as well as gastric PH:

- The removed stomach was cut open along the greater curvature and the contents were collected in centrifuge tubes and centrifuged at 200 × *g* for 10 min. The resultant supernatant fluid is transported to a test tube where PH was determined by a PH-meter [13].
- The stomach was then washed with warm saline, and the inner surface was photographed and the area of gastric ulcers in mm^2 was calculated. Next, the gastric mucosal tissues were removed, frozen in liquid nitrogen and stored at – 80˚C [14].
- Gastric tissue samples from each group were fixed in 10% formalin for 24 h. The specimens were then embedded in paraffin, sectioned and stained with hematoxylin and eosin (H & E) before being evaluated by light microscopy [15].

2-Measurement of gastric tissue MDA, SOD and GSH-Px levels:

- Tissue malondialdehyde (MDA) (mmol/l) was determined by the double heating method of Draper and Hadley [16].
- Tissue SOD and GSH-Px activities were measured by using Ransod and Ransel and Randox Laboratories GmbH commercial kits, respectively with the Shimadzu UV-1601 spectrophotometer [17, 18].

3-Determination of plasma TNF-α and IL-1B levels:

Blood samples in EDTA-containing vials were centrifuged at 1000 × *g* for 10 min at 4˚C. The levels of IL-1 and TNF- were determined by ELISA according to the manufacturer's instructions from Assay Designs, Ann Arbor, MI, Bender Med Systems [19, 20].

Results:

Table 1: Effect of pretreatment with quercetin (50mg/kg) for one week on the number of gastric ulcers, the mean area of ulcers (mm^2) and gastric PH. All values are expressed as Mean ± Standard deviation (SD) for 10 rats in each group; control group (group 1), indomethacin (group 2) and indomethacin + Quercetin (group 3).

	Control	IND	IND + QUE
No. of gastric ulcers	0	10 ± 1.49	1.4 ± 0.84
Mean area of ulcer (mm^2)	0	28.8 ± 2.53	5.14 ± 1.75
PH	3.6 ± 0.62	1.53 ± 0.36	3.66 ± 0.57

Table (1) and figures (1a) clarify that the number of gastric ulcer increases from 0 in the control group to 10 ± 1.49 in the indomethacin ulcerated group ($P < 0.001$) while pretreatment with quercetin in indomethacin ulcerated rats decreases this number to 1.4 ± 0.84 ($P < 0.001$). The mean area of ulcers (mm^2) increases from 0 in the control group to 28.8 mm^2 ± 2.53 in the indomethacin group ($P < 0.001$). Indomethacin-induced mean ulcer area was decreased in quercetin treated rats to 5.14 mm^2 ± 1.75 ($P < 0.001$). PH decreases from 3.6 ± 0.62 in the control group to 1.53 ± 0.36 in the indomethacin group ($P < 0.001$). Treatment with quercetin before induction of gastric ulcer with indomethacin increases it to 3.66 ± 0.57 ($P < 0.001$).

The gastric damage was also confirmed by macroscopic and histological examination (figures 1b and 1c). The gastric mucosa was normal in control group. In indomethacin group, it was extensively damaged involving the cells lining the gastric pits or into the gland area but this was partly protected by administration of quercetin.

Figure (1a):

