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Palm tocotrienol-rich fraction supplementation suppressed stress-induced gastric oxidative stress in rats

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ABSTRACT

Palm tocotrienol-rich fraction (TRF), an extract from palm oil containing both tocotrienol and tocopherol, is known for its antioxidant effect. The present study investigated the effect of palm TRF on the stomach using a water immersion restraint stress (WRS) model. Forty-two male Sprague-Dawley rats were divided into three groups and were administered 60 mg/kg/d of palm TRF, α-tocopherol or vehicle only for 28 days. At the end of the treatment period, half of the rats from each group were subjected to WRS for 3.5 hours, after which the rats were sacrificed. The stomachs were then examined for lesions and measured for thiobarbituric acid reactive substance content and xanthine oxidase activity. Rats exposed to WRS showed gastric mucosal lesions. Pretreatments of the palm TRF and α-tocopherol reduced the occurrence of the lesions. WRS also increased gastric malondialdehyde content and xanthine oxidase activity significantly, but the increase in the oxidative stress parameters were significantly suppressed by both vitamin E pretreatments. However, the effects of palm TRF and α-tocopherol on the parameters measured were not different. In conclusion, palm TRF has the ability to protect against gastric mucosal injury, likely via its ability to inhibit oxidative stress.

Keywords: Tocotrienol, water immersion restraint stress, lipid peroxidation, gastric lesion, xanthine oxidase

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INTRODUCTION

Reactive oxygen species (ROS) are cytotoxic and mediate tissue damage by injuring cellular membranes and intracellular components (Hall et al., 2010). It has been suggested that ROS are implicated directly in stress-induced acute gastric mucosal lesions. ROS and lipid peroxidation induced by ROS has been shown to be involved in the pathogenesis of gastric mucosal lesions induced by water-immersion restraint stress (WRS) (Kwichien et al., 2008). WRS caused a decrease in gastric mucosal blood flow (Brzozowski et al., 2008) and this led to increased production of xanthine oxidase in the stomach. The increase in xanthine oxidase activity would then promote lipid peroxidation (Ohta et al., 2010). Treatment with various antioxidants such as vitamin E (Ohta et al., 2010), tea catechin (Hamaishi et al., 2006) and soy genistein (Takekawa et al., 2006) reduced WRS-induced gastric lipid peroxidation. Palm tocotrienol rich fraction (TRF) is a vitamin E extract from palm oil. Our previous study has shown that palm TRF was protective against WRS induced gastric lesion (Ibrahim et al., 2008). It is well known for its antioxidant properties (Asmadi et al., 2005; Kamisah et al., 2009). Therefore, the present study was designed to compare the effect of palm TRF and α-tocopherol on gastric oxidative stress in rats that were exposed to water immersion restraint stress (WRS).

MATERIALS AND METHODS

Animals, reagents and diet

The male Sprague-Dawley rats with initial body weight of 200-250 g used in this study were obtained from the Laboratory Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia. They were kept on a regular night/day cycle, with natural light for a period of 12 hours (0700 to 1900 hr). Throughout the feeding period, all rats were habituated to handling to reduce stress-related disturbances. The rats were housed in large cages with wire-mesh bottoms to prevent coprophagy. Food and water were given *ad libitum* throughout the experiment.

All chemicals and enzymes were of highest grade obtainable from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated. The palm tocotrienol-rich fraction used in this study was prepared by Malaysian Palm Oil Board (Gapor et al., 1993), comprising 21% α-tocopherol, 17% α-tocotrienol, 4% γ-tocopherol, 33% γ-tocotrienol and 24% δ-tocotrienol.

