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Effect of Various Doses of Palm Vitamin E and Tocopherol on Factors Affecting Gastric Lesions in Rats

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Abstract: This study examined the effects of various doses of palm vitamin E and tocopherol with and without aspirin on gastric acid, malondialdehye (MDA) and mucus in rats. The study was divided into two phases: phase 1 determined the effects of various doses of palm vitamin E and tocopherol on the factors affecting mucosal integrity. Sixty-four male rats of Sprague-Dawley species (200-250 g) were randomly divided into eight groups. Group I was fed a normal diet (control), Group II, III and IV were fed a diet supplemented with palm vitamin E in a dose of 60, 100 and 150 mg kg^{-1} food, respectively. Whereas rats in Group V, VI, VII and VIII were fed with tocopherol in a dose of 20, 30, 50 and 100 mg kg^{-1} food, respectively for 4 weeks. The rats were killed after four weeks of feeding for the determination of gastric malondialdehyde (MDA), acid and mucus content. There was a significant decrease in gastric MDA and gastric acid in all the palm vitamin E supplemented groups compared to control. However, these doses of palm vitamin E had no significant effect on gastric mucus. Tocopherol in a dose of 100 mg kg^{-1} food causes a significant increase in gastric mucus and decrease gastric acid content compared to control and other tocopherol groups. Phase 2 study determined the effect of multiple doses of palm vitamin E and tocopherol on gastric parameters and gastric lesions index post aspirin exposure. Fifty-six rats of the same weight and species were randomized into seven groups. Group I was fed a normal diet, Group II to IV were fed with palm vitamin E enriched diet in a dose of 60, 100 and 150 mg kg^{-1} food, respectively. Whereas Groups V to VII were fed with tocopherol enriched in a dose of 20, 30 and 50 mg kg^{-1} food, respectively. After four weeks of feeding with the respective diets the rats were challenged with a single intragastric dose of 400 mg kg^{-1} body weight aspirin suspended in propylene glycol. The rats were killed 6 h post-aspirin exposure for the determination of gastric lesion index and gastric parameters as mentioned in phase I study. Both vitamins E exhibits similar efficacy but less potency on gastric lesions indexes in rats and it was accompanied by a significant decrease in gastric MDA content. We conclude that the effect of vitamin E on gastric parameters before and after aspirin administration is not the same and not dose-dependent. Palm vitamin E reduces MDA concentration with and without aspirin administration and at the same time reduces gastric acid secretion without aspirin challenge. Tocopherol reduced gastric MDA post aspirin administration. However, low dose tocopherol reduces MDA and high dose tocopherol reduces acid and increase gastric mucus in the absence of aspirin administration.

Key words: Palm vitamin E, dose, tocopherol, aspirin, gastric lesion, rat

INTRODUCTION

Over 30 million people worldwide use non-steroidal anti-inflammatory drugs (NSAIDs) daily. Numerous human studies have shown that the use of NSAIDs is associated with various gastroduodenal mucosal lesions[1,2]. The mechanism by which aspirin and other NSAIDs produce acute and chronic gastroduodenal mucosal injury are incompletely understood[3]. It has been suggested that the mechanism of aspirin-induced gastric lesion be mediated through lipid peroxidation[4].

Alpha tocopherol (vitamin E) is a naturally occurring antioxidant in biological systems and is present in the cell membrane of various tissues including the intestine and stomach[5]. Vitamin E prevents free radical-induced injury by blocking the free radical chain reaction. The formation of experimental gastric lesions may be reduced through decreasing free radicals and minimizing lipid

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peroxidation[4]. Previous studies have shown that deficiencies in vitamin E have resulted in peptic ulceration[6] and that vitamin E supplementation to the diet has protective effects on the gastric mucosa[7,8].

Serbinova and Packer[9] have shown that tocotrienol to be a more potent antioxidant than alpha tocopherol. Palmvitee is a vitamin E concentrate from palm oil and it contains approximately 80% tocotrienol and 20% tocopherol[10].

