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A Comparative Study on the Protective Role of Obestatin, Quercetin and their Combination on the Injured Rat Gastric Mucosa Induced by Stress

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Abstract

Background: Modern life contains a lot of nervous stresses which considered as the origin of many diseases.

Aim of Study: Comparison between the protective effect of obestatin, quercetin and their combination on the stress-induced gastric ulcer in rats.

Material and Methods: Sixty healthy adult Wistar male albino rats weighed 180-220g were used. Ten rats were considered as normal control (Group I). Stress-induced gastric ulcers were produced in the other 50 rats. These rats were divided into 5 groups: Group II (with no medication), Group III (pretreated with misoprostol), Group IV (pretreated with obestatin), Group V (pretreated with quercetin) and Group VI (pretreated with both obestatin and quercetin). All medications were used for 2 weeks before induction of ulcer.

Results: In all groups the following parameters were measured; TBARS, antioxidant enzymes activities, myeloperoxidase activity in gastric tissue, ulcer index, and plasma tumor necrosis factor-a. Comparing the results of Groups IV and V to that of Group II showed that, the use of obestatin or quercetin separately can ameliorate the condition to some extent with variable superiority. Also, the combined pretreatment of these rats with obestatin and quercetin produced more significant improvement; approaching normal; of all the tested parameters than the use of each drug separately. Moreover, the results in Group VI were far superior over the use of the standard drug misoprostol in Group III.

Conclusion: The combined use of obestatin and quercetin as a prophylactic measure against the development of stress-induced gastric ulcer is more effective than the use of each drug separately.

Key Words: *Gastric ulcer – Quercetin – Obestatin – Stress.*

Introduction

STRESSORS have a major influence upon mood, health, performance, and wellbeing. Severe life stressors frequently predate the onset of functional gastrointestinal illnesses. The stomach is extremely sensitive to various stress stimuli and peptic ulcer has often been described as a stress disease **[1]**.

In these instances, the stress ulcers are generated due to many mechanisms as frequent elevations in glucocorticoids and poor gastric blood flow **[2]**.

Oxidative stress takes place in all of us but is increased by things like a poor diet, high levels of stress, a lack of sleep and exposure to chemical toxins. Most of the mucosal lesions such as ulcers and or inflammatory changes accompanying stress were found to be associated with the excessive generation of reactive oxygen species or reactive nitrogen species and decreased activity of antioxidative enzymes **[3]**.

Several approved drugs are working for the protection of that illness. Although these medications are effective, they produce many unwanted effects thus limiting their usage. In recent years, there has been a growing interest in alternative therapies, due to their professed relative lower adverse effects, ease of availability and affordability **[4]**.

Obestatin (OB) is a hormone released from the stomach deriving from the same peptide precursor as ghrelin. OB exhibits potent anti-inflammatory and antioxidant activities **[5]**. Many studies specified that OB could be considered as a gastroprotective agent against the formation of acute gastric mucosal injury induced by various ulcerogens **[6-8]**.

The major bioflavonoid in our diets, Quercetin (QC) is commonly found in fruits and vegetables, especially onions, citrus, apples and green tea. QC which is recognized as an antioxidant and believed to protect against several degenerative diseases by preventing lipid peroxidation; has been studied for

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its gastroprotective and ulcer healing-promoting actions [9,10]. The free radical-scavenging, antioxidant and anti-inflammatory properties of this compound may be partly related to its anti-ulcer effects [10].

In the present study, we aimed to compare the potential protective role of obestatin, quercetin and their combination on the injured rat gastric mucosa induced by stress.

Subjects and Methods

Experimental animals:

This study was conducted on sixty healthy adult Wistar male albino rats, weighing between 180-220g. They were obtained from the animal house of Faculty of Sciences, Tanta University. Handling of the animals was carried out in accordance with the ethical guidelines for investigations and was approved by the Local Ethical Committee for the Care and Use of Laboratory Animals, Faculty of Medicine, Tanta University from June 2018-June 2019.

The animals were housed in group cages with free access to food (nutrient solid meal) and tap water and maintained under controlled conditions of illumination (light-dark cycle of 6:30AM-6:30PM) and temperature (21-27ºC). The experiments were performed between 7:00AM and 4:00PM. In all experiments, the animals were kept in cages with raised floors of wire mesh to prevent coprophagy and were maintained in a specific pathogen-free condition at the housing facilities. They were housed under the standard conditions in strict accordance with the institutional and national official guidelines.

