Tocotrienols and "-Tocopherol Reduced Acute and Chronic Lung Lipid Peroxidation Induced by Paraquat in Rats

A.Y. Asmadi¹, A. Adam², W.Z. Wan Ngah³, M. Norazlina¹, Y. Kamisah¹, M.F. Nur-Azlina¹, M.S. Qodriyah¹, N.S. Ahmad¹, M.T. Gapor´ and A. Marzuki⁵
¹Department of Pharmacology and ²Department of Biochemistry, Faculty of Medicine, ³Department of Pharmacy, Faculty of Allied Health Science, Universiti Kebangsaan Malaysia, ¹Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia ³Faculty of Pharmacy, Universiti Teknologi MARA, Shah Alam, Selangor, ⁴Agro Product Unit, Engineering and Processing Research Division, Malaysian Palm Oil Board, P.O.Box 10670, 50720 Kuala Lumpur, Malaysia
E-mail: draayusof@yahoo.com

Abstract: The effect of dietary tocotrienols and "-tocopherol acetate supplementation on paraquat-induced lung toxicity was investigated in rats. The administration of a single dose of paraquat (20 mg/kg, i.p.) to rats increased lung malondialdehyde (MDA) levels and produced lung edema. Tocotrienols (150 mg/kg pellet) or "-tocopherol (34 mg/kg pellet) supplementation of two months significantly reduced lung MDA levels in rats exposed to paraquat but lung edema was unaffected. However lower doses of tocotrienols in the diet did not significantly lower lung MDA levels compared with the unsupplemented controls. Our results showed that dietary supplementation of tocotrienols and "-tocopherol provides protection against paraquat toxicity possibly through their antioxidant property.

Key words: Tocotrienol, "-tocopherol, paraquat, lipid peroxidation, lung, rats

Introduction
Paraquat (PQ), a widely used herbicide, is extremely toxic to plants and animals. PQ poisoning, whether occurring accidentally or intentionally may affect the lungs, liver, brain, kidneys and other organs (Smith and Heath, 1976). Early mortality of patients with PQ poisoning occurs as a result of vascular collapse, and delayed mortality is mainly due to progressive pulmonary fibrosis (Rivero et al., 1992; Hong et al., 2000; Koo et al., 2002) PQ toxicity has been found to be mediated by the production of free radicals (Kappus, 1986), which resulted in oxidative damage to cells. PQ is reduced to cation radicals, which are reoxidized by molecular oxygen to the superoxide anion (Tampo et al., 1999; Margolis et al., 2000). This resulted in the production of other reactive oxygen species that are highly reactive to cellular macromolecules, leading to oxidative stress (Bus and Gibson, 1984). Since PQ toxicity involves free radicals, it can be attenuated by antioxidants such as vitamin C and E, melatonin and iron chelators and antioxidative enzymes such as superoxide dismutase (Suntres, 2002). Vitamin E is one of the important lipid soluble antioxidants that scavenge free radicals (Burton, 1994). There are two types of naturally occurring vitamin E, which are the tocopherols and the tocotrienols. The latter can be found abundantly in palm oil. It has been reported that tocotrienols have a higher antioxidant activity than "-tocopherol (Kamat and Devasagayam, 1995; Kamat et al., 1997).

Under oxidative stress conditions, vitamin E concentration in the lungs is increased (Ikeda et al., 2003), which implies mobilization of the vitamin E to the lungs (Elsayed et al., 1990). It has also been demonstrated that tocopherol protects against acute effects of PQ induced lung injuries (Suntres and Shek, 1996). So far, studies using tocopherol or tocotrienols on the chronic effects of PQ induced lung injuries are limited. Therefore, in this study we investigated the outcomes of dietary a mixture of tocotrienols and "-tocopherol supplementation on acute and the chronic effects of PQ induced lung injury.

Materials and Methods
Animals, reagents and diet: Male Wistar rats, weighing 200-250 g were obtained from Laboratory Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia. The rats were kept in polycarbonate cages and food and water ad libitum. "-Tocopherol acetate and paraquat dichloride (methyl viologen) were purchased from Sigma Chemical Co. (St. Louis, MO). The tocotrienols (T) used was a mixture of 57% "-tocotrienol, 16% "-tocotrienol and 27% "-tocotrienol supplied by the Malaysian Palm Oil Board. All other reagents were the highest grade available.
commercially. Tocotrienols- and tocopherol-enriched diets were prepared by dissolving the appropriate doses of the vitamin E in acetone and then adding drops of the acetone onto the pellet, after which it was evaporated under vacuum. Control pellets were added acetone without vitamin E.