Experimental design

Sixty male Sprague-Dawley rats were divided into three equally sized gropus. The first group served as the control group which was only given olive oil (vehicle). The second group was administered 60 mg/kg/day palm tocotrienol-rich fraction (TRF), whilst the third received 60 mg/kg/day α-tocopherol by oral gavage for 28 days. The vitamin E dose was chosen based on our previous study, which showed the ability of this dose to reduce gastric lesions occurrence (Fahami et al., 2005). After 28 days of pretreatment, each group was further subdivided into another two groups. One subgroup was subjected to water-immersion restraint stress at the end of the treatment period, by placing them each in a plastic restrainer followed by immersion in water for 3.5 hours (Nishida et al., 1997), while another subgroup was not exposed to any stress. The rats were then sacrificed. The stomach was opened along the greater curvature and examined for lesions and kept for further biochemical measurements.

The experimental procedure and humane animal handling were conducted in accordance with the national guidelines for the care and use of laboratory animal, and were approved by the Institutional Animal Care and Use and Medical Research Ethics Committee (Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia).

Assessment of gastric lesions

Gastric lesions were measured as previously described (Wong et al., 2002). Gastric lesions were measured under a dissecting microscope at the magnification of 3X. Lesion size in mm was determined by measuring each lesion along its greatest diameter. A petechial lesion is equal to 1 mm lesion. The total lengths in each group of rats were averaged and expressed as the lesion index.

Biochemical analysis

The xanthine oxidase activity in the gaster was measured according to the method described by Terao et al. (1992). Gastric lipid peroxidation measured as thiobarbituric acid reactive substance (TBARS) content (Ledwozyw et al., 1986) and protein concentration (Lowry et al., 1951) were determined following the established methods.

Statistical analysis

The results are expressed as means ± standard error of the mean (s.e.m.) for seven rats. The data were analyzed statistically using ANOVA and Tukey's post-hoc test (SPSS Inc. USA). Normal distribution of variables was examined by Kolmogrov-Smirnov test. A p value of less than 0.05 was considered statistically significant.

RESULTS

Gastric lesions

No focal lesions in the gastric mucosa were seen in all groups of non-stressed rats. However, in rats subjected to the 3.5-hour WRS, gastric mucosal lesions were developed. Macroscopic lesions were observed, mostly averaging 1-2 mm in diameter, or in the form of petechial hemorrhages, confined to the glandular part of the stomach. Palm TRF pretreatment for 28 days decreased the gastric lesions by 52% (P < 0.001) and 40% by α-tocopherol (P < 0.001) in the WRS group. The gastric lesions indices were similar in the palm TRF and the α-tocopherol stressed groups (Table 1).

Table 1: The gastric lesion index (mm) in rats (n=7 per group) that were pretreated with palm TRF or α-tocopherol (60 mg/kg/day) for 28 days and exposed to water-immersion restraint stress for 3.5 hours.

	Non-stressed	Stressed
Control	0 ± 0	60.1 ± 5.73*
Palm TRF	0 ± 0	31.6 ± 2.08*#
α-Tocopherol	0 ± 0	24.3 ± 1.41*#

*Significantly different from the non-stressed groups respectively, #significantly different from the stressed control (ANOVA followed by Tukeys test, p<0.05).
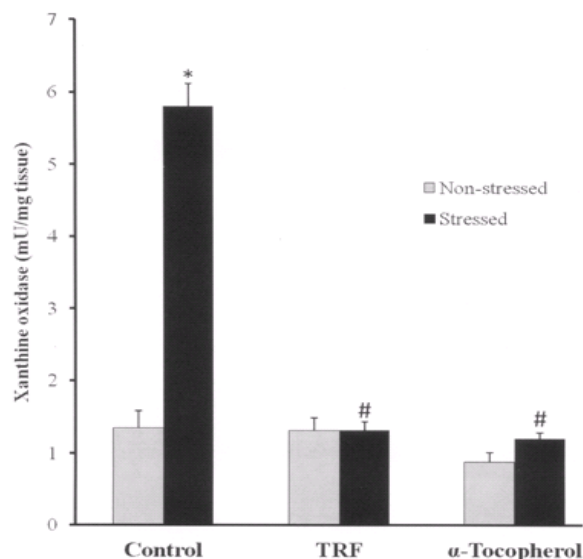
Fig 1. The xanthine oxidase activity in the stomach of rats that were pretreated with palm tocotrienol-rich fraction (TRF) or α-tocopherol (60 mg/kg/day) for 28 days and exposed to water-immersion restraint stress for 3.5 hours. Bars represent mean ± s.e.m. (n=7). *Significantly different from the non-stressed group, #significantly different from the stressed control (ANOVA followed by Tukeys test, p<0.05).

