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Comparative effect of palm vitamin E and ranitidine on the healing of ethanol-induced gastric lesions in rats

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Abstract

The effect of palm vitamin E on the healing of ethanol-induced gastric lesion was compared with ranitidine. Fifty-six male rats of Sprague-Dawley species (200–250 g of weight) were randomly divided into three groups ($N = 14$). Gastric mucosal injury was induced by orogastric tube administration of 0.5 ml 100% ethanol. Immediately after induction, Group I (k) rats was fed with a normal diet (control), group II (p) was fed palm vitamin E enriched diet (150 mg/kg food), Group III(r) was treated with ranitidine 30 mg/kg body weight intraperitoneally and Group IV (p + r) was fed with palm vitamin E and treated with ranitidine 30 mg/kg body weight intraperitoneally of the same dose. The rats were killed at the end of 1 week and 3 weeks of treatment or feeding. The rate of gastric healing was faster in palm vitamin E treated group compared to control and ranitidine treated groups as shown by a lower mean ulcer index. The effect was seen as early as the first week of treatment whereas ranitidine did not show any healing effect even after 3 weeks of therapy. Neither gastric acidity nor gastric mucus production are involved in gastroprotective effect of palm vitamin E. The most probable mechanism is via reducing lipid peroxidation process as shown by a significant decrease in gastric MDA

Keywords: palm vitamin E, ranitidine, ethanol, gastric lesion, rat

Free radicals are involved in several pathological processes such as inflammation and tissue damage caused by chemicals including gastric mucosal injury induced by nonsteroidal anti-inflammatory agents or alcohol (Yoshikawa *et al*. 1993; Tuwaijri & Dhohyan 1995; Pihan *et al*. 1987). Alpha tocopherol (vitamin E) is a naturally occurring antioxidant in the biological system existing in the cell membranes of various tissues. It prevents free radical induced injury by blocking the free radical chain reaction. Palm vitamin E is a vitamin E concentrate derived from palm oil. It contains 21.9% tocopherol and 78.1% tocotrienol (Gapor *et al*. 1989; Cottrell 1991) which have been proven to have a better antioxidant property compared to conventional vitamin E (α–tocopherol) (Packer & Serbinova 1994). It has been reported that vitamin E supplemented diet prevents the formation of gastric ulcers in rats (Pihan *et al*. 1987; Yoshikawa *et al*. 1993; Tuwaijri & Dhohyan 1995). Palm vitamin E being a better antioxidant should have a similar if not better effect on ethanol induced gastric lesion in rats. Our earlier study (Kamsiah *et al*. 1996) have reported that the mean ulcer index at 1 week post ethanol injury was significantly lower in the rats fed with palm vitamin E 3 weeks before ethanol exposure.

Our finding suggests that palm vitamin E appears to promote healing of ethanol induced gastric lesions. However, the mechanism of how vitamin E promotes ulcer healing is not yet established. Gastric mucous injury is associated with various factors which include free radicals, hyperacidity, weakening of mucosal protective barrier like decreasing in mucus production or inhibition of prostaglandin E secretion, etc. It is possible that the effect of palm vitamin E on ulcer healing may be mediated via its effect on gastric acid secretion, free radicals formation or activation of mucosal protective barriers like increasing mucus production. The present study was carried out to determine whether palm vitamin E heals gastric mucosal injury compared to ranitidine, the conventional anti ulcer agent. This study also investigated the possible mechanisms of palm vitamin E promoted healing of ethanol induced gastric lesions.

Materials and methods

The study was divided into two phases. In the 1st phase of the study, 56 male rats of Sprague-Dawley species (200–250 gm of weight) were randomly divided into four groups (n = 14). Gastric mucosal injury was induced by giving 0.5 ml 100% ethanol by an orogastric tube. Immediately after induction, Group I (k) rats was fed with a normal diet (control), group II (p) was fed palm vitamin E enriched diet (150 mg/kg food), Group III(r) was treated with ranitidine 30 mg/kg body weight intraperitoneally and Group IV (p + r) was fed with Palm vitamin E enriched diet and treated with the same dose of ranitidine. The rats were killed after 1 week of feeding. Mean ulcer index, gastric tissue contents of malondialdehyde (MDA), gastric acid and mucus were measured. The second phase of the study was similar to the 1st phase study. We used similar groups of animals given similar diet and treatment except that the rats were killed after 3 weeks of feeding or treatment. The same parameters as 1st phase were repeated. The lower end oesophagus and pylorus were clamped and the stomach was removed. Samples of gastric juice were collected and centrifuged at 3000 r.p.m. for 10 min. Aliquots of each sample were titrated with 0.01N NaOH to a pH of 7.0.The hydrogen ion concentration was calculated as described by (Shay *et al.* 1954). The gastric mucous was exposed by cutting the stomach along the greater curvature, washed with saline and laid on a flat wooden board. The severity of gastric mucosal lesions expressed as ulcer index was determined semiquantitatively as described by Berry *et al.* (1988) which were graded as follows: 5 = multiple ulcers following almost entire length of gastric fold, 4 = lesions which followed approximately 80% of the folds, 3 = ulcers 1–4 mm in length on 80% of the folds, 2 = at least 2 ulcers approximately 2 mm in length, 1 = the presence of 1 ulcer and generalized erythema and 0 = no visible damage.

