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Comparison of Anti-Oxidant Activity of Different Brands of Esomeprazole Available in Iraqi Pharmacies

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ABSTRACT
Peptic ulcer involves mucosal injury in the gastrointestinal tract that has several underlying pathologies. Oxidative stress, defined as imbalance between oxidants and antioxidants inside the body, has been found to play an important role in the pathogenesis of peptic ulcer. Results from previous study by the same research team[1] showed that esomeprazole has a good antioxidant effect compared to other proton pump inhibitors used for the treatment of peptic ulcer. Therefore, this study was carried out to investigate the difference in antioxidant effect of various brands of esomeprazole available in Iraqi community pharmacies. Three brands of esomeprazole were investigated for their antioxidant effects by measuring their ability to scavenge the α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical and compared to pure esomeprazole and vitamin C as a standard antioxidant. The antioxidant activity was expressed as IC_{50} values and as ascorbic acid equivalent antioxidant capacity (AEAC). IC_{50} values of 2 esomeprazole brands (Nexium and Gordex) were comparable to that of vitamin C and pure esomeprazole. In addition, the AEAC of Nexium and Gordex were statistically not different from vitamin C, while that of Esofag was lower than vitamin C. Nexium and Gordex have an adequate antioxidant activity to be comparable to vitamin C, and hence provide better options for the treatment of peptic ulcer. The work was conducted at the College of Pharmacy, University of Mosul.

Keywords: Esomeprazole, DPPH, *H. pylori*, oxidative stress, peptic ulcer

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INTRODUCTION

Peptic ulcer is a condition of injury to the mucosa of the stomach or the proximal portion of the duodenum that is caused by the gastric acid. The oesophagus may also be affected. Genetics, stress, diet, drugs and bacterial infection (*Helicobacter pylori*), among others, are all predisposing factors.[2] Patients usually suffer from epigastric pain, nausea, stomach pain, dyspepsia, and bloating.[3] There is growing evidence that stress (both physical and psychological) and *Helicobacter pylori* infection contribute to the pathogenesis of peptic ulcer through oxidative stress.[4] The term oxidative stress describes the state of imbalance between oxidants and antioxidants inside the body with a favour of the oxidants and resultant damage to cellular components.[5] Oxidants in the form of reactive oxygen species (ROS) are produced in large quantities in the gastric mucosa in response to stress and infection leading to injury.[4]

Proton pump inhibitor (PPI) have been in the market for about three decades now and since their presentation, they have proven to be vital, effective and safe for the management of peptic ulcer.[6] These drugs inhibit both basal and stimulated acid secretions by inhibiting H,K-ATPase, thus contributing to the healing of peptic ulcers.[7] Moreover, PPIs beneficial effects in peptic ulcer have been linked to their antioxidant properties adding to the protection of gastric mucosal cells.[8,9] Compared to other PPIs, esomeprazole is more effective and at least equally safe.[10,11]

In a previous work carried out by our group to explore the antioxidant effects of different PPIs (omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole), esomeprazole, together with omeprazole, exhibited the best *in vitro* antioxidant potential.[1] This result, combined with the fact that most studies conduct comparisons among different drugs belonging to the same class, motivated the group to investigate the antioxidant effects of different brands of esomeprazole available in community pharmacies, possibly to provide a recommendation to physicians of the added benefit of the antioxidants effect in the treatment of peptic ulcer.

MATERIALS AND METHODS

Chemicals

Ascorbic acid (vitamin C) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) were supplied from Sigma–Aldrich (Germany). Pure esomeprazole was supplied from Sigma–Aldrich (Germany). Different brands of esomeprazole were obtained from different suppliers as follows: Nexium; AstraZeneca (UK), Esofag; Micro Labs (India) and Gordex; Julphar, UAE.

Preparation of samples

Ascorbic acid was used as control and prepared as 5 mg/ml stock solution in water. All studied samples of esomeprazole were prepared as 1 mg/ml in water. To explore the scavenging activity, serial of dilutions of the control and each studied brand were freshly prepared at 5, 7.5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μg/ml.