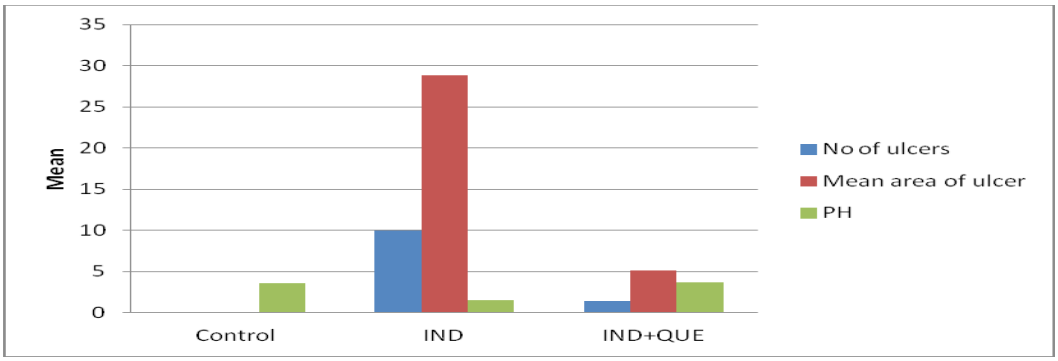
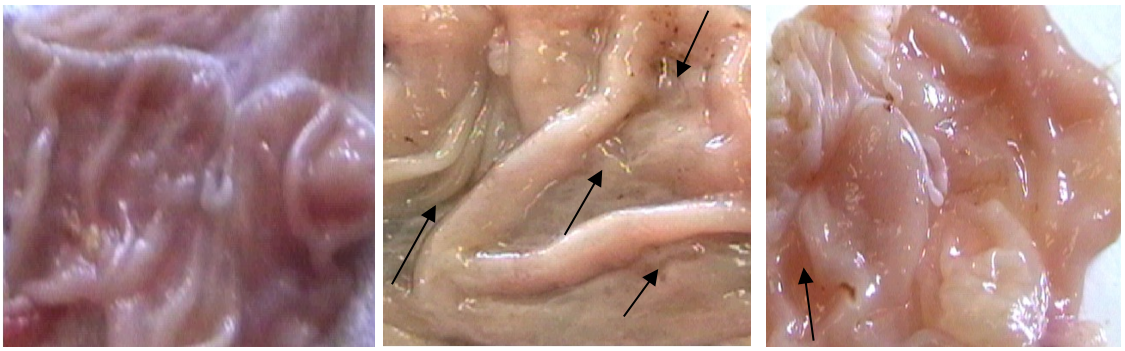


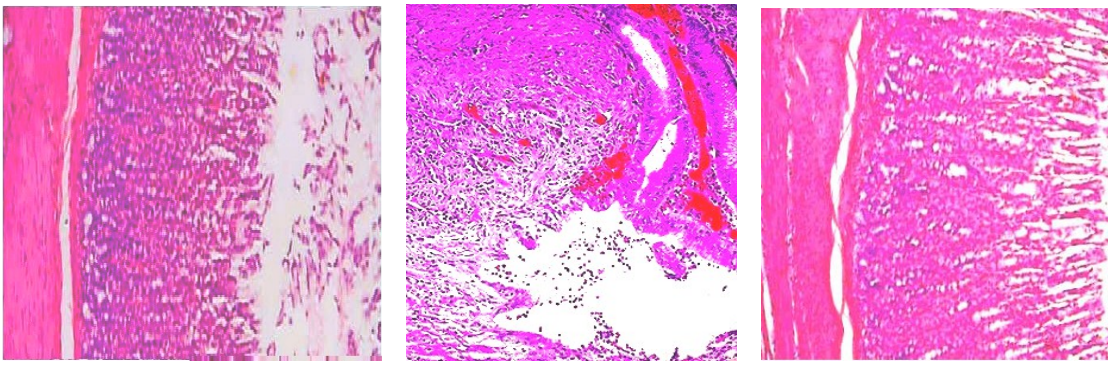
Figure (1b) macroscopic picture:



Normal gastric mucosa **INDO ulcerated group**

Quercetin treated group

Figure (1c) microscopic picture:



