Palm vitamin E and the healing of ethanol-induced gastric lesions

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The main focus of the study was to examine the effect of palm vitamin E (a tocotrienol-enriched fraction of palm oil) on the healing of ethanol-induced gastric mucosal lesions. The study was divided into three sections. Study 1 determined the gastric content of vitamin E after dietary supplementation with palm vitamin E for 3 weeks. Seven rats were fed a normal diet and another 7 were fed a palm vitamin E-enriched diet (150 mg/kg food). The gastric content of vitamin E levels were higher in rats fed with a palm vitamin E-enriched diet ($P < 0.01$). Study 2 determined the time-dependent effects of palm vitamin E on gastric lesions and gastric acidity postethanol administration. Two groups of rats were fed either a normal rat diet or a palm vitamin E-enriched diet (150 mg/kg food). After 3 weeks, the control and a treated group received a single intragastric dose of 100% ethanol. Assessment of gastric lesions after 1 week showed a lower gastric lesion index in the palm vitamin E group compared with the controls ($P < 0.05$) but there was no difference in the gastric acid content after 1 week between the two groups. Study 3 determined the effects of palm vitamin E on the gastric tissue content of malondialdehyde (MDA), PGE, and gastric acidity without ethanol administration. The MDA content was lower in the palm vitamin E-treated group ($P < 0.05$). However, the gastric acid and PGE content in both groups did not differ. The findings suggest that feeding with a palm vitamin E-enriched diet (150 mg/kg food) for 3 weeks resulted in a significant concentration of vitamin E in the gastric tissue. It was concluded that palm vitamin E may promote the healing of ethanol-induced gastric lesions through minimizing the lipid preoccupation process in the gastric mucous.

Key words: palm vitamin E, gastric lesions, gastric mucous.

Introduction

The mechanism of gastric mucosal protection against the variety of insults by endogenous and exogenous agents is a complex one and appears to be multicomponential. The mucosal components to which this protective function is most often assigned are the surface mucus, bicarbonate zone, prostaglandin content, the cell membranes of gastric epithelium, and the mucosal blood flow.1,2

Administration of 100% ethanol (0.5 mL/200–250 g) in rats has previously been shown to produce acute gastric mucosal injury. Free radicals have been implicated in the development of mucosal damage induced by ethanol.4 Previous studies have shown that deficiencies in vitamin E have resulted in peptic ulceration and that vitamin E supplementation to the diet has a protective effect on the gastric mucosa, especially from ethanol-induced lesions.8,9 Although there have been reports that vitamin E decreases gastric mucosal prostaglandin,10 there are others which have attributed the protective effects of vitamin E to increased prostaglandin and glutathion synthesis.8 Vitamin E (tocopherol) has been shown to prevent the formation of experimental gastric ulcers, achieved through decreasing free radicals and thus minimizing lipid peroxidation.11

Palm vitamin E contains approximately 20% tocopherol and 80% tocotrienol.12 In 1996, Afaf and Appleqvist13 showed tocotrienol to be a more potent antioxidant compared with tocopherol. In view of this, it was of interest to us to determine the effects of palm vitamin E on gastric lesions and some other factors that are important in the healing of gastric lesions such as prostaglandin, gastric acidity and lipid peroxidation.

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Accepted 3 May 1999
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Materials and methods

Animals and diet
This study used Sprague-Dawley male rats weighing between 200 and 250 g. The rats were fed either a commercially prepared normal rat diet or an identical basic diet enriched with palm vitamin E for 3 weeks. The compositions of the normal diet are shown in Table 1. The total vitamin E content in the basic diet was 25.2 mg/kg food (unpublished data). Animals were housed in cages with wide mesh wire bottoms to prevent coprophagy. This study was approved by the ethics committee of our institution.

The palm vitamin E enrichment was in the dose of 150 mg/kg rat pellet. The diet was prepared by dissolving 150 mg of palm vitamin E in a sufficient amount of acetone, pouring it over 1 kg of rat pellet and allowing the acetone to evaporate. The normal rat pellets were treated with acetone only.

Study design
The experimental work was divided into three studies: Study 1, Study 2 and Study 3.

Study 1: Determination of gastric tissue content of total vitamin E after 3 weeks of dietary supplementation with palm vitamin E. The aim of this study was to determine the concentration of total vitamin E content in the gastric tissue after 3 weeks of feeding with dietary supplementation of 150 mg of palm vitamin E per 1 kg of commercially prepared normal rat diet. Fourteen rats were divided into two groups; seven were fed a normal diet and another seven were fed with the palm vitamin E-enriched diet. After 3 weeks of feeding, the rats were killed. The isolated stomach was immediately immersed in liquid nitrogen and thereafter stored at –70°C until the time of analysis. The sample preparation and the analysis of vitamin E was performed using high-performance liquid chromatography (HPLC) according to a method described by Lang et al.14 The HPLC system used was Gilson with a fluorescent detector (Gilson Medical Electronics, Inc., Villiers le Bel, France). Vitamin E was detected at an excitation wave of 294 nm and an emission wave of 330 nm. The concentration of vitamin E was determined using a standard curve.