Estimation of scavenging activity

Due to the delocalisation of a spare electron over the molecule, the DPPH radical is stable and thus prevents the formation of dimer. Such radical is used for quantifying the ability of antioxidants to quench the DPPH radical in the DPPH radical scavenging potential assay. DPPH's dark purple colour will be diminished and converted to yellow, accompanied with decreasing sample's absorbance at 517

nm when agents that have antioxidant potential are present in the sample. Therefore, in this study esomeprazole antioxidant activity was analysed using DPPH-assay process for scavenging of free radicals.[12,13] In short, for the preparation of 0.1 mM solution, DPPH powder was dissolved in 95% methanol. After which, 1 mL of DPPH solution was added to 3 ml of each diluted drug at various concentrations to produce a mixture of the studied sample and DPPH. At room temperature, the mixture was vigorously shaken and incubated in the dark for 30 min, and the absorbance was determined against a blank by spectrophotometer (UV-VIS Shimadzu) at 517 nm. Vitamin C has been used as a standard compound for comparison. Each experiment was performed in triplicate and the percentage of scavenging activity of each drug for radicals was calculated using the equation below:

*Percentage of radicals scavenging activity (% RCA)= [(Absorbance of control – Absorbance of sample) / Absorbance of control]*100*

For each studied sample, the levels of IC_{50} (μg/mL) were determined, reflecting the appropriate concentration of the compound necessary to produce 50 percent scavenging activity for the DPPH radicals relative to the reference agent. The lowest the reaction mixture absorbance, the greater the scavenging ability for free radicals.

To express antioxidant activity of the studied brands as mg of ascorbic acid "or called, ascorbic acid equivalent antioxidant capacity (AEAC)", the reduction in absorbance was expressed as mg of ascorbic acid equivalents/100 gm of fresh solution of each sample and calculated by using the equation below:

AEAC (mg AA/100 gm) = IC_{50} (AA) / IC_{50} (S)10^5*

Where "AA" stands for ascorbic acid, and S is the sample

Statistical analysis

d tests were performed in triplicate for each studied brand in addition to the reference substance. The results data are represented as mean ± standard deviation (SD). The paired t-test from the student has been used to evaluate the antioxidant potential of each brand as compared to control where indicated. Differences at $P < 0.05$ were deemed statistically significant. One-way analysis of variance (ANOVA) was used for multiple AEAC statistical comparisons, and the significance of the variation among means was calculated as indicated by Tukey's post hoc test. Difference was found statistically significant at $P < 0.05$. The GraphPad Prism 6 program has been utilized for statistical analysis.

RESULTS

The antioxidant potential of different brands of esomeprazole was evaluated based on their capacity to quench the DPPH free radicals when compared to a standard reference agent. The *in vitro* assay of antioxidant capability of various esomeprazole samples revealed the presence of antioxidant potential at a varying degree.

The IC_{50} (50% inhibitory concentration) values of each of the studied brands are illustrated in table 1. The obtained data reveals that the IC_{50} for different esomeprazole samples fluctuates between 14.5 μg/ml and 21.7 μg/ml, where pure esomeprazole showed the lowest IC_{50} in comparison to the rest of the studied samples. However, Nexium and Gordex showed IC_{50} values of 15.5 and 15.6 μg/ml respectively, which are also statistically comparable to vitamin C with no significant difference. The only exception is Esofag that showed IC_{50} value of 21.7 μg/ml, which is significantly lower than the reference substance. The percentage of inhibition of free radicals was detected in all of the studied samples, however at different free radicals scavenging potential in a concentration-dependent means up to the given concentration. This variability in scavenging potential reflects difference in antioxidant potential of the studied samples. In spite of this, all of the studied brands showed a maximum value of scavenging activity at 100 μg/ml (figure 1). Various brands of esomeprazole exhibited different scavenging activity in comparison to the reference agent (vitamin C) in concentration-dependent manner, where Gordex started to be comparable to vitamin C at 10 μg/ml and reached the plateau at concentrations between 30-40 μg/ml. However the rest of the studied compounds; pure esomeprazole, Esofag an Nexium were comparable to standard at 30-40 μg/ml and reached their maximum scavenging activity for DPPH free radicals at higher concentrations (figure 1).

To confirm the previous results, calculation of ascorbic acid equivalent antioxidant capacity (AEAC) of the studied esomeprazole brands was performed and the results pointed that the ACAE values for pure esomeprazole, Gordex and Nexium (100908±33380, 85955±9432 and 87527±11337 mg AA/100 g respectively) were comparable to ascorbic acid. On the other hand, Esofag ACAE value (63836±19069 mg AA/100 g) revealed different result, which is significantly lower than vitamin C, (figure 2). This indicates that the pure esomeprazole, Gordex and Nexium have higher free radical scavenging potential and antioxidant effect in comparison to Esofag.

DISCUSSION

The unravelling of the differences in the antioxidant effect of PPIs will provide researchers with potential new guide on the preferences of choosing the right compounds for treating acid related diseases. Our current research showed that there is also a difference in antioxidant activity between different generic forms of esomeprazole, which proved to have the highest antioxidant activity among other proton pump inhibitors tested in our previous study.[1] This ability of the generic forms of esomeprazole to inhibit the DPPH radical to different extents may indicate a potential interaction between the drug and the excipients.