Normal gastric mucosa **INDO ulcerated group**

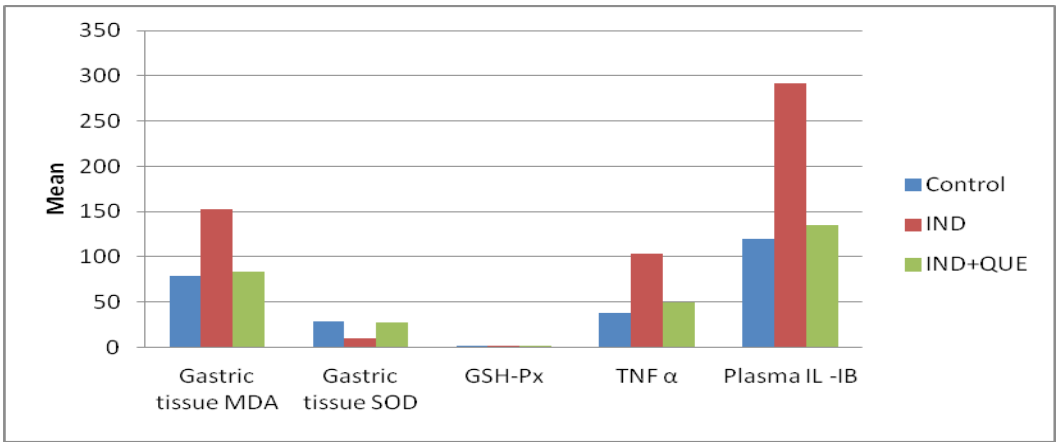
Quercetin treated group

Table 2: Effect of pretreatment with quercetin (50mg/kg) for one week on gastric tissue malondialdehyde (MDA) in μmol/g protein, superoxide dismutase (SOD) in U/mg protein, glutathione peroxidase (GSH-Px) in U/mg protein and the plasma levels of tumor necrotic factor alpha (TNF-α) in pg/ml and interleukin 1B (IL-1B) pg/ml. All values are expressed as Mean ± Standard deviation (SD) for 10 rats in each group; control group (group 1), indomethacin (group 2) and indomethacin + Quercetin (group 3).

	Control	IND	IND + QUE
Gastric tissue MDA	78.9 ± 4.95	152.9 ± 5.57	83.7 ± 5.4
Gastric tissue SOD	28.95 ± 2.29	9.68 ± 1.33	27.88 ± 2.71
Gastric tissue GSH-Px	0.412 ± 0.043	0.185 ± 0.032	0.385 ± 0.044
Plasma TNF-α	38.2 ± 3.86	103.5 ± 6.77	49.3 ± 8.52
Plasma IL-1B	119.3 ± 3.74	291.5 ± 9.04	135.25 ± 8.65

Table (2) and figure (2) clarify that the gastric tissue malondialdehyde was increased from 78.9 ± 4.95 μmol/g protein in the control group to 152.9 ± 5.57 in the indomethacin ulcerated group ($P < 0.001$), while treatment with quercetin decreases it to 83.7 ± 5.4 ($P < 0.001$). Superoxide dismutase decreases from 28.95 ± 2.29 U/mg protein in the control group to 9.68 ± 1.33 in the indomethacin group ($P < 0.001$). Pretreatment with quercetin increases it to 27.88 ± 2.71 ($P < 0.001$). Glutathione peroxidase decreases from 0.412 ± 0.043 U/mg protein in the control group to 0.185 ± 0.032 in the indomethacin ulcerated group ($P < 0.001$), while pretreatment with quercetin increases it to 0.385 ± 0.044 ($P < 0.001$). Plasma TNF-α increases from 38.2 ± 3.86 pg/ml in the control group to 103.5 ± 6.77 in the Indomethacin ulcerated group ($P < 0.001$). Pretreatment with quercetin decreases it to 49.3 ± 8.52 ($P < 0.001$). Plasma IL-1B increases from 119.3 ± 3.74 pg/ml in the control group to 291.5 ± 9.04 in the indomethacin ulcerated group ($P < 0.001$) and this was decreased to 135.25 ± 8.65 by administration of quercetin ($P < 0.001$).

Figure (2):



Discussion:

Oxygen derived free radicals have been implicated in the pathogenesis of a wide variety of clinical disorders and gastric ulceration in human and experimental animals [21].

Most of the available drugs are thought to act on the offensive factors which neutralize acid secretion like antacids, H2 receptor blockers or interfere with acid secretion as proton pump blockers [22].

Quercetin is a phenolic compound widely distributed in the plant kingdom. It is found in frequently consumed foods as apples, berries, onions, tea and vegetables. Quercetin is reported to have many beneficial effects on human health including cardiovascular protection, anticancer activity, antiulcer effects, antiallergic activity, cataract prevention, antiviral activity and anti-inflammatory effects [23].