Study 2: Determination of time-dependent effects of palm vitamin E on gastric lesions and gastric acidity postethanol administration. The aim of Study 2 was to determine the time-dependent effects of palm vitamin E on gastric acidity and experimentally induced gastric lesions. Forty-two rats were randomized into two groups and received either a normal rat diet (n = 21) or a rat diet enriched with palm vitamin E (150 mg/kg) (n = 21). After 3 weeks of feeding, the rats were challenged with a single dose of 0.5 mL of 100% ethanol administered via an orogastric tube. Rats from each group were sacrificed at intervals of 1 h, 24 h and 1 week postethanol exposure. The lower end of the oesophagus was clamped and the stomach was removed. Samples of gastric juice were collected and centrifuged at 1500 g for 10 min. Aliquots of each sample were titrated with 0.01N NaOH to a pH of 7.0. The concentration of hydrogen ion was calculated as described by Shay et al.15

The gastric mucosa was then exposed by incising the stomach along the greater curvature. The mucosal surface of the stomach was washed with normal saline and laid on a flat wooden board with the mucosal surface facing up. The gastric mucosa was observed for lesions and was graded according to the following scale, previously described but with minor modifications:16 0 = no visible damage; 1 = the presence of one lesion and generalized erythema; 0.5 = the presence of dot haemorrhages; and 0 = no visible damage.

Study 3: Determination of palm vitamin E effects on factors affecting mucosal integrity (without the induction of lesions). The aim of Study 3 was to determine the effects of palm vitamin E on various parameters that are important in maintaining gastric mucosal integrity. Fourteen rats were fed a normal diet with (n = 7) or without (n = 7) palm vitamin E (150 mg/kg food). Gastric lesions were not induced in these groups of rats. After 3 weeks of feeding on the respective diets, gastric acidity and gastric tissue content of malondialdehyde (MDA) and PGE2 were measured.

Measurement of gastric acidity
The measurement of gastric acidity was done according to the method described above in Study 2.

Measurement of prostaglandin E2
Samples of gastric mucosal tissue were prepared for prostaglandin analysis according to the method described by Redfern et al.17 The tissue samples were homogenized in 20 volumes of 100% ethanol using a glass homogenizer on ice. Cold water was added to this mixture to make the concentration of ethanol 15%. The mixture was then centrifuged at 400 g for 10 min. The supernatant obtained was transferred to another tube and 10 mL of acetic acid was added to make a pH of 3.0. The extraction of PGE2 was performed using an Amprep C 18 cartridge (Amersham International, UK) and the content was analysed using a kit (Prostaglandin E2 assay system, code RPA 530; Amersham International).

Measurement of malondialdehyde
Tissue samples weighing 0.2 g from the corpus region were homogenized using a glass homogenizer (Potter S: B Braun, Germany). The content of gastric tissue MDA was then determined using the method described by Ledwozyw et al.18 The gastric tissue content of protein was determined by the

**Table 1. Composition of basic diet (Gold Coin)**

<table>
<thead>
<tr>
<th>Substances</th>
<th>Percentage by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein content</td>
<td>22.0</td>
</tr>
<tr>
<td>Crude fibre content</td>
<td>5.0</td>
</tr>
<tr>
<td>Crude fat content</td>
<td>3.0</td>
</tr>
<tr>
<td>Moisture</td>
<td>13.0</td>
</tr>
<tr>
<td>Ash</td>
<td>8.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.8–1.2</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.6–1.0</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>49</td>
</tr>
</tbody>
</table>

Additives: Vitamin A, D, E, C, K, B12, thiamine, riboflavin, pantothenic acid, niacin, pyridoxine, folic acid, choline and microminerals.
Lowry et al. method and the MDA was expressed in terms of gram protein.\textsuperscript{19}

\textbf{Statistical analysis}

The results obtained were analysed using ANOVA. Student’s \textit{t}-test was used where appropriate and a \( P \) value of less than 0.05 was taken as significant. Results are expressed as mean ± SEM

\textbf{Results}

\textit{Study 1}

\textbf{Dietary supplementation of palm vitamin E.} The rats that received a normal rat diet enriched with palm vitamin E (150 mg/kg) for 3 weeks had a significantly higher content of total vitamin E in the stomach (17.60 ± 3.0 \textit{vs} 13.30 ± 2.40, \( P < 0.01 \)) compared with rats that were fed with a normal diet (Fig. 1).

\textit{Study 2}

\textbf{Effect of palm vitamin E on gastric acidity postethanol administration.} There was no difference in the concentration of gastric acid between the control and treated groups 1 h postethanol administration (14.14 ± 0.83 \textit{vs} 13.25 ± 3.95) (Fig. 2). The concentration of gastric acid decreased significantly in the palm vitamin E-treated group compared with controls 24 h after ethanol exposure (7.5 ± 2.97 \textit{vs} 28.05 ± 0.60). The gastric acid concentration was, however, significantly higher in the group treated with palm vitamin E killed 1 week postethanol exposure compared with the control group (36.0 ± 0.93 \textit{vs} 16.60 ± 2.84).