Pharmaceutical companies utilize different kinds of excipients to ensure a good product quality with reasonable price. Both physician's and patient's choices of a certain pharmaceutical product depend on many factors, of which the patient estimated benefit from the product being the most potent. Practically, comparisons between two or more generic products of the same drug is performed through bioavailability studies; either relative or absolute, and the products are assumed to be bioequivalent if the plasma concentration time curves of the tested and the reference products do not differ significantly, such studies do not

focus on the effect of various other factors on the pharmacological activity of the drug in question.[14]

The antioxidant effect of a specific excipient cannot explain by itself the observed differences in the total antioxidant outcome of a generic esomeprazole product; the ratio of the excipient to esomeprazole in most pharmaceutical products is relatively high, which supports the idea about the importance of such additives and their interaction with the principal compound(s) especially in modern pharmaceutical products.[15] Excipients were usually regarded as inert essences in pharmaceutical preparations which are in general untruthful, the impact of excipients on medicinal products was traditionally underestimated.[16] Pharmaceutical industry fields are of continuous need for new excipients to placate the requirements of the countless types of formulations and the great need of the modern production machines. The presence of reactive functional groups in the basic structure of various excipients used in the pharmaceutical preparations could play a crucial part in the interaction between the excipient and the active ingredient both physically and chemically, such an interaction may have a detrimental effect on the therapeutic action of the drug especially if the interaction is not thoroughly analysed.[15]

Based on this scenario, the antioxidant activity of the tested products, which are well-documented gastric acid suppressants, was assayed according to their ability for scavenging DPPH radical. The method utilizes the fact that antioxidant effect could be measured directly by quantifying the scavenging capacity of the investigated product for DPPH free radical. Analysing the antioxidant potential of the tested products was performed by estimation of their IC_{50} values for their scavenging of free radicals and comparing the results with a reference compound, in addition to expression of the antioxidant activity of the studied compounds as mg of ascorbic acid; the ascorbic acid equivalent antioxidant capacity (AEAC). The proposed masking potential for the antioxidant activity by excipient in one of the studied esomeprazole products could be explained by mechanisms alike modification of the microenvironment required for the optimum action of the antioxidants by one of the excipients, or production of free radicals by a weak antioxidant which lead to oxidation of the primary antioxidant as well as the possibility of formation of less active antioxidant by the more powerful antioxidant.[17] Therefore, based on the results of this study, analysis of the final antioxidant outcome of esomeprazole generic products might provide better insight on the overall therapeutic action for peptic ulcer patients as the tested products showed different antioxidant activities.

The significance of this study depends on the documented role of free radicals and reactive oxygen species in the pathogenesis of peptic ulceration, and when a drug has the potential to quench the free radicals, in addition to its acid suppression properties, this could provide a valuable option for health care professionals to manage this disease. However, most studies compare between different drugs of the same class and do not pay a proper attention to variation in the pharmacological effect of the different brands of the same drug. So, this study provides the first ever comparison between the antioxidant capacities of different esomeprazole brands available in community pharmacies. Still the present study did not reveal the exact hindering role for each excipient present in the formulation that showed the least antioxidant potential, instead it just revealed the overall masking effect for the excipients and; therefore, additional *in vitro* and *in vivo* studies are needed to unveil the effect of all excipient on the antioxidant potential, in addition studying the acid suppression properties of this brand in comparison to others. Also, running a survey about the preferred esomeprazole containing product by the doctor/patients is needed to compare the results with the finding of the present study.

In conclusion, different brands of esomeprazole show variable antioxidant activities. Some of these activities are comparable to that of pure esomeprazole and vitamin C and this can be used to take advantage of the added therapeutic benefit from the antioxidant potential in the treatment of peptic ulcer.

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Table 1: The values of IC_{50} (µg/ml) of Vitamin C and different studied esomeprazole samples.

Drug	IC_{50} (µg/ml)
Vitamin C	13.4±2.4
Pure esomeprazole	14.5±4.6
Gordex	15.6±2.4
Esofag	21.7±3.6*
Nexium	15.5±3.3

The results are expressed as mean±SD, for 3 different experiments. The results are considered statistically significant (*P < 0.05) where indicated by using paired t-test.