Experimental design:

The animals were randomly divided into six groups; each group contained 10 rats.

- *Group I:* This group was designated as the negative control (where animals are not treated with any reference drug (saline-vehicle treated); each rat was given a single subcutaneous (SC) dose of saline and about 15 minutes later another dose was given.
- *Group II:* Served as the positive control; in which Cold-Water-Restraint Stress (CWRS)-induced gastric ulcer was applied by technique produced by Senay and Levine [11].
- *Group III:* In which we used misoprostol for two weeks before induction of the ulcer. Misoprostol was suspended in 0.5ml of 1% carboxymethyl cellulose and was given orally by oral gastric tube in the dose 50 µg/kg according to Elgarawany et al., [12].
- *Group IV:* In this group, the rats were given OB in a dose of 40 µg/Kg body weight, intraperitoneally according to Khalefa [6] for two weeks before induction of the ulcer.
- *Group V:* The rats in this group were given QC in a dose of 50mg/Kg, orally according to Alkushi & Elsawy [13] for two weeks before induction of the ulcer.
- *Group VI:* In which OB and QC were administrated together as shown above.
- At the end of the experiment, the effects of different drugs on CWRS-induced gastric ulcer were studied.

Experimental model for stress-induced gastric ulcer:

- This model employs the restraint technique produced by Senay and Levine [11]. In the current study, we preferred induction of gastric ulcers by Cold-Water-Restraint Stress (CWRS) because it resembles human peptic ulcers, both grossly and histopathologically.
- The procedure for inducing ulcers included animals being fasted for a period of 24-36 hours prior to the experiment. Ulcers are then induced by placing animals individually in a restricted cage and immersing them vertically in a water tank gradually to the level of the xiphoid inside the restraint cold ventilated refrigerator at a temperature of 2-3ºC for 3 hours.

Blood and organs sampling:

At the end of the experimental period, blood samples were taken from retro-orbital venous plexus immediately in heparinized capillary tubes under light ether anesthesia. Thereafter, the blood was centrifuged at 3000rpm for 15 minutes to separate plasma for different biochemical assays. The animals were then decapitated under ether anesthesia. Laparotomy was performed and the stomachs were dissected out. The excised stomach was placed on an ice bearing surface and was opened along the greater curvature and examined for ulceration. Thereafter, tissue samples (from the stomach) were rapidly excised and stored at –20ºC for subsequent biochemical assays.

Determination of Ulcer Index (UI):

The lesions were examined according to a modified scoring system developed by Parmar and Desai [14]. The average scores for each group were calculated and expressed as the ulcer index. The

ulcer index was calculated by severity of gastric mucosal lesions 1mm or less, 1-2mm and more than 2mm and graded as 1, 2 and 3 score, respectively. Then the UI was calculated by using the formula: UI = 1 X (number of lesions of grade 1) + 2 X (number of lesions of grade 2) + 3 X (number of lesions of grade 3). Then, the overall score was divided by a factor 10, which was designated as ulcer index.

Biochemical measurements:

Thiobarbituric acid reactive substance (TBARS) in gastric tissue was estimated by the method of Ohkawa et al. [15]. Superoxide Dismutase (SOD) activity in gastric tissue was measured by the method of Marklund and Marklund [16]. Catalase (CAT) activity in gastric tissue was measured according to the method of Aebi [17]. The activity of glutathione peroxidase (GSH-Px) in gastric tissue was determined according to the method of Lawrence and Burk [18].

Tumor Necrosis Factor-α (TNF-α) level was assessed using commercially available ELISA kits according to manufacturer's instructions (Titer Zyme EIA kit, Assay Designs, Inc., Ann Arbor, MI, USA) Cannon et al. [19].

Myeloperoxidase (MPO) activity in gastric tissue was assayed according to the method of Pulli et al. [20]. The MPO activity was measured by following the oxidation of O-dianisidine dihydrochloride by H_2O_2. Results were expressed as U/g tissue.

Chemicals:

OB and QC were purchased from Sigma-Aldrich Co., USA, while misoprostol (Cytotec) was obtained from Amoun Pharmaceutical Company S.A.E, Egypt.

