Introduction

Almost 16 million people were reported worldwide to be blind and about 50 million have impaired vision as a result of cataract (Thylefors et al., 1995). In developing countries, including India and Kenya, blindness resulting from cataract appeared earlier in life and is more prevalent than in developed countries (Taylor, 2000). Cataract formation is associated with aging, eye injury, smoking, diabetes, excessive exposure to light and steroid (Jacob, 1999). While the mechanism of initiation and progression of cataractogenesis is being investigated, there is still no consolidated theory, which can predict the course of development. It is believed that oxidative stress appears to play a key role in cataract formation, which can cause oxidation on lens lipid and protein (Brown, 2001).

Vitamin E has long been known to be highly efficient as an antioxidant to protect the body from free radical damage, which is well accepted as the first line of defense against lipid peroxidation. Vitamin E naturally occurring tocopherol and tocotrienol isomers present in palm oil. Some of the tocopherol isomers present in palm oil, the tocotrienol, are not normally present in the other edible oils (Packer, 1991).

Some reported vitamin E effectively inhibits and delays cataract formation (Sun-Yuh & Cristina, 2000). Thus the present study aims to determine the effects of palm vitamin E supplementation on oxidative status in steroid-induced cataract formation.

Materials and Methods

The study consisted of thirty-six male New Zealand white rabbits (1.5-2.0 kg) were housed on a 12:12 light/dark cycle. Food and water was available ad libitum. The rabbits were divided into six groups. The groups A and B were given olive oil by oral feeding, while rabbits in groups C and D were supplemented with palm vitamin E at 3 mg/kg bw; E and F were supplemented with palm vitamin E at 15 mg/kg bw diluted in olive oil. However group B, D and F, prednisolone acetate 1% (PA1%) at 1 mg/kg was instilled onto the eyes to induce cataract. Blood was taken to measure levels of MDA and vitamin E in plasma and antioxidant enzyme activities of SOD, GPx and CAT in erythrocyte at week 0 and subsequently every two weeks until cataract was formed in one of the groups, upon which, all rabbits were slaughtered. Ophthalmoscopy was performed every week to observe any opacity in crystalline lens, which indicates early cataract formation.

MDA in plasma was measured by the method of Ledwozyw et al. (1986). MDA was measured using a spectrofluorometer and measured at EX 515 nm and EM 553 nm. MDA standard of known concentration was prepared from 1,1,1,3-tetraethoxypropane. SOD activity was determined according to method by Beyer & Fridovich (1987), which is based on the inhibition by SOD of the nitroblue tetrazolium (NBT) produced by superoxide radical generated by riboflavin. The enzyme assay system composed of 50 mM phosphate buffer; pH 7.0, 0.117 mM riboflavin and 1.18 M mixed substrate. Reaction was initiated by H2O2, and fall in absorbance was measured at 340 nm due to oxidation of NADPH. CAT activity was determined according to method by Aebi (1984). The enzyme assay system composed of 50 mM phosphate buffer; pH 7.0 and 30 mM H2O2.
The decomposition of substrate, \( \text{H}_2\text{O}_2 \), was measured using spectrophotometer at 240 nm. Vitamin E was determined method by Meydani et al. (1987) using high performance liquid chromatography (HPLC). Separations were carried out on a silica column using a mobile phase of hexane: isopropanol, 99:1 at a flow rate 1.5 ml/min. The vitamin E was detected at \( \text{EX} 292 \text{ nm} \) and \( \text{EM} 340 \text{ nm} \).

Statistical analysis was carried out using ANOVA, taking \( p<0.05 \) as its significance value.

**Results**

**Ophthalmoscopy examination**

Ophthalmoscopy examination showed formation of cataract in PA 1% treated group (groups B) and PA 1% treated group with supplemented 3 mg/kg palm vitamin E (group D) at 10th week. Supplementation with 15 mg/kg palm vitamin E to PA 1% treated group (group F) effectively prevented steroid-induce cataract formation.