The content of MDA in the stomach was determined using the method described by Ledwozyw *et al.* (1986). Gastric mucus was quantitatively measured as described by Corne *et al.* (1974). In this technique the Alcian blue which binds specifically to mucus was used. The Glandular portion of stomach was isolated and immersed in the Alcian blue solution (10 ml 0.1% weight/volume) for 2 h. The unbound Alcian blue was removed from the stomach by washing twice in sucrose solution (0.25 M). After thorough washing, the mucus bound dye in gastric glandular tissue was eluted using 10 ml magnesium chloride solution (0.5 M). The tissue was soaked in the solution for 30 min. After 30 min of soaking, the mixtures were shaken vigorously for 1 minute. The procedure was repeated four times, following which 4 ml of the solution was taken and mixed with 4 ml of diethylether and vortexed vigorously. The emulsion was then centrifuged at 3600 r.p.m. for 10 min. The absorbance of the aqueous phase of the solution was measured using spectrophotometer at 605 nm.

Statistics

Data are expressed as mean ± SEM Statistical significance ($P < 0.05$) was determined by ANOVA followed by student's t-test.

Results

Effect of palm vitamin E on mean ulcer index

The mean ulcer index of group (p) and (p + r) sacrificed at 1 week post ethanol exposure was significantly lower (0.36 ± 0.14, 0.79 ± 0.24 $P < 0.05$) compared to control (1.64 ± 0.39), and ranitidine (1.36 ± 0.32) treated groups. However there was no significant difference in mean ulcer index between the group (k) and (r) in the rats killed at 1 week after ethanol exposure (1.64 ± 0.39 *vs*. 1.36 ± 0.32) (Figure 1).

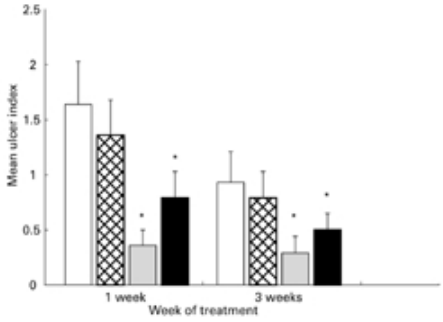

Figure 1

Effect of palm vitamin E on mean ulcer index. □ Control (k); ▨ Ranitidine (r); ■ Palm vitamin E (p); ▪ Palm vitamin E and ratinidine (p + r). *$P < 0.05$; compared to control and ranitidine group

The overall mean ulcer index observed at 3 weeks post ethanol injury was lower compared to mean ulcer index at 1 week post ethanol injury for all the groups (Figure 1). The mean ulcer index at 3 weeks was again significantly lower in group (p) and group (p + r) compared to control (0.29 ± 0.15, 0.5 ± 0.26 *vs*. 0.93 ± 0.28). However there was no significant difference in the mean ulcer index in group(r) compared to control (k).

Effect of palm vitamin E on gastric acid concentration

There was no significant difference in gastric acid secretion between the control (k); palm vitamin E (p), ranitidine(r) and palm vitamin E plus ranitidine (p + r) treated groups killed at 1 week after exposure to ethanol (Figure 2).

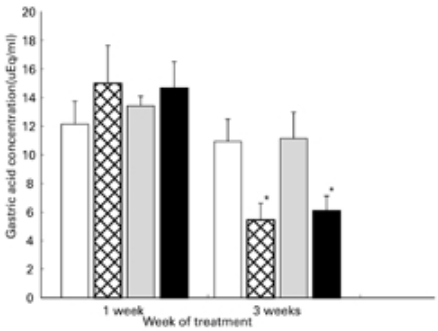

Figure 2

Effect of palm vitamin E on gastric acid concentration. □ Control (k); ▨ Ranitidine (r); ■ Palm vitamin E (p); ▪ Palm vitamin E and ratinidine (p + r). *$P < 0.05$; compared to control and Palm vitamin E group

After 3 weeks of treatment the gastric acid concentration decreased significantly ($P < 0.05$) in the ranitidine(r) and ranitidine plus vitamin E (p + r) groups compared to control (k) and palm vitamin E (p) groups. However there was no significant difference in gastric acid concentration in palm vitamin E group (p) compared to control (k).