Gastric xanthine oxidase

In rats subjected to WRS, there was more than four-fold increase in the gastric xanthine oxidase activity. Palm TRF or α-tocopherol pretreatment had completely suppressed this increase significantly. This enzyme activity was similar in all groups that were not exposed to stress (Figure 1).

Gastric lipid peroxidation

Exposure to WRS for 3.5 hours augmented gastric lipid peroxidation, measured as thiobarbituric acid reactive substance (TBARS) significantly in rats. Pretreatment with palm TRF or α-tocopherol inhibited this increase. In rats that were not exposed to stress, the gastric TBARS contents were similar in all groups. However, pretreatment with the antioxidants, palm TRF or α-tocopherol did not further decrease the parameter content (Figure 2).
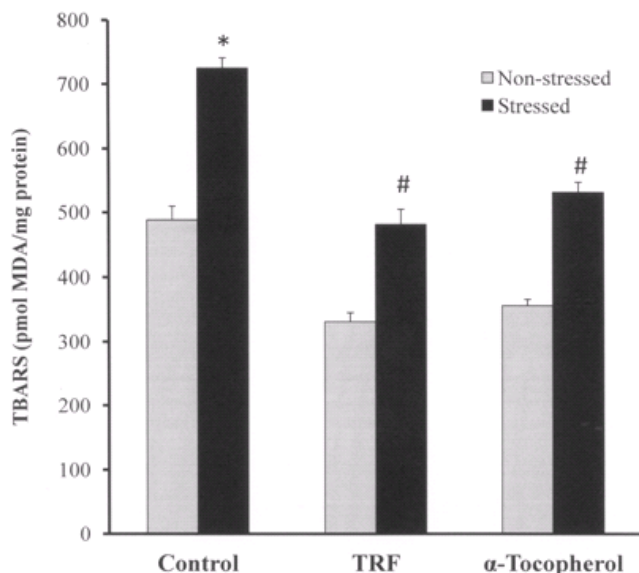

Fig 2. The effect of palm tocotrienol-rich fraction (TRF) or α-tocopherol supplementation (60 mg/kg/day) for 28 days on gastric thiobarbituric acid reactive substance (TBARS) content in rats exposed to water-immersion restraint stress (WRS) for 3.5 hours. Bars represent mean ± s.e.m. (n=7). *Significantly different from the non-stressed group. #significantly different from the stressed control (ANOVA followed by Tukeys test, p<0.05).

DISCUSSION

Water immersion restraint stress (WRS) is an established model for inducing stress in experimental animals. It was demonstrated to consistently produce stress-induced gastric lesions (Falalyeyeva et al., 2010; Yesilada et al., 2010). In the present study, WRS produced remarkable hemorrhagic gastric lesions. It was shown that WRS increased gastric contractions (Ibrahim et al., 2011) and later this would cause a temporary restriction to gastric blood flow to the mucosa and produced ischemic damage, resulting in the development of gastric lesions (Brzozowski et al., 2008).

Pretreatment with either palm TRF or α-tocopherol at 60 mg/kg body weight for 28 days reduced this occurrence of gastric lesion by 47% and 60%, respectively. The finding is in agreement with the study reported by Ohta et al., (2006). Prostaglandin E$_2$, the substance that maintains the gastric mucosal integrity (Guslandi, 1987), gastric acidity and gastrin level were found to be decreased in the gastric of rats that were exposed to WRS but pretreatments with palm vitamin E (also known as palm TRF) and α-tocopherol were able to reverse the detrimental effects of WRS (Ibrahim et al., 2008). These substances play important roles in maintaining the integrity of the gastric mucosa. Palm TRF or α-tocopherol had been shown to prevent the increase in WRS-induced gastric contractions (Ibrahim et al., 2011). This may explain the protective effect of vitamin E in reducing the formation of gastric lesions. Related to this, tocotrienol was found to exert better effect than α-tocopherol (Nur Azlina and Nafeeza, 2008). These findings suggest that vitamin E possesses anti-ulcerogenic property.