The present study was designed to demonstrate gastric ulcer protective activity of quercetin in rats and the possible mechanisms implicated in it.

In the present study, the gastro protective effectiveness of the quercetin was evident from significant reduction in the number and mean area of the gastric ulcer in quercetin treated rats as compared with indomethacin ulcerated rats. The protective effect of quercetin in preventing ulcer development can be due to their protein precipitation as quercetin helps in precipitating microproteins on the ulcer site therefore forming a

protective layer over the lining mucosa thus protects the underlying mucosa from gastric acid secretion, toxins and other irritants [24].

Gastric acid plays an important permissible role in NSAIDs associated mucosal injury [25]. In our study, there was a significant deccrease in gastric PH indicating increased gastric acidity and acid output in ulcerated animals and gastric PH was increased by treating the rats with Quercetin which is a highly desirable for gastroprotection and antiulcer effect. This protective effect may be due to its direct inhibitory action on the acid producing cells. Quercetin also has antihistaminic properties preventing the release of histamine from gastric mast cells and inhibiting the gastric H^+ – K^+ proton pump, diminishing acid gastric secretion. Moreover, it increases prostaglandins E_2 and I_2 synthesis by gastric mucosa. They inhibit the secretion of gastric acid and stimulate mucus and hydrophobic surfactant like phospholipids secretion in gastric epithelial cells [26, 27].

Also it was reported that quercetin exhibit ulcer healing effect in indomethacin induced gastric ulcerated rats by several mechanisms such as increasing mucous secretion evidenced by higher hexosamine and carbohydrate over protein ratio, increasing mucosal resistance, increasing mucosal blood flow and epithelialization rates. These actions were referred to the triterpenoid saponins and flavonoids components of quercetin. Quercetin intake also reduces cell shedding and increase DNA content of gastric mucosal cells indicating gastric mucosal renewal ability [28].

In our study we tried to explore the antioxidant effect of quercetin in indomethacin ulcerated rats by measuring gastric tissue content of malondialdehyde (MDA) concentration which is considered as an indicator of lipid peroxidation which is a well known example of oxidative damage that affects cell membranes, lipoproteins and other lipid containing structures under conditions of oxidative stress and gastric antioxidant enzyme activity by measuring superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Our results showed that there was significant increase in MDA concentration in indomethacin ulcerated group with reduction in the levels of antioxidant enzyme (SOD, GSHPx). We also found that pretreatment with quercetin in indometheacin ulcerated rats significantly decreased the elevated MDA and increased the

reduced antioxidant enzyme activities. Our results were agreed with other findings that revealed that the preventive effects of quercetin is due to inhibition of lipid peroxidation by its antioxidant nature indicating that quercetin has an antioxidant effect [26, 28, 29].

NSAIDs induces gastric ulcer by inhibiting cyclooxygenase enzyme complex that convert free eicosapolyenoic acids like arachidonic acid to cyclic endoperoxides which is the key intermediate in prostaglandin synthesis [29]. In animal experiments, quercetin inhibits production of inflammation producing enzymes (cyclooxygenase (COX) [30] and lipoxygenase (LOX) [31] and decreasing NF-kappaB activation, nitric oxide synthase (NOS) overexpression [32, 33, 34] and TNF-α [35, 36].

So in our study we aimed to find out the anti-inflammatory efficacy of quercetin against gastric ulcer induced by indomethacin that was assessed by measuring plasma levels of TNF-α and IL-1B. Our results showed that there was a significant increase in both TNF-α and IL-1B levels in the plasma in indomethacin ulcerated group and this was significantly decreased in quercetin group.

Conclusion:

Flavonoids as quercetin represent a highly diverse class of secondary metabolites with potentially beneficial effects on human health. These compounds protect the gastrointestinal mucosa from lesions produced by various experimental ulcer models and against different necrotic agents. Several mechanisms of action may be involved in this protective effect including antioxidant, anti-secretory and anti-inflamatory effects. Quercetin may represent an attractive therapeutic option for preventing and healing NSAIDs induced gastric ulcers but we recommend for further studies on its anti-inflammatory effects against gastric ulcer.

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