



Combination treatment of lycopene and hesperidin protect experimentally induced ulcer in laboratory rats

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ABSTRACT

Aim: Lycopene, a carotenoid and hesperidin, a flavonoid are naturally occurring in vegetables and fruits. Synergistic effect of a combination of carotenoid and flavonoid has been reported due to its antioxidant activity. Therefore, the present study was aimed to evaluate the protective effect of this combination on pylorus ligation induced ulcers in rats. **Materials and Methods:** Thirty Wistar albino rats were divided into five groups ($n = 6$). Rats were fasted for 24 h before pylorus ligation. After 24 h of fasting the rats were treated with hesperidin (100 mg/kg) and lycopene (2 mg/kg) and their combination 1h prior to surgery. After an hour under ether anesthesia pylorus ligation was performed, after 5 h the animals were sacrificed, stomach was dissected, and gastric contents were collected and measured. Total acidity and pH of gastric content was estimated. Ulcer index was calculated, and *macroscopic* examination of the stomach was carried out. **Results:** The sham operated rats showed a significant increase in pH, volume of gastric content and total acidity and ulcer index. The rats pretreated with lycopene and hesperidin showed significant improvement in the ulcer conditions. However, rats treated with a combination of lycopene and hesperidin showed more significant restoration of gastric function as compared to sham operated rats. Moreover, a significant difference was also noted in rats treated with a combination as compared to lycopene and hesperidin treatment alone. **Conclusion:** Thus experimentally the combination was seen to treat ulcers by anti-secretory, neutralizing, cytoprotective and mainly due to its antioxidant property.

KEY WORDS: Hesperidin, lycopene, pylorus ligation, ulcer index

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INTRODUCTION

Peptic ulcer has been recognized as the most important problem in developing countries. Even though the etiology of ulcers is still debated, it is accepted that ulcers are caused due to imbalances between the mucosal defensive and aggressive factors [1]. The incidence of ulcer has shown a significant increase in the past decade. According to the recent survey it shows that peptic ulcer disease remains a relatively common condition worldwide, with annual incidence ranging from 0.10% to 0.19% diagnosed by physician and 0.03-0.17% diagnosed during hospitalization [2]. Traditional treatment options available are mucoprotectives, antacids, alginates, motility stimulants, and acid suppressants and anti-reflux surgery is done in severe cases [3]. In spite of this, the rational therapy of ulcers is elusive and research advances in search for more potent drugs. Since ulcer is a disease that widely affects the population daily and if not treated can be fatal; studying it and finding a solution for the same is the need of the hour. It has become a matter of grave concern and hence the world shifts towards the safe and efficacious herbal drugs.

The present drug lycopene is a carotenoid and is a constituent of many fruits and vegetables [4]. It has been scientifically reported as an antioxidant [5], hepatoprotective [6], anti-

inflammatory [7], anti-diabetic [5], anti-proliferative [8] and anti-cataract [9]. Hesperidin is a bioflavonone glycoside found abundantly in citrus fruits and reported to possess antioxidant [10], anticonvulsive [11], hypoglycemic [12], sedative [13] and anti-cancer [14] activity. Moreover, several studies show the synergistic antioxidant activities of carotenoids and bioflavonoids [15,16]. The synergistic effects of the two components have a concatenate effect together, but may have only a fraction of efficacy when assessed individually.

Therefore, the aim of the present was designed to study the combination treatment of hesperidin and lycopene for antiulcer activity in laboratory rats.

MATERIALS AND METHODS

Animals

Thirty Wistar albino rats of either sex weighing 200-250 g were obtained from National Institute of Biosciences, Pune. The animals were acclimatized at standard laboratory conditions maintained at temperature $23 \pm 2^\circ\text{C}$ with relative humidity $55 \pm 10\%$ and 12 h light and dark cycles. Animals had free access

to water and laboratory feed. The animals were deprived of food 24 h before the study. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC).

Drugs and Chemicals

Hesperidin was obtained as a gift sample from NANS Products (Mumbai, India) and lycopene from Zedip Formulations (Ahemdabad, India). All other chemicals and reagents were purchased from local supplier of Pune.