A remarkable increase in xanthine oxidase activity was observed in response to WRS in our study. WRS caused a significant reduction in gastric blood flow as demonstrated in many studies (Brzozowski et al., 2008; Kwiecien et al., 2008; Konturek et al., 2010). This can lead to ischemia-reperfusion-like change which promotes the conversion of xanthine dehydrogenase to xanthine oxidase (Ohta et al., 2009). Increased activity of the latter enzyme would augment the production of reactive oxygen species such as hydroxyl radical (Yasukawa et al., 2004). Similarly, increased activity of xanthine oxidase in WRS model was also reported in other studies (Ohta et al., 1999; Ohta et al., 2010). Kwichien et al. (2002) reported that the exposure of 3.5 hours of WRS led to an increase in oxidative metabolism, comparable to that observed in ischemia-reperfusion model of gastric injury.

The xanthine oxidase activity in rats subjected to WRS and pretreated with palm TRF or α-tocopherol was demonstrated to be similar to that of the non-stressed groups. It suggests that in the presence of stress, vitamin E prevented the production of the enzyme in the gastric. In consequence, the free radicals formation was prevented. As mentioned earlier, xanthine oxidase promotes production of free radicals. Involvement of free radicals has been proposed as one of the mechanisms in the development of stress-induced gastric ulcers. Free radicals will promote lipid peroxidation and this process can be assessed by the production of its stable end product, malondialdehyde which is usually measured as thiobarbituric acid reactive substance (TBARS). WRS increased gastric lipid peroxidation significantly compared to non-stressed rats in the current study. Being an antioxidant, both palm TRF and α-tocopherol were able to reduce this increase.

Tocotrienol has been shown in many studies to be a potent antioxidant (Gupta and Chopra, 2009; Tiwari et al., 2009). It is found abundantly in palm oil and differs from tocopherol by the presence of three double bonds in its phytyl chain (Azzi and Stocker, 2000). In all parameters measured in this study, the effects of the TRF were similar to that of α-tocopherol. In vivo, α-tocopherol is discriminatively transported into the liver by α-tocopherol transfer protein (Zingg, 2007). This resulted in almost 90% of the vitamin E being retained in the body is in the form of α-tocopherol in rats even though they were fed diets that contained quite a similar proportion of tocotrienol to tocopherol (Kamisah et al., 2005).

Even though palm TRF and α-tocopherol were able to completely inhibit the increases in gastric xanthine oxidase activity as well as TBARS production in this study, these antioxidants were not able to totally suppress the formation of gastric lesions. This proves that formation of the gastric lesions is not mediated by oxidative insult alone. Many other factors are involved in the development of stress, and even more in stress-induced gastric ulcers such as changes in stress hormones (Nur Azlina and Nafeeza, 2008), gastric motility (Ibrahim et al., 2011), mucus and bicarbonate secretions (Takayama et al., 2011), gastric acidity as well as inhibition of gastric mucosal prostaglandin synthesis (Brzozowski et al., 2008; Ibrahim et al., 2008) and reduction in gastric mucosal blood flow (Brzozowski et al., 2008).

Stress is a part of life and all individuals experience it once or more in their life. Therefore, in humans, these findings can be translated that both palm TRF and α-tocopherol may be taken as supplements to prevent or reduce the risk of stress-induced gastric lesion formation, to improve quality of life.

In conclusion, palm tocotrienol-rich fraction and α-tocopherol afford gastroprotective against formation of gastric ulcers in rats exposed to water immersion restraint stress, an effect probably associated with the suppression of oxidative changes via their antioxidant mechanisms.

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