Statistical analysis:

Data were processed using the statistical package for the social sciences version 20 (SPSS Inc., Chicago, Illinois, USA) program. Descriptive statistics were used as means (M) and SD, frequency distribution, and comparisons. One-way analysis of variance test was used to compare between groups, followed by the post hoc test (least significant difference) for intergroup comparisons. We considered differences to be statistically significant, if p-values were less than 0.05.

Results

Effect of induction of the gastric ulcer and the prophylactic usage of misoprostol, OB, QC and combined OB & QC on tissue levels of thiobarbituric acid reactive substance and enzyme activities of superoxide dismutase, catalase and glutathione peroxidase in the gastric mucosa (Table 1): The results of our study showed that the induction of gastric ulcer in Group II resulted in a significant increase in TBARS level in gastric mucosa tissue and this indicates increased oxidative stress. In contrast, there was a significant decrease of SOD, CAT, and GSH-Px antioxidant enzyme activities in gastric mucosa tissue in the same group as compared with the control group.

In Group III, treatment of rats with misoprostol before induction of stress caused significant decrease of TBARS level (p=0.001) associated with significant increase of SOD (p=0.026), CAT (p= 0.014), and GSH-Px (p=0.015) antioxidant enzyme activities in gastric mucosa tissue as compared with Group II (ulcer control group) indicating improvement of the oxidative stress.

On the other hand, the pretreatment with OB in Group IV resulted in significant decrease in TBARS level (p<0.001) together with significant increase of SOD (p=0.007), CAT (p=0.001) and GSH-Px (p=0.001), antioxidant enzyme activities in gastric mucosa tissue when compared with Group II. These results are similar to that in Group III but it is more significant.

In Group V, the usage of QC as a prophylactic measure against gastric ulcer produced similar changes to that occurred in Group III and IV but is more significant as compared to Group II (p <0.001).

The combined administration of both OB and QC in Group VI caused more significant changes than those produced in Group III, and like those of Group V but with the restoration of the antioxidant enzymes activities to near-normal values (p <0.001).

Effect of induction of the gastric ulcer and the prophylactic usage of misoprostol, OB, QC and combined OB & QC on ulcer index and plasma TNF-α level (Table 2): In Group II, the induction of the gastric injury by stress produced an ulcer with an index of 8.19 ± 1.04. The treatment with misoprostol in Group III caused a significant decrease in the ulcer index (p=0.003). In Group IV, the usage of OB also produced a significant decrease in the ulcer index (p<0.001). The same change is produced in group V in which the rats were treated with QC (p<0.001). These previous changes were compared to the ulcer index of Group II. Furthermore, a prominent significant reduction (p<0.001) in ulcer index occurred in Group VI (as

compared to Group II) in which the rats with gastric ulcer were treated with a combination of OB & QC.

In (Table 3), the results of plasma TNF-α level in different groups showed that the induction of gastric ulcer produced a significant elevation in its level as compared to the control group (p<0.001). In Group III, pretreatment of rats with a gastric ulcer with misoprostol resulted in significant lowering of plasma TNF-α level as compared to Group II (p=0.015). Also, pre-treatment of the gastric ulcer with OB in Group IV produced a significant decrease in plasma TNF-α level (p<0.001). In addition, a less significant reduction in plasma TNF-α level was noticed in Group V treated with QC when compared to Group II (p=0.002). The use of a combination of OB and QC in Group VI caused more significant decrease of plasma TNF-α level (approaching level in control group) as compared also to the gastric ulcer group (Group II) (p<0.001).

Effect of induction of the gastric ulcer and the prophylactic usage of misoprostol, OB, QC and combined OB & QC on myeloperoxidase (MPO) level in gastric tissue (Table 3): The results of Group II showed that, the induction of gastric ulcer produced significant increase in Myeloperoxidase (MPO) level in gastric tissue as compared to Group I (control group) (p<0.001). In Group III, the pre-treatment of rats with a gastric ulcer with misoprostol resulted in a significant decrease in MPO level in gastric tissue as compared to Group II (gastric ulcer group) (p=0.006). The pre-treatment of the rats with OB in Group IV caused significant reduction in MPO level in gastric tissue as compared to Group II (p=0.002). In Group V, the rats with gastric ulcers were treated with QC and this produced also a significant reduction in MPO level in gastric tissue as compared to Group II (p<0.001). Finally, in Group VI the combined pre-treatment of rats with both OB and QC produced more significant decrease (approaching near normal) in MPO level in gastric tissue (p<0.001).