**Effect of palm vitamin E on SOD activity**

At 10th week, SOD activity in PA 1% treated group (group B) showed significant increase \( (p<0.05) \) compared to 0 week. Supplementation with 3 and 15 mg/kg palm vitamin E to group treated with PA 1% (groups D and F) increased significantly \( (p<0.05) \) SOD activity compared to PA 1% treated group (group B) at 10th week (Figure 2).

**Effect of palm vitamin E on GPx activity**

At 10th week, GPx activity in PA 1% treated group (group B) and groups supplemented with 15 mg/kg (groups E and F) increased significantly compared to control group and groups supplemented with 3 mg/kg palm vitamin E (groups C and D) (Figure 3).
Effect of palm vitamin E on CAT activity

At 10th week, supplementation 3 and 15 mg/kg palm vitamin E to PA 1% treated groups (group D and F) increased significantly (p<0.05) CAT activity compared to PA 1% treated group (group B) (Figure 4).

![Figure 4](image)

**FIGURE 4** CAT activity (U/ mg Hb) in treated groups. Data are means ± SEM (n= 4-6). p<0.05 (1- vs. week 0, a- vs. gp. A, b- vs. gp. B, c- vs. gp C, d- vs. gp D, f- vs. gp F)

Effect of palm vitamin E on vitamin concentration

At 10th week, supplementation with 15 mg/kg palm vitamin E to PA 1% treated group (group F) increased vitamin E concentration significantly (p<0.05) compared to PA 1% treated group (group B) and PA 1% treated group with supplemented 3 mg/kg palm vitamin E (group D) (Figure 5).

![Figure 5](image)

**FIGURE 5** Vitamin E concentration (ug/ml) in treated groups. Data are means ± SEM (n= 4-6). p<0.05 (1- vs. week 0, a- vs. gp. A, b- vs. gp. B, c- vs. gp C, d- vs. gp D)

Discussion

Oxidative damage could result not only from an increase in ROS, but also from a decrease in antioxidant capacity (McIntosh et al., 1998). Steroid was reported to alter the activities of antioxidant defenses system in the liver, which can result in the elevation of lipid peroxide (LPO) in humoral component such as liver (Lee et al., 1998).

The present study showed lipid peroxidation may play an important role in the pathogenesis of cataract, shown as an increased MDA level in PA 1% treated group (group B). Kosano et al. (2000) had found increased lipid peroxidation in the liver, blood and lens on chick embryo given glucocorticoid. In association with the suppression of cataract formation, supplementation of 15 mg/kg palm vitamin E decrease the steroid-induced elevation of MDA in plasma, of which vitamin E is the major non-enzymatic antioxidant, possibly reacting with peroxy radical to inhibit the propagation cycle of lipid peroxidation (Ohta et al., 1996).

Antioxidant enzymes such as SOD, GPx and CAT are the most important enzymes that prevent oxidative attacks by reactive oxygen species. The present study showed SOD and CAT activities were decreased and GPx activity was increased in blood of PA 1% treated group (group B). Cekic et al. (1999) also showed the same result. SOD and CAT activities decreased as a result of oxidative stress involved in cataract formation. The increase of GPx activity showed that body defence mechanism response to the effects of this oxidation process by activating the increase in synthesis of GPx (Nourmohammadi et al., 2001).

Supplementation with 15 mg/kg palm vitamin E to PA 1% treated group (group F) caused an increase in GPx, SOD and CAT activities in blood. These results showed possibly there exists a synergistic effect between palm vitamin E and antioxidant enzymes. Ghatak et al. (1996) showed supplementation of vitamin E greatly improved response on antioxidant enzymes such as SOD, GPx and CAT.

Supplementation of palm vitamin E was expected to increase in plasma vitamin E concentration. The higher concentration of vitamin E may delay or inhibit progression of cataract (Robertson et al., 1991). In the group treated with PA 1%, the increase vitamin E concentration may causes oxidative stress modulate TTP caused hepatic TTP and its mRNA are increased (Tamai et al., 2000).
In conclusion, supplementation with palm vitamin E appears to slow down the formation of steroid-induced cataract in rabbits. This appears to correlate with the reduction in oxidative stress as shown in the lower MDA level in the supplemented group with palm vitamin E.

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References