Experimental Design

Wistar albino rats were divided into five groups ($n = 6$). The animals were fasted for 24 h with water ad libitum before pylorus ligation [17]. To prevent cannibalism and coprophagy they were housed singly in cages with raised bottoms of wide wire mesh. After 24 h of fasting the rats were treated with hesperidin and lycopene 1 h prior to surgery. Group I rats received a vehicle (2% gum acacia solution) at a dose of 10 ml/kg. Group II and Group III rats were treated with hesperidin and lycopene at a dose of a 100 mg/kg and 2 mg/kg; respectively. Group IV were treated with a combined dose of lycopene (2 mg/kg) and hesperidin (100 mg/kg). Group V rats were administered with a standard drug omeprazole at a dose of 10 mg/kg. Then after an hour under ether anesthesia one inch midline abdominal incision was given below the xiphoid process. The pylorus was then carefully lifted and ligated without damaging its blood supply. The stomach was replaced, and the abdominal wall was sutured. After 5 h of ligation, the animals were sacrificed, and the stomach was dissected out. The contents of the stomach were drained by cutting it through the greater curvature. The gastric contents were then centrifuged at 3000 rpm for 10 min and the supernatant was collected and measured.

Estimation of Gastric pH

A volume of 1 ml of the gastric juice was diluted with 1 ml of distilled water and the pH of the solution was measured using a pH meter (EQUIPTRONICS - EQ-614) [18].

Estimation of Total Acidity

An aliquot of 1 ml of gastric juice diluted with 1 ml distilled water was taken in a conical flask and titrated against 0.01N NaOH with phenolphthalein as an indicator till a permanent pink color is obtained [19]. The volume of NaOH was then noted. The total acidity expressed as mEq/L was then calculated by the following formula:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times N \times 100 \text{mEq/L}}{0.1}$$

Ulcer Index

The stomach mounted was then scored from 0 to 3 on the basis of severity as normal colored stomach (0); red coloration (0.5); spot ulcer (1); hemorrhagic streak (1.5); deep ulcers (2)

and perforations (3). Mean ulcer score for each animal will be expressed as ulcer index [20].

$$U_I = U_N + U_S + U_p \times 10^{-1}$$

Where, U_I = Ulcer index; U_N = Average number of ulcers per animal; U_S = Average number of severity score;

U_p = Percentage of animals with ulcer.

Statistical Analysis

All the data were expressed as Mean \pm standard error of the mean. The statistical analysis was done by one-way ANOVA, followed by Tukey's test. $P < 0.05$ was considered as minimum level of significance.

RESULT

Effect of Combination Treatment of Lycopene and Hesperidin on Gastric Content, Total Acidity and pH

As shown in Table 1, a significant reduction in gastric content and total acidity as well as increase in gastric pH was observed in rats treated with lycopene ($P < 0.05$, $P < 0.001$ and $P < 0.01$; respectively) and hesperidin ($P < 0.01$, $P < 0.001$ and $P < 0.001$; respectively) as compared to control rats. Further, the rats treated with the combination of hesperidin and lycopene showed more significant results ($P < 0.001$, $P < 0.001$ and $P < 0.001$) compared to control rats. Moreover, significant reduction in gastric content and total acidity as well as increase in pH was observed in combination treated rats when compared to lycopene treated rats ($P < 0.01$, $P < 0.001$ and $P < 0.001$; respectively), but as compared to hesperidin treatment it shows the significant improvement only in gastric content and total acidity ($P < 0.05$ and $P < 0.01$; respectively). No significant difference was found in combination treated rats as compared to omeprazole treatment.