Table (1): Effect of induction of the gastric ulcer and the prophylactic administration of misoprostol, OB, QC and combined OB & QC on tissue levels of Thiobarbituric Acid Reactive Substance (TBARS) and enzyme activities of Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GSH-Px) in the gastric mucosa.

	Group I (control)	Group II (induction of g. ulcer)	Group III (g. ulcer + misoprostol)	Group IV (g. ulcer + obestatin)	Group V (g. ulcer + quercetin)	Group VI (g. ulcer+ obestatin & quercetin)
TBARS (nmol/l/mg protein)	1.37±0.85	3.94±0.79*	2.85±0.52†	2.15±0.78**	1.91±0.66††	1.33±0.57¶
SOD (U/mg protein)	6.81±1.35	3.75±1.42*	4.98±0.81†	5.27±1.30**	6.26±1.26††	6.54±1.00¶
CAT (U/mg protein/min)	1.55±0.85	0.61±0.36*	1.1±0.20†	1.26±0.42**	1.37±0.86††	1.43±0.87¶
GSH-Px (U/mg protein/min)	121.3±13.25	67.7±14.97*	82.60±7.40†	87.7±15.91**	98.7±5.1 6††	110.7±8.62¶

Data are expressed as mean ± SD.

* : p<0.05 gastric ulcer untreated group compared with the control group.

† : p<0.05 gastric ulcer group treated with misoprostol compared with the gastric ulcer untreated group.

** : p<0.05 gastric ulcer group treated with OB compared with the gastric ulcer untreated group.

†† : p<0.05 gastric ulcer group treated with QC compared with the gastric ulcer untreated group.

¶ : p<0.05 gastric ulcer group treated with OB & QC compared with the gastric ulcer untreated group.

Table (2): Effect of induction of the gastric ulcer and the prophylactic usage of misoprostol, OB, QC and combined OB & QC on ulcer index and plasma TNF-α level.

	Group I (control)	Group II (induction of g. ulcer)	Group III (g. ulcer + misoprostol)	Group IV (g. ulcer + obestatin)	Group V (g. ulcer + quercetin)	Group VI (g. ulcer+ obestatin & quercetin)
Ulcer index	0	8.19±1.04*	7.20±0.85†	1.53±0.80**	1.99±0.69††	1.19±0.18¶
Plasma TNF-α (pg/ml)	74.3±6.98	128±8.51*	119.95±7.22†	87.45±5.50**	1 17.30±7. 13††	78.10±7.29¶

Data are expressed as mean ± SD:

* : p<0.05 gastric ulcer untreated group compared with the control group.

† : p<0.05 gastric ulcer group treated with misoprostol compared with the gastric ulcer untreated group.

** : p<0.05 gastric ulcer group treated with OB compared with the gastric ulcer untreated group.

†† : p<0.05 gastric ulcer group treated with QC compared with the gastric ulcer untreated group.

¶ : p<0.05 gastric ulcer group treated with OB & QC compared with the gastric ulcer untreated group.

Table (3): Effect of induction of the gastric ulcer and the prophylactic usage of misoprostol, OB, QC and combined OB & QC on Myeloperoxidase (MPO) level in gastric tissue.

	Group I (control)	Group II (induction of g. ulcer)	Group III (g. ulcer + misoprostol)	Group IV (g. ulcer + obestatin)	Group V (g. ulcer + quercetin)	Group VI (g. ulcer+ obestatin & quercetin)
Myeloperoxidase (U/g tissue)	2.43±0.17	4.76±0.73 *	3.99±0.75†	3.91±0.66**	2.88±0.55††	2.50±0.56¶

Data are expressed as mean ± SD:

* : $p<0.05$ gastric ulcer untreated group compared with the control group.

† : $p<0.05$ gastric ulcer group treated with misoprostol compared with the gastric ulcer untreated group.

** : $p<0.05$ gastric ulcer group treated with OB compared with the gastric ulcer untreated group.

†† : $p<0.05$ gastric ulcer group treated with QC compared with the gastric ulcer untreated group.