**Experimental design:** Two hundred and ten rats were divided into six groups and fed either normal rat diet (control), "-tocopherol-enriched diet (TF, 34 mg/kg food) or three different doses of tocotrienols-enriched diets (T1, 30 mg/kg food; T2, 60 mg/kg food; or T3, 150 mg/kg food) for two months. At the end of the feeding period, half of the rats from the control group (PQ) and all the rats in the vitamin E-supplemented groups were exposed to a single dose of paraquat (PQ, 20 mg/kg body weight, intraperitoneally). The remaining half of the control group (C) was only given normal saline. The rats were then sacrificed on days 1, 3, 7, 14 and 28 post-PQ exposures. The lungs were removed for malondialdehyde (MDA) (Ledwozyw et al., 1986) and relative water content (Pearce et al., 1965) determinations. The animal care and handling, as well as experimental procedure were approved by the faculty’s animal ethics committee.

**Statistical analysis:** Results were expressed as mean ± standard error. Statistical comparison was made by one-way ANOVA and followed by Least Significant Difference (LSD). P < 0.05 was considered significant.

**Results**

**Lung malondialdehyde concentration:** PQ administration increased lung malondialdehyde (MDA) concentrations at all time points determined (Fig. 1). However, supplementation of TF and tocotrienols at the dose of 150 mg/kg food (T3) were able to prevent this increase. A significant reduction in MDA concentrations was also observed at smaller doses of tocotrienols (30 and 60 mg/kg food) groups after 7 days exposure to PQ, but not after 14 days.

**Relative water content:** Exposure to PQ significantly increased lung water content at days 1 and 3 but not at days 7, 14 and 28 (Fig. 2). Supplementation with TF and all doses of tocotrienols did not have any effect on the lung water content at all time points studied.

**Discussion**

The main target organ in PQ toxicity is the lung. As expected, PQ caused an increase in lung MDA levels, indicating an increase in lipid peroxidation in the lungs. Our results showed that the MDA levels were remained high even after 28 days post-PQ exposure. Other researchers have also shown similar findings where PQ administration increased lipid peroxidation in the lungs (Melchiorri et al., 1996; Mustafa et al., 2002). However, these studies only measured lung MDA levels.
24 hours post-PQ exposure. Since we used a lower dose of PQ (20 mg/kg body weight) compared to other studies (50 mg/kg body weight) (Melchiorri et al., 1996; Cheng et al., 1999), we were able to study the chronic effect of the oxidant up to 28 days post-exposure. The dose was selected based on our preliminary study (unpublished results) to ensure prolongation of the rats’ survival.

Dietary supplementation of TF (34 mg/kg food) and tocotrienols at the dose of 150 mg/kg (T3) were able to prevent the increase in MDA levels due to PQ at all time points. The protective effect of tocopherol on PQ toxicity has been reported previously (Suntres and Shek, 1996; Cheng et al., 1999), but studies involving tocotrienols are limited. The protective effects of tocotrienol against oxidant-induced injuries in other organs such as liver (Kamat et al., 1997), heart (Kamisah et al., 2000) and bone (Soelaiman et al., 2004) have been documented. Lung MDA levels tend to decrease in groups receiving lower doses of tocotrienols (30 and 60 mg/kg food) at days 1 and 3, even though the reduction was not significant. The antioxidative effects of tocotrienols at these doses might be overwhelmed by PQ toxicity. At day 7, the deleterious effect of PQ was overcome by the tocotrienols which is reflected by the low MDA levels. However at days 14 and 28, these doses of tocotrienols were no longer able to reduce the MDA levels. The rapid clearance of tocotrienol from the plasma (Fairus et al., 2004) may be the reason for this observation.

Edematous lung is reflected by relative water content. In this study, lung water content was increased as early as day 1 after PQ administration but returned to normal at day 7. This suggested that edema is an early response to PQ, i.e. within 24 hours after PQ administration, which has also been shown in other studies (Greenberg et al., 1978; Suntres and Shek, 1996). Our findings also suggest that the animals recovered from lung edema on day 7 even if left untreated.

Dietary vitamin E pretreatment (tocotrienols and "-tocopherol) of two months did not appear to reduce the edema in the rats. A study by Suntres and Shek (1996) also showed that pretreatment of animals with "-tocopherol liposomes did not alter significantly the PQ-induced changes in lung weight. PQ-induced lung edema could be due to mechanisms other than direct injury of pulmonary endothelial cells which could not be prevented by antioxidants.

In conclusion, a high dose of tocotrienols (150 mg/kg pellet) and "-tocopherol (34 mg/kg pellet) are effective in reducing lipid peroxidation process caused by PQ up to 28 days post-exposure.

Acknowledgement
This study was funded by a grant from Malaysian Ministry of Science, Technology and Innovations (IRPA Grant No. 03-07-03-089).

References
Asmadi et al.: Vitamin E, paraquat and lipoperoxidation