Our earlier studies[11,12] have reported that palm vitamin E given at the dose of 150 mg kg^{-1} food for three weeks either before or after the induction of gastric lesions were able to accelerate the healing of ethanol-induced gastric injury in rats. In another study we also found that palm vitamin E and tocopherol in a dose of 300 mg kg^{-1} food given for duration of 8 weeks were equally effective in preventing aspirin-induced gastric lesions in rats[13]. We undertake this study to ascertain the mechanism of how palm vitamin E and tocopherol protect aspirin-induced gastric lesions and to see whether the effect of vitamin E on factors affecting gastric lesion are dependent on dose and the type of vitamin E used.

MATERIALS AND METHODS

Study design: The study was divided into 2 phases: Phase II and I.

Phase I study: The objective of phase I study was to determine the effects of various doses of palm vitamin E and tocopherol on factors that are important in maintaining gastric musical integrity. In this study, Sixty four rats were fed a normal rat chow (control, n=8) or palm vitamin E enriched diet in the dose of 60, 100, 150 and 300 mg kg^{-1} rat chow. Whereas rats in Group V, VI, VII and VIII were fed with tocopherol in a dose of 20, 30, 50 and 100 mg kg^{-1} food, respectively for 4 weeks. The diet was prepared in the same way as was described before[12]. The compositions of the normal rat chow are shown in Table 1. After 4 weeks of feeding on the respective diets, the rats were killed for the determination of gastric MDA, gastric acid concentration and gastric mucus content.

Measurement of gastric acidity: The measurement of gastric acidity was done according to the method described by Shay *et al.*[14]. 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 0.01 N NaOH to a pH of 7.0. The concentration of hydrogen ion was calculated as described by Shay *et al.*[14].

Table 1: Composition of basic rat diet (Gold Coin)

Substances	Percentage by weight
Crude protein content	22.0
Crude fibre content	5.0
Crude fat content	3.0
Moisture	13.0
Ash	8.0
Calcium	0.8-1.2
Phosphorus	0.6-1.0
Nitrogen free extract	49

Additives: Vitamin A, D, E, C, K, B12, thiamin, riboflavin, pantothenic acid, niacin, pyridoxine, folic acid, choline and microminerals

Measurement of malondialdehyde (MDA): Tissue samples weighing 0.2 g from the corpus region was homogenized using a glass homogenizer (Potter). The content of gastric tissue MDA was then determined using the method described by Ledwozyw *et al.*[15]. A sample of 0.5 mL was acidified with 2.5 mL 1.22 mol L^{-1} trichloroacetic acid in 0.5 mol L^{-1} HCl. The mixture was left to stand for 15 min. After this time, 1.5 mL of 0.6% thiobarbituric acid in 0.05 mol NaOH was added. The samples were incubated in a 100°C water bath for 30 min. Subsequently it was cooled under running tap water and 4 mL of n-butanol was added. After thorough mixing, the mixture was centrifuged for 10 min at 1500 g. The absorbance of the upper phase was read at 535 nm using a spectrophotometer.

Measurement of gastric mucus concentration: Gastric mucus was quantitatively measured as described by Corne *et al.*[16]. In this technique, Alcian blue, which binds specifically to mucus, was used. The glandular portion of stomach was isolated and immersed in 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. 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 rpm for 10 min. The absorbance of the aqueous phase of the solution was measured using spectrophotometer at 604 nm.

Phase 2 study: The aim of phase 2 study was to determine whether treatment with multiple doses of palm vitamin E and tocopherol were able to prevent aspirin-induced gastric lesions. Fifty-six male rats of sprague-Dawley species weighing between 200 to 250 g were randomized to seven groups. Group I (n=8) was fed a normal rat chow, whereas Groups II, III and IV were fed with a normal rat chow supplemented with palm vitamin E in a dose 60, 100

and 150 mg kg^{-1} food, respectively for 4 weeks. Rats in Groups V, VI and VII were fed with normal rat chow supplemented with tocopherol in a dose of 20, 30 and 50 mg kg^{-1} food, respectively for 4 weeks. After 4 weeks of feeding, the rats were challenged with a single dose of 400 mg kg^{-1} body weight of aspirin administered via an orogastric tube. The rats were killed at 6 h post-aspirin exposure for determination of gastric lesions, gastric acidity and MDA content.