Effect of Combination Treatment of Lycopene and Hesperidin on Ulcer Index

Rats treated with individual doses of lycopene and hesperidin significantly decreased the ulcer index (2.21 and 2.00 vs. 3.917;

Table 1: Effect of combination treatment of lycopene and hesperidin on gastric content, total acidity and pH

Group	Gastric contents (ml)	Total acidity (mEq/L)	pH
Control	9.46 \pm 0.33	78.36 \pm 0.77	2.46 \pm 0.15
Lycopene (2 mg/kg)	7.02 \pm 0.81*	67.18 \pm 1.13 [ⓐ]	4.18 \pm 0.27 [#]
Hesperidin (100 mg/kg)	6.42 \pm 0.45 [#]	59.83 \pm 1.19 [ⓐ]	5.40 \pm 0.40 [ⓐ]
Lycopene (2 mg/kg)+ Hesperidin (100 mg/kg)	4.36 \pm 0.20 ^{ⓐ,Ⓢ}	51.17 \pm 2.57 ^{ⓐ,Ⓢ,^}	6.23 \pm 0.31 ^{ⓐ,Ⓢ}
Omeprazole (10 mg/kg)	3.27 \pm 0.32 [ⓐ]	47.33 \pm 1.89 [Ⓢ]	5.91 \pm 0.26 [ⓐ]

* $P < 0.05$, [#] $P < 0.01$, [ⓐ] $P < 0.001$ versus control, [ⓐ] $P < 0.01$, [Ⓢ] $P < 0.001$ versus Lycopene (2 mg/kg), [Ⓢ] $P < 0.05$, [^] $P < 0.01$ versus Hesperidin (100 mg/kg)

$P < 0.05$) as compared to control rats. Rats treated combination of lycopene and hesperidin showed more significant reduction in ulcer index compared to control rats ($P < 0.01$) [Figure 1].

Effect of Combination Treatment of Lycopene and Hesperidin on Macroscopic Changes

As shown in Figure 2, marked deep ulcers with perforations were observed in the stomach of control rats (A). However, lycopene (B) and hesperidin (C) treatment at individual doses reduced the severity of the ulcers with mild red coloration and spot ulcers. The perforations and hemorrhagic streaks were not observed in the rats treated with the combined dose of lycopene and hesperidin (D). The omeprazole treated rats were also found to ameliorate these macrophagic changes (E).

DISCUSSION

Peptic ulcer is considered as one of the modern age epidemics which causes a high rate of morbidity particularly in non-

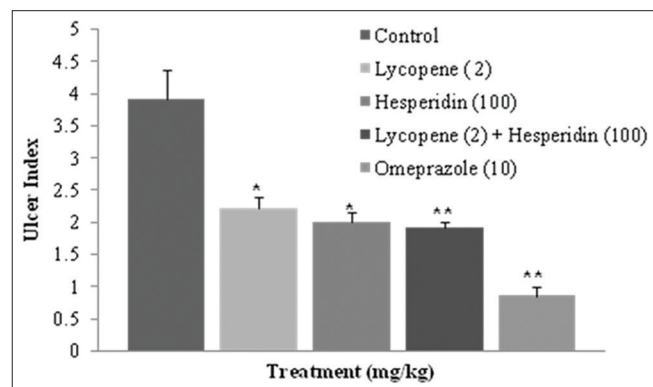


Figure 1: Effect of combination treatment of lycopene and hesperidin on Ulcer index. * $P < 0.05$ and ** $P < 0.01$ versus control

industrialized nations. As reported previously, it is mainly caused due to the imbalance between aggressive and defensive factors [1]. The deleterious effects of smoking and the excessive use of non-steroidal anti-inflammatory drugs inducing ulcers have also been reported. Stress is another factor that triggers the formation of ulcers [21]. The current treatment of ulcers is the “Triple therapy.” This makes the use of a proton pump inhibitor or H₂ antagonist (reduce gastric acid secretion) combined with either two different antibiotics or an antibiotic and a bismuth salicylate [3]. However, these drugs produce several side effects like gynecomastia [22], thrombocytopenia [23], nephrotoxicity and hepatotoxicity [24]. To overcome these side effects and provide a safe and efficacious therapy, a paradigm shift to natural herbal remedies is necessary, and the combination of carotenoids and bioflavonoids is one of such approaches.