\textbf{Effect of palm vitamin E on gastric lesions postethanol administration.} The lesion index in rats given a palm vitamin E-enriched diet was not different than that observed in the control group at 1 h and 24 h after ethanol administration (Fig. 3). In both groups, the lesion index observed at 24 h was higher than that observed at 1 h. The lesion indices at 1 h and 24 h in the palm vitamin E group were 21.7% and 16% lower compared with controls.

The lesion index of the palm vitamin E-treated group was significantly lower than the control group after 1 week postethanol exposure (\( P < 0.05 \)) (Fig. 3). In both groups, the lesion index improved at 1 week compared with 24 h postethanol exposure. However, the degree of reduction in the lesion index was bigger in the palm vitamin E-treated group compared with the control group (97% \textit{vs} 87%).

\textit{Study 3}

\textbf{Effects of palm vitamin E on gastric acidity (without lesion induction).} There was no difference in the gastric acid concentration of the palm vitamin E group compared with controls (50.64 ± 9.82 \textit{vs} 58.33 ± 4.80) at the end of the 3-week feeding period.

\textbf{Effects of palm vitamin E on gastric MDA (without lesion induction).} The gastric tissue content of MDA was lower in the palm vitamin E-treated group compared with controls (\( P < 0.05 \)) (Fig. 4). A 38% difference in gastric tissue content of MDA was observed between the groups.

\textbf{Effects of palm vitamin E on gastric PGE\textsubscript{2} content (without lesion induction).} There was no difference in the content of gastric tissue PGE\textsubscript{2} between the palm vitamin E-treated group and the controls.
anti-inflammatory drugs and reperfusion ischemia. We have shown that vitamin E can improve gas-

tric ulcers and inflammation caused by ethanol, non-steroidal anti-inflammatory drugs and reperfusion ischemia.11,20 We found that palm vitamin E in the dose of 150 mg/kg food fed to rats for 3 weeks achieves significantly higher levels of vitamin E compared with controls. In this study, we observed that the lesions in the palm vitamin E-treated group improved significantly after 1 week. The lesions in both groups were comparable at the earlier time intervals. These findings suggest that palm vitamin E can accelerate the healing of gastric lesions. However, it does not appear to be able to prevent the formation of these lesions induced by ethanol.

It is not clear if palm vitamin E can suppress acid secretion. At the end of the feeding period, whether or not gastric lesion was induced with ethanol, the gastric acid concentration of the rats treated with palm vitamin E was not different to that of the controls. The gastric acid concentration measured at two other time intervals (i.e. 24 h and 1 week) does suggest that palm vitamin E may have an impact on the gastric acid concentration. An initial decrease in gastric acid concentration was observed 24 h postethanol administration in the rats treated with palm vitamin E. The lesion index at this point was still high despite a reduction in gastric acid concentration. On the contrary, we observed an increase in the gastric acid concentration at the 1 week interval. It cannot be totally excluded that this increase was due to a rebound effect after an initial reduction in gastric acid secretion. Despite the high gastric acid concentration, the gastric lesions improved. This suggests that the healing effect of palm vitamin E is not mediated by a reduction of gastric acid concentration.

In this study, the high MDA content in the control stomachs suggests previous findings that ethanol-induced injury is mediated through the lipid peroxidation process. It has been shown that free radicals and lipid peroxidation play a role in the pathogenesis of ethanol-induced gastric injury. Although MDA can be generated in vitro during tissue sampling, the positive correlation between lipid peroxidation and lesion index strongly supports the contention that lipid peroxidation has a role in the ethanol-induced gastric lesions.

We found that MDA levels to be lower in the palm vitamin E-treated group. The reduced MDA levels accompanying the improved gastric lesions in this group suggest that palm vitamin E probably accelerates the healing of gastric lesions by retarding the lipid peroxidation process. Bjorneboe and Bjorneboe showed that alcohol reduces the antioxidant status of the stomach, making it more vulnerable to gastric injury.21

The levels of PGE2 did not differ significantly in the palm vitamin E-treated group compared with the control group. This suggests that palm vitamin E, in the dose that was used in this study, did not influence the gastric PGE2 levels remarkably but indicated a tendency that it might improve the level, recording an approximate 30% increase in PGE2 levels compared with controls. This aspect requires further studies investigating the effects of different doses of palm vitamin E on prostaglandins.

We conclude that palm vitamin E improves the healing of ethanol-induced gastric lesions but could not prevent the injury. The healing properties of palm vitamin E are probably mediated through retarding the lipid peroxidation process. We conclude that administering palm vitamin E prior to ethanol challenge accelerates the healing of ethanol-induced gastric lesions. The effects of different doses of palm vitamin E on gastric acid secretion and prostaglandins requires further investigation.

References