¶ : $p<0.05$ gastric ulcer group treated with OB & QC compared with the gastric ulcer untreated group.

Discussion

Stress has long been suspected to play a role in the etiology of many diseases. The exact pathogenesis and the effect of chronic exposure to environmental stressors on gastric mucosal integrity have not been fully described [2].

Although many drugs are available for protection against gastric ulcer, all these drugs have side effects and limitations. This study was designated to compare between two unusual lines of prevention against that type of lesion and studying the success of their combination.

Oxidative stress takes place in all of us but is increased by things like a poor diet, exposure to chemical toxins, a lack of sleep, and high levels of stress.

The results of our study showed that the application of stress for induction of gastric ulcer in Group II resulted in a significant increase in oxidative stress which is showed by increased TBARS level in gastric mucosa tissue and a significant decrease of SOD, CAT, and GSH-Px antioxidant enzyme activities as compared with the control Group I. These changes suggest oxidative damage by stress and correlate well with the increase in the severity of ulceration. Our results are in accordance with many studies which reported that stress-induced gastric ulceration is a consequence of the oxidative damage of the gastric mucosa [1-3].

Exploring the results of the present study also revealed that the administration of OB for two weeks before exposure to stress produced a significant decrease in TBARS level associated with a significant increase of SOD, CAT, and GSH-Px antioxidant enzyme activities in gastric mucosal tissue. The previous results indicate a significant improvement of the oxidative stress as compared to Group II.

There is accumulating evidence suggested that excessive inflammation plays critical roles in the pathophysiology of stress-related diseases. Application of stress in Group II resulted in the presence of gastric ulcer with a high ulcer index, an elevation of the plasma level of TNF-α and myeloperoxidase (MPO) level in gastric tissue as compared to Group I (Table 2).

OB was considered by many as a good protective agent for gastric injury caused by stress. The beneficial effects of OB on the protection of gastric mucosa against acute injury could be related to the anti-inflammatory properties of this peptide and due to suppression of MPO activity, and lipid peroxidation in the gastric tissue which increased in stress [6-8]. This is in accordance with our results and with a previous study proved that OB exerted a potent inhibition of pro-inflammatory mediators and augmentation of endogenous antioxidants [21].

Tumor necrosis factor-α is a cell signaling protein involved in systemic inflammation and acute phase reaction. The central role of TNF-α in inflammation has been demonstrated by the ability of agents that block the action of TNF-α to treat a wide range of inflammatory conditions, including gastric mucosal injury [22].

About the effect on plasma TNF-α, our results demonstrated that OB has a significant effect on the reduction of its level (Table 2). This is in accordance with a study performed by Ibrahim et al. that mentioned that OB lessens the level of end-product lipid peroxidation and reduction in mucosal expression of pro-inflammatory IL- 1b and TNF-α [23]. Further support for the potent anti-inflammatory effects of obestatin comes from the fact that obestatin inhibited the activation of NFkB, which is considered as a critical signaling molecule in inflammation and pathogenesis of stress damage [23].

Another study showed that administration of OB significantly ameliorates clinical and histopathological severity of chronic colitis via the suppression of polymorphonuclear leukocyte infiltration and inhibition of inflammatory conditions. This was associated with a reduction of lipid peroxidation and enhancement of glutathione synthesis which has a critical role in oxidative stress **[5]**.

Gastric hypoxia and ischemia lead to mucosal acidification and injury which implicated in the pathogenesis of stress ulcer. Gastric mucosal microcirculation and Nitric Oxide (NO) play a crucial role in the regulation of regional blood flow **[24]**.

Another explanation of the antiulcerogenic effect of OB is by its vasorelaxant effects via activation of a specific endothelial NO signaling cascade. This mechanism regulates mucus-alkaline secretion, which acts as a physical barrier against gastric acid and pepsin, appear to be controlled by various factors including prostaglandins and NO **[25]**.

Also, administration of OB increases gastric mucosal blood flow and cell proliferation and decreased gastric motility and consequently its secretion; this effect has been associated with a reduction in gastric ulcer index **[7]**.

It is known that some of the major stimuli that can induce apoptosis are; cytoplasmic stress, presence of hormones such as corticosteroids and an excessive presence of free radical. OB has been shown to prevent apoptosis by interacting with specific mechanisms proposed as integral components of the antiapoptotic cascades **[26]**. In addition, OB displays a variety of cellular effects, by regulating metabolic cell functions, increasing cell survival and proliferation and inhibiting apoptosis and inflammation in different cell types **[27]**.