Determination of gastric lesion: The gastric mucosa was then 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 semi quantitatively as described by Berry *et al.*[17] which were graded as follows: 5 = multiple ulcers following almost the entire length of gastric fold, 4 = lesions which followed approximately 80% of the folds, 3 = ulcer 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 generalised erythema and 0 = no visible damage. Gastric acid and MDA were measured as was described in phase I study.

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

RESULTS

Phase I study
Effect of vitamin E on gastric acid with and without aspirin administration: In the absence of aspirin, palm vitamin E in a dose of 60, 100, 150 and 300 mg kg^{-1} body weight caused a significant reduction in gastric acid compared to control ($p<0.05$). However, Tocopherol in a dose of 100 mg kg^{-1} body weight caused a significant reduction in gastric acid concentration compared to control in the absence of aspirin administration ($p<0.05$) (Fig. 1). On the other hand both tocopherol and palm vitamin E in a dose of 20, 30, 50 mg kg^{-1} body weight and 100 kg^{-1} body weight, 60, 100 and 150 mg kg^{-1} body weight, respectively had no significant effect on gastric acidity in the presence of aspirin (Fig. 2).

Effect of palm vitamin E on gastric MDA: Tocopherol in a dose of 20, 30 and 50 mg kg^{-1} body weight caused a significant reduction in gastric MDA post aspirin exposure ($p<0.05$). In the absent of aspirin administration only tocopherol in a dose of 20 mg kg^{-1} body weight was able to reduce MDA significantly compared to control (Fig. 3).
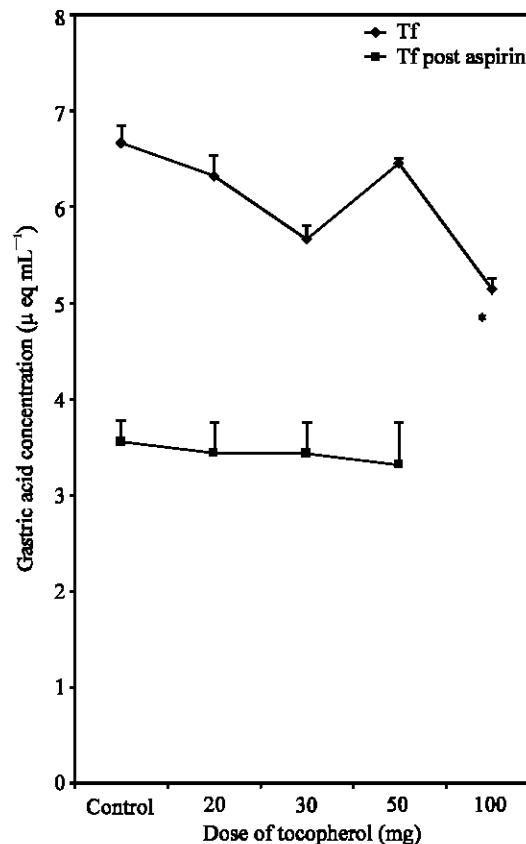

Fig. 1: Effect of tocopherol E on gastric acidic with and without aspirin (*$p<0.05$ compared to control). Tf=Tocopherol

The gastric tissue content of MDA was significantly lower in the palm vitamin E treatment groups compared to control ($p<0.05$). The significant reduction in gastric MDA content was observed with and without aspirin administration in palm vitamin E-treated group. There was no significant difference in gastric MDA among the palm vitamin E-treatment groups (Fig. 4).

Effect of palm vitamin E on gastric mucus content: There was no significant difference in the gastric mucus concentration among the palm vitamin E-treated groups compared to control. However, tocopherol in a dose of 100 mg kg^{-1} body weight increase gastric mucus significantly compared to control (Fig. 5).