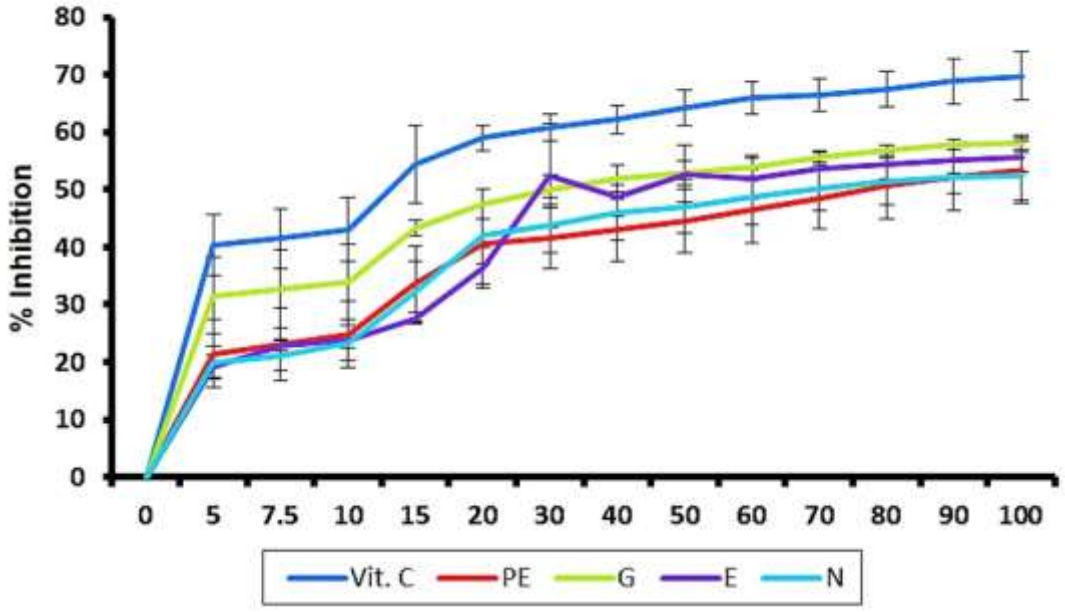

Figure 1: Percentage of free radicals inhibition (percentage of scavenging activity) of Vitamin C and different studied esomeprazole samples via DPPH assay. The results are expressed as mean±SD, n=3. Vit. C=Vitamin C, PE=Pure Esomeprazole, G=Gordex, E=Esofag and N=Nexium. (Column width)

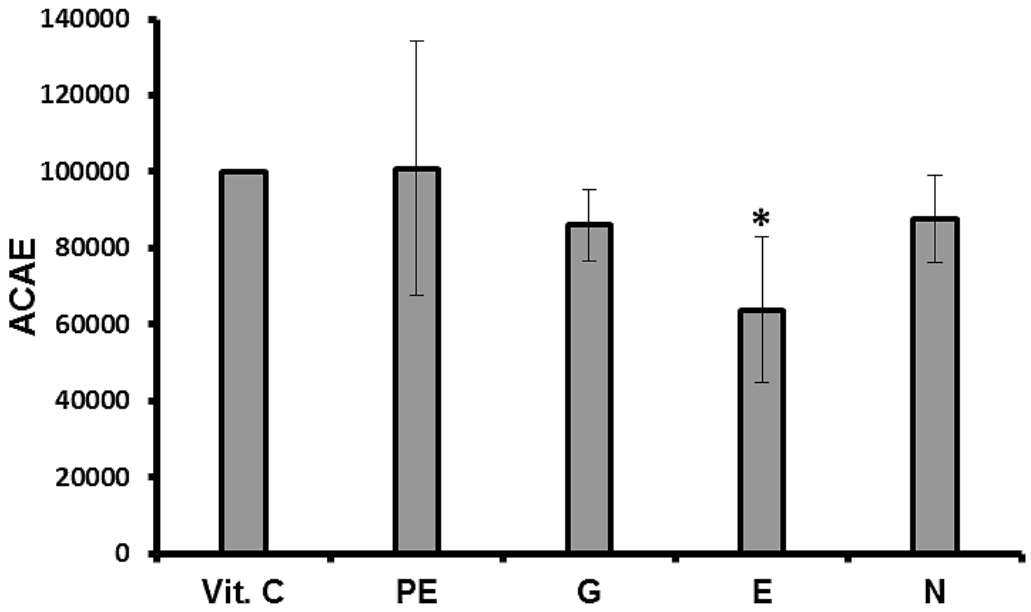

Figure 2: Ascorbic acid equivalent antioxidant capacity (AEAC) of various esomeprazole samples. The results stand for various antioxidant potentials of the samples when presented as mg ascorbic acid (AA) equivalents per 100 g of each drug (AEAC). The results are shown as mean±S.D. for 3 different experiments. Each bar in the figure is significantly different were indicated (*P < 0.05; in comparison to vitamin C). Vit. C=Vitamin C, PE=Pure Esomeprazole, G=Gordex, E=Esofag and N=Nexium. (Column width)