In the present study, the pylorus ligation model was used. Pylorus ligation induced ulcer model is mainly used to check the effect of drugs on gastric secretions. Pylorus is the end portion of the stomach after which the duodenum of the small intestine begins. Due to the ligation of the pylorus, there is formation of stress conditions in the membrane. Stress generated can impair the ion transport system thus disturbs the H⁺/K⁺ ATPase pump and hence results in the production of excessive hydrochloric acid that leads to the development of ulcers. Further, stress may also cause inactivation of prostaglandin synthetase leading to decreased biosynthesis of prostaglandin and thereby reduced the thickness of the protective gastric mucosa [25].

The generation of reactive oxygen species (ROS) is another major reaction of stress induced ulcers [25]. Lycopene and hesperidin are powerful antioxidants, scavenge these ROS and prevent lipid peroxidation of the membrane thereby preventing ulcers [16]. In the present study, we have observed the individual dose of lycopene (2 mg/kg) and hesperidin (100 mg/kg) significantly decreases the gastric secretions and total acidity

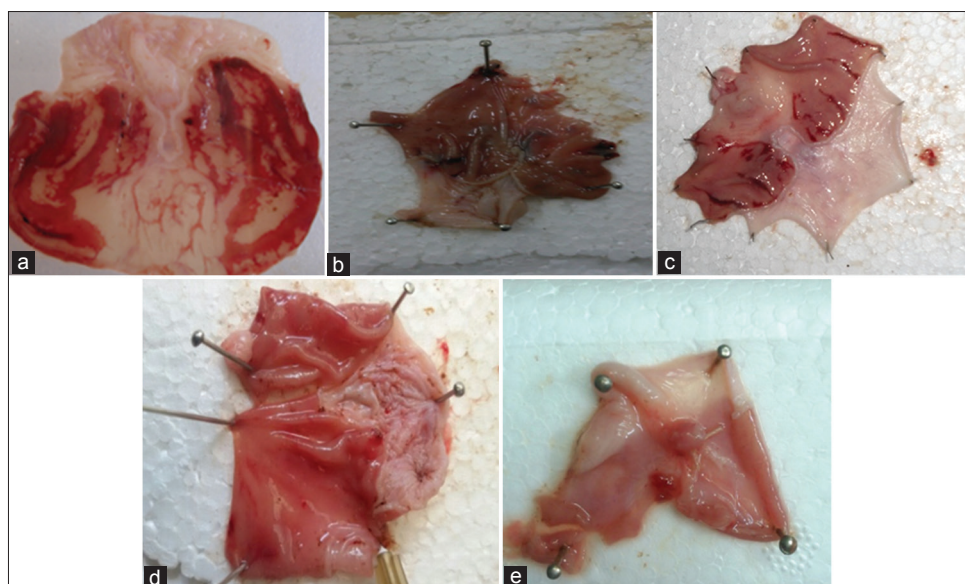


Figure 2: Effect of combination treatment of lycopene and hesperidin on macroscopic changes in the pylorus ligation in rats, a: Control; b: lycopene (2 mg/kg); c: Hesperidin (100 mg/kg); d: hesperidin (100 mg/kg) + lycopene (2 mg/kg) and e: omeprazole (10 mg/kg)

as well as increases gastric pH due to the restoration of the normal gastric acid conditions. Liang *et al.*, reported the possible mechanism for the antioxidant synergism of flavonoids and carotenoids due to their interaction on the basis of their water/lipid partition coefficient as well as fast electron transfer reaction from (iso)flavonoid or anion to carotenoid radical cation [26,27]. This electron transfer is facilitated by the flavonoids. In the agreement of the previous reports, we observed more significant improvement in lycopene and hesperidin combination treated rats when compared to control rats, as well as rats treated with lycopene and hesperidin alone. The individual effects of both the phytochemicals are effective, but they are proven to have a synergistic effect as antioxidants, and hence they potentiate the anti-ulcer effect when combined [15].

Thus, the present study suggests interplay between anti-secretory, cytoprotective, neutralizing and the antioxidant properties of a combination of lycopene and hesperidin.

CONCLUSION

Therefore, in conclusion, our results showed synergism in anti-ulcer activity of lycopene and hesperidin. A pharmacologically and analytically well standardized extracts of lycopene and hesperidin together can be cheap and very effective herbal alternative for the treatment of ulcers.

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