Phase 2 study
Effect of palm vitamin E and tocopherol on aspirin-induced gastric lesions: The gastric lesions index of the palm vitamin E and tocopherol-treated groups were significantly lower compared to control at 6 h after aspirin administration ($p<0.05$). The lowest gastric lesion index was observed in the group treated with either 100 mg kg^{-1} food of palm vitamin E or 30 mg kg^{-1} food of tocopherol
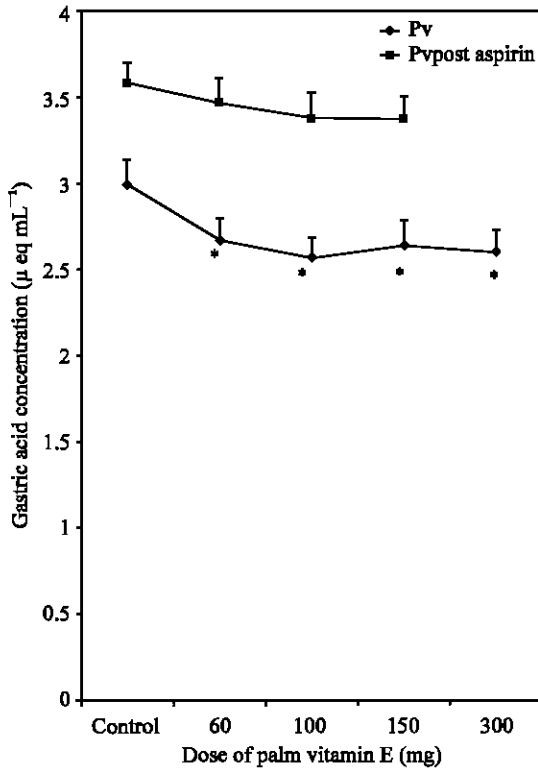
Fig. 2: Effect of palm vitamin E on gastric acid with and without aspirin (*p<0.05 compared to control). Pv=Palm vitamin E
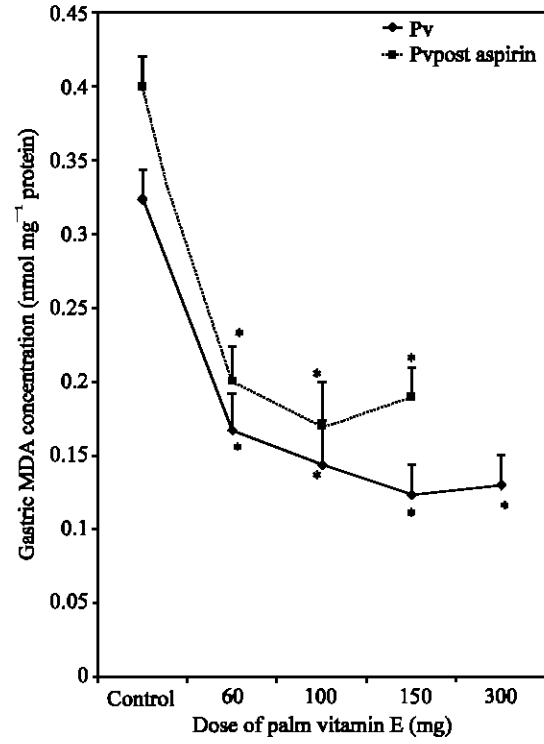

Fig. 4: Effect of palm vitamin E on gastric MDA with and without aspirin (*p<0.05 compared to control). Pv= Palm vitamin E
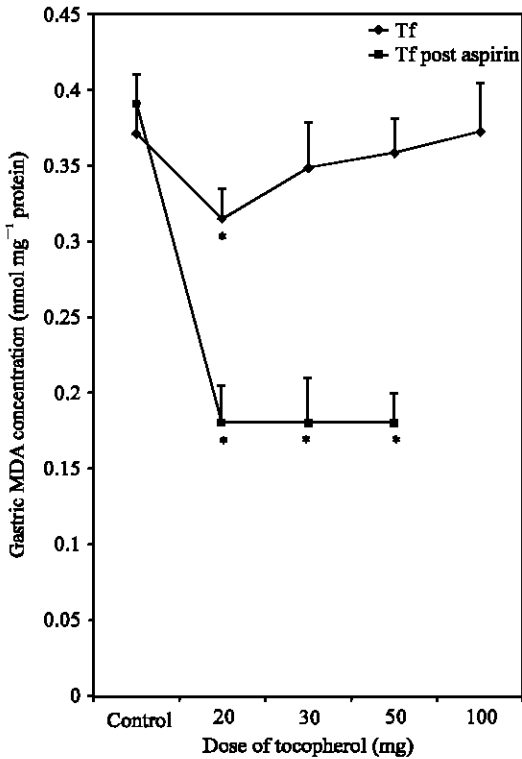