QC has received considerable attention because there are no known drug interactions or long-term adverse effects from its use and highly effective promising new adjunctive management for protection and healing of common gastric ulcers **[28]**.

The results of the present study showed that the pretreatment of rats with QC caused significant decrease of TBARS level associated with a significant increase of SOD, CAT and GSH-Px antioxidant enzyme activities in gastric mucosa tissue as compared with Group II. The previous outcomes indicate the inhibitory effect of QC on lipid peroxidation and oxidative stress.

It was proved that QC is one of the most potent scavengers of free radicals and its antioxidant potential is four times that of Vitamin E **[29]**. However, the antioxidant properties of QC, which is the most important mechanism of its anti-ulcer activity, involves also maintenance of mitochondrial integrity, size and functions, inhibition of oxidizing enzymes and reduction of lipid peroxidation **[29,30]**.

Furthermore, exploring the results showed that the use of QC for two weeks, before application of stress and ulcer production, comparable changes occurred as in Group III and IV. It is to be noted that the results are more significant as compared to Group II. Our results also were in accordance with the results of a study conducted by Farzaei et al. **[31]**. They proved that QC increases the synthesis of local prostaglandins, regulates the immune system's response to outside stressors, inhibits low-density lipoproteins oxidation and acting as a vasodilator **[31]**. Also, QC can decrease the number of gastric mast cells and prevent the release of its histamine and inhibits the gastric H+/K+ proton pump and consequently diminishing gastric acid secretion **[32]**.

Moreover, our results showed that the administration of QC produced a significant decrease in MPO level in gastric tissue (Table 3). Likewise, justifying gastric MPO activity, which is attributed to neutrophil infiltration **[33]**, the elevation of nitrite/nitrate which shows NO production, this can be added among the protective mechanisms of QC **[34]**.

Another research showed that anti-inflammatory foods containing QC can help in the management of a great number of inflammatory health problems. QC can reduce inflammation by scavenging free radicals inhibiting inflammatory leukotriene production and increases glutathione (GSH) level **[35]**.

QC substantially alleviated the mucosal damage and apoptosis by modulating lysosomal enzyme activities through decreasing its iron and the subsequent its membrane permeabilization during protection against ethanol-induced gastric ulcer **[36]**.

Many researchers suggested that stress acts on the hypothalamus and activates Hypothalamic-pituitary-Adrenal (HPA) axis, causing adrenergic discharge which produces gastric mucosal ischemia and injury **[37]**. Also, HPA axis cause an increase in cortisol levels which increase gastrin release and allow the gastric lining to thin and become more susceptible to the development of ulcers with a decrease in mucosal regenerative activity **[38]**.

QC can fight these effects during times of extended stress as it suppresses the release of corticotropin-releasing factor which is necessary for cortisol formation and discharge and modulates DNA binding activities of glucocorticoid receptors **[39]**.

Furthermore, two more studies showed that administration of OB and QC can induce anxiolytic effects as a help to decrease stress symptoms and consequently can suppress gastric injuries precipitated by stress **[40,41]**.

In the current study, we utilized misoprostol because it's the recommended model of the application while evaluating mucosal and cytoprotective agents. Misoprostol which is a prostaglandin E1 analog acts upon gastric parietal cells, inhibiting the secretion of gastric acid which leads to decreased proton pump activity. Although other classes of drugs are more effective for the treatment of acute peptic ulcers, misoprostol is only indicated for use as a qualified protective agent **[12,42]**.

The results of the present study showed that the pretreatment of stress-induced gastric ulcer using misoprostol in Group III caused a significant decrease in the ulcer index as compared to Group II. In addition, a less significant reduction in plasma TNF-a level was noticed in Group V treated with QC when compared to Group II. Taken together, our results demonstrated that the administration of misoprostol, produced similar changes on all parameters to that produced by QC and OB but these changes are less prominent.

In Group VI, the results revealed that the combined pretreatment with OB and QC caused synergistic improvement in the oxidative stress induced by stress. They decreased the oxidative stress marker TBARS and increased the activity of antioxidant enzymes SOD, CAT, and GSH-Px significantly in the gastric tissue. The increased activities of gastric antioxidant enzymes in Group VI might be explained by the synergistic effect of both QC and OB.