Effect of palm vitamin E on gastric malondialdehyde (MDA) content

The gastric MDA content was significantly lower in rats fed with palm vitamin E (p) and palm vitamin E plus ranitidine (p + r) compared to control (k) and ranitidine (r) treated groups at the end of 1 week and 3 weeks of treatment (Figure 3). However there was no significant difference in gastric MDA content between the control (k) and ranitidine (r) treated groups (Figure 3).

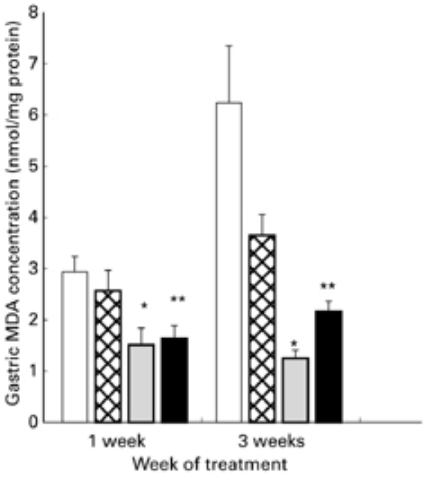

Figure 3

Effect of palm vitamin E on gastric malondialdehyde (MDA). □ Control (k); ▨ Ranitidine (r); ▪ Palm vitamin E (p); ▪ Palm vitamin E and ratinidine (p + r). *$P < 0.05$; compared to control and ranitidine group

Effect of palm vitamin E on gastric mucus concentration

There was no significant difference in gastric mucus concentration among all the treated groups after 1 week and 3 weeks of treatment (Figure 4).

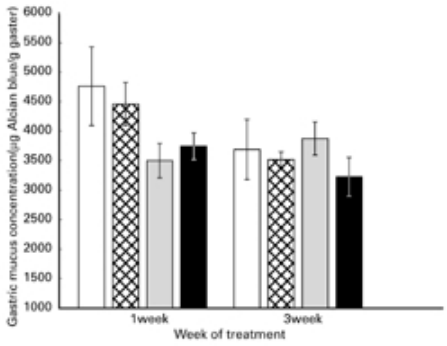

Figure 4

Effect of palm vitamin E on gastric mucus concentration. □ Control (k); ▨ Ranitidine (r); ▩ Palm vitamin E (p); ▪ Palm vitamin E and ratinidine (p + r).

Discussion

The pathogenesis of ethanol induced gastritis is complex and remain poorly understood. Pihan *et al.* (1987) had reported that there was a similarity between Aspirin and ethanol induced gastric lesion in rats. There is substantial evidence to support the claim that reactive oxygen species are involved in gastric injury followed by ethanol and aspirin exposure. In the present study we found that the mean ulcer index obtained after 1 week and 3 weeks of treatment with palm vitamin E and palm vitamin E Plus ranithidine were significantly lower compared to control and group treated with ranitidine alone. This finding suggests that palm vitamin E given alone or in combination with ranitidine is able to promote the healing of ethanol induced gastric lesions. The magnitude of reduction in the mean ulcer index appear to be greater during the initial stage of treatment as shown by a greater reduction in mean ulcer index at 1 week (78%) compared to 3 weeks (68%) of treatment. The reason for these differences was not clear. There is a possibility that gastric lesion develop over a course of a week and is maximal between 1 and 2 week after which the natural healing will take place irrespective of treatment (Anthony *et al.* 1995). The effect of ranitidine on ethanol induced gastric injury was not seen at 1 week as well as at 3 weeks of treatment. The reason for inability of ranitidine to reduce ulcer index in this study did not surprise us as it takes four to six weeks for ranitidine to heal peptic ulcer in human. We assume that ranitidine may have an effect on ulcer index if the duration of the study was extended to a longer period of times such as 4–6 weeks. The lower mean ulcer index observed in all groups at 3 weeks of treatment compared to 1 week post treatment indicated that the healing of ethanol induced gastric injury must have taken placed after 3 weeks of injury. This finding was in agreement with the finding of Anthony *et al.* 1995.The rates of healing seem to be faster in the presence of palm vitamin E. The ulcer healing which occurred in the absence of a significant difference in gastric acid and mucous secretion suggest that the healing effect of palm vitamin E was not mediated by a reduction in gastric acid secretion neither it by interfering with mucous secretion. Palm vitamin E reduced lipid peroxidation as reflected by a significant reduction in gastric MDA content. These finding suggest that gastroprotection of palm vitamin E may be mediated by reduction of lipid peroxidation process induced by ethanol.

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