Fig. 3: Effect of tocopherol on gastric MDA with and without aspirin (*p<0.05 compared to control). Tf=Tocopherol
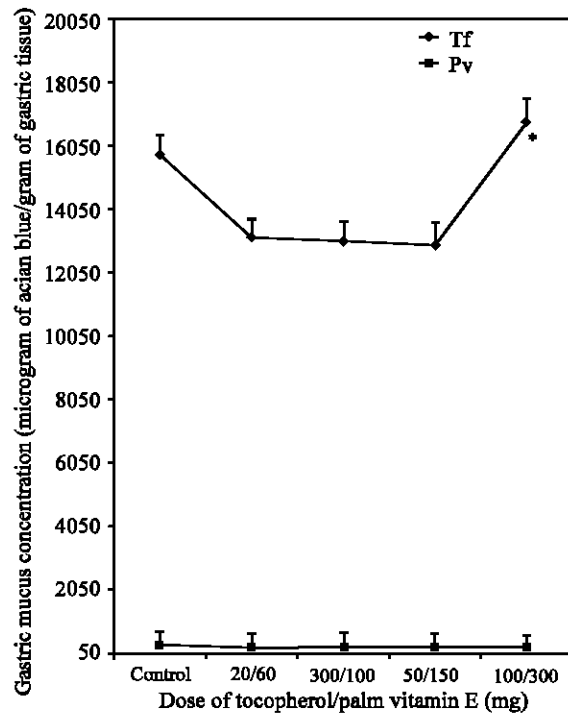

Fig. 5: Effect of vitamin E on gastric mucus concentration (*p<0.05 compared to control and other treatment group). Tf=Tocopherol, Pv=Palm vitamin E
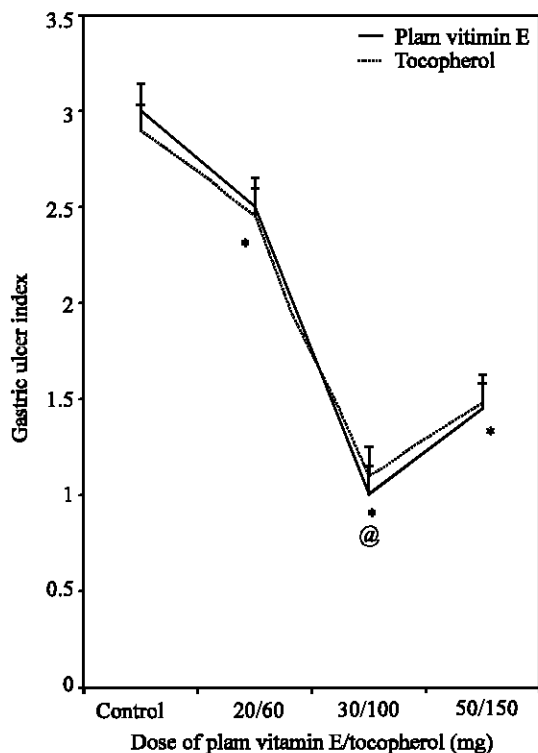
Fig. 6: Effect of vitamin E on gastric ulcer index (p<0.05 compared to control, @p<0.05 compared to the other treatment group)

(p<0.05). However, there was no significant difference in gastric lesion index between the palm vitamin E and tocopherol-treated groups throughout the study period (Fig. 6).

DISCUSSION

Many reports have shown that vitamin E can improve gastric lesions induced by non-steroidal anti-inflammatory drugs[1-3,8].