Similarly, pretreatment with both OB and QC produced a significant decrease in the ulcer index when administered to the rats. Joining both agents together caused highly significant outcomes.

So, in total, QC ameliorates the production of the gastric ulcer by its more potent antioxidant effects and its effect on MPO enzyme which are more powerful than that of OB. On the other hand, OB protects gastric mucosa through its anti-inflammatory activity which is stronger than that of QC.

Finally, the use of combined pre-treatment with both OB and QC in Group VI produced the most significant results of all the tested parameters (approaching normal in many of them).

Conclusion:

The results of the present study made clear that the injurious effects of stress on the gastric mucosa pass mainly through oxidative stress, inflammation, and ischemia. Additionally, it discusses the effective role of QC, OB, and their combination in protection against this type of mucosal injury. Also, we can conclude that the possible combined use of OB and QC as a defensive strategy against gastric mucosal damage induced by stress. Lastly, we can verify that the combined use of both supplements is more effective than their separate use or the use of common antiulcer protective agents. The use of both agents acting as a synergistic combination because QC has the upper hand in combating oxidative stress and MPO enzyme activity while OB is superior as anti-inflammatory and vasodilator agent.

Finally, the present study suggests that the natural combined products including OB and QC could be considered as a new approach shield against occurrences of gastric injury especially stress-induced type.

Conflict of interest:

The authors declare no conflict of interest.

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دراسة مقارنة بين الدور الوقائى للآوبستاتين والكيرسيتين ومزيجهما على إصابة الغشاء المخاطى للمعدة الناجم عن الضغط العصبى فى الفئران

خلفية: الحياة الحديثة تحتوى الكثير من الضغوط العصبية التى تعتبر أصل العديد من الأمراض.

الهدف: مقارنة بين التأثير الوقائى للآوبستاتين والكيرسيتين ومزيجهما على قرحة المعدة التى يسببها الضغط العصبى فى الفئران.

الطريقة: تم إستخدام ستين من ذكور الفئران البيضاء البالغة التى يتراوح وزنها بين ١٨٠-٢٢٠جم. وأعتبرت عشرة من الفئران كمجموعة ضابطة (المجموعة الأولى). تم إحداث قرحة بالمعدة وذلك عن توقيع الضغط العصبى على الفئران الخمسين الأخرى. تم تقسيم هذه الفئران إلى ٥ مجموعات: المجموعة الثانية (الفئران التى لديها قرحة المعدة مع عدم وجود تقديم دواء)، المجموعة الثالثة (تمت معالجتها بالميزوبروستول)، المجموعة الرابعة (تم إستخدام الآوبستاتين)، المجموعة الخامسة (تم إستخدام الكيرسيتين) والمجموعة السادسة (تمت معالجتها بكل من الآوبستاتين والكيرسيتين. تم إستخدام جميع الأدوية لمدة إسبوعين قبل إحداث القرحة.

النتائج: فى جميع المجموعات تم حساب القياسات التالية، TBARS، أنشطة إنزيمات مضادات الأكسدة، نشاط المايلوبروكسيديز فى أنسجة المعدة، مؤشر القرحة، عامل نخر ورم البلازما .

أظهرت مقارنة نتائج المجموعتين الرابعة والخامسة ونتائج المجموعة الثانية أن إستخدام الأوبستاتين أو الكيرسيتين بشكل منفصل يمكن أن يحسن الحالة إلى حد ما مع التفوق المتبادل. وكذا أنتجت المعالجة المدمجة للمركبين تحسناً ذو دلالة إحصائية أوضح قد تقترب من الطبيعى أفضل من إستخدام كل منهما على حدة. علاوة على ذلك، كانت النتائج فى المجموعة السادسة أعلى بكثير من إستخدام الميزوبروستول القياسى فى المجموعة الثالثة.

الخلاصة: يعتبر الإستخدام المشترك للآوبستاتين والكيرسيتين كإجراء وقائى ضد تطور قرحة المعدة التى يسببها الضغط العصبى أكثر فعالية من إستخدام كل دواء بشكل منفصل وكذا أفضل من العلاجات السابق إستخدامها لنفس الهدف.