In the current study, tocopherol reduced the gastric tissue content of MDA significantly compared to the control after aspirin administration. Where as palm vitamin E reduced the gastric tissue content of MDA significantly compared to the control with and with out aspirin administration. The effect of vitamin E on gastric MDA post aspirin exposure was not dependence on dose. This is because both low and higher dose of vitamin E were effective in reducing gastric MDA content. However, the effect of tocopherol on Gastric MDA content in the absence of aspirin challenge appear to be dose dependence, as only low dose of tocopherol which 20 mg kg^{-1} food was effective in reducing gastric MDA. The reason for this is not clear. There is a possibility that high dose of tocopherol may act as pro-oxidant that encourage free radical formation. Palm vitamin E on the

other hand does not behaving as pro-oxidant even at higher doses. This finding suggest that aspirin administration generate free radical formation, vitamin E given prophylactically before chemical insult was able to protect free radical generation.

Besides inhibiting free radical formation, Palm vitamin E and high dose tocopherol (100 mg kg^{-1} food) appears to reduce gastric acid secretion, as the gastric acid concentration was significantly lower in the palm vitamin E–treated group and tocopherol 100 mg kg^{-1} food compared to control. The effect of vitamin E on gastric acid secretion was nullified by aspirin administration. The reason for this is not clear. There is a possibility that the magnitude of rise in gastric acid induce by aspirin is so great that palm vitamin E and high dose tocopherol was unable to reduce it. Both tocopherol and palm vitamin E did not interfere with gastric mucous concentration however; there is a tendency that high dose of tocopherol (100 mg kg^{-1} food) increase gastric mucus secretion. However, this effect of tocopherol was not studied after aspirin administration.

Palm vitamin E and tocopherol given 4 weeks before aspirin exposure were able to prevent aspirin-induced gastric lesions as the gastric lesion index in the palm vitamin E and tocopherol treated group were significantly lower compared to control. In this study, we found that the effect of 60, 100 and 150 mg kg^{-1} food of palm vitamin E on ulcer index and gastric parameters were equipotent to tocopherol dose of 20, 30 and 50 mg kg^{-1}, respectively. This study suggested that palm vitamin E in a dose of 100 mg kg^{-1} food and tocopherol in a dose of 30 mg kg^{-1} food appear to be more effective in preventing aspirin-induced gastric lesions than in the other doses as the gastric lesion index in these two groups were significantly lower compared to the other groups. Even though tocotrienol is more potent antioxidant than tocopherol[9], this study showed that palm vitamin E, although rich in tocotrienol, is less potent than tocopherol in its effect on gastric lesion index and gastric MDA post aspirin administration. This is because a higher dose of palm vitamin E is required to manifest the same effect on gastric lesion index and gastric MDA after aspirin administration. The reason for this is not clear. It is possible that the effect of tocotrienol in palm vitamin E is attenuated by the higher fraction (20%) of tocopherol. This finding was in contrast to our earlier study[11,12] in which palm vitamin E in a dose of 150 mg kg^{-1} food was unable to prevent ethanol-induced lesions. This discrepancy may be attributed to the severity of gastric lesions. It thus appears that ethanol-induced gastric lesions were more severe compared to aspirin-induced lesions, thus requiring larger doses of palm vitamin E (in excess of

150 mg kg^{-1} food) for prevention. Aspirin-induced lesions on the other hand only required palm vitamin E or tocopherol as low as 60 and 20 mg kg^{-1} food, respectively.

The preventive effect of vitamin E on aspirin-induced lesions was only associated with a significant reduction in gastric MDA content. This again suggests that the probable mechanism of prevention be is via reducing lipid peroxidation. This result is in agreement with our previous finding[11-13] that the most likely mechanism of healing and the preventive effect of palm vitamin E on ethanol- induced and aspirin-induced lesions is via the reduction of lipid peroxidation process. Even though palm vitamin E and high dose tocopherol may have beneficial effect on gastric acid and mucus however, this effect was not seen post aspirin administration. We conclude that the effect of palm vitamin E on gastric parameters before and after aspirin administration is not the same and not dose-dependent. Palm vitamin E reduces MDA concentration with and without aspirin administration and at the same time reduces gastric acid secretion without aspirin challenge. Tocopherol, in the other hand may react as pro-oxidant in the absence of chemical insult. Only high dose of tocopherol reduces acid and increase mucus secretion in the absence of aspirin administration. However, both vitamins E exhibits similar efficacy but less potency on gastric lesions indexes in rats and the probable mechanism of prevention be is via reducing lipid peroxidation.

ACKNOWLEDGMENT

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