Palm vitamin E and glucosamine sulphate in the treatment of osteoarthritis of the knee

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ABSTRACT

الأهداف: تقييم أثر فيتامين هـ في تخفيف الأعراض لدى المرضى المصابين بالتهاب عظمي مفصلي في الركبة (OA) مقارنة مع عقار سولفيت الجلوكوزامين.

الطريقة: أجريت هذه الدراسة والتي كانت عبارة عن دراسة مفتوحة المركز الطبي – جامعة كيبانجسان ماليزيا خلال الفترة من مارس 2006م إلى نوفمبر 2007م. تم توظيف 79 مريض لتلقي إما عقار سولفيت الجلوكوزامين عبر الفم بمقدار 1.5 جرام أو 400 ملجم من فيتامين هـ عبر الفم لمدة 6 أشهر. تم تقييم الأعراض باستعمال مدخلات الالتهاب العظمي المفصلي لجامعتي انتاريو الغربية وماكماسترWOMAC ، ونقاط أنالاجو البصرية VAS.

النتائج: أكمل عدد 64 مريضاً التجربة (فيتامين هـ عدد33=n، وعقار سولفيت الجلوكوزامين عدد n=31). وبعد 6أشهر من العلاج. ظهر على كلتا المجموعتين تحسن ملحوظ في نقاط WOMAC ونقاط (VAS) خلال الوقوف والمشي. لم يكن هنالك فرقاً ملحوظاً لدى نقاط WOMAC ونقاط (VAS) بين المجموعتين. ماعدا حدوث ردة فعل حساسية بسيطة غير مريحة في البطن لدى مريض واحد. لم يتم الإبلاغ عن حدوث أثار عكسية خطيرة أخرى. سجل سيروم مولونديلهيد ارتفاع ملحوظ في مجموعة جلوكوزماين مقارنة مع المجموعة التي تم معالجتها بفيتامين هـ في نهاية هذه الدراسة. سجل مصل فيتامين هـ ارتفاع ملحوظ في مجموعة فيتامين هـ مقارنة مع جلوكوزماين.

خاتمة: تشير نتائج الدراسة أن تلقي جرعة 400 ملجم من فيتامين هـ عبر الفم بشكل يومي له دور عظيم في تقليل الأعراض لدى المرضى المصابين بالتهاب عظمي مفصلي في الركبة. كما أن له أثر كسولفيت الجلوكوزامين في تقليل الأعراض و إزالة الآثار الجانبية الخطيرة. المزيد من الدراسة مطلوبة لتحري آلية فعله بجانب أثر مضاد التأكسد.

Objectives: To assess the efficacy of oral palm vitamin E in reducing symptoms of patients with osteoarthritis (OA) of the knee compared to oral glucosamine sulphate.

Methods: This open study was carried out at the Universiti Kebangsaan Malaysia Medical Center, Kuala Lumpur, Malaysia between March 2006 and November 2007. Seventy-nine patients were recruited to receive either 1.5g oral glucosamine sulphate or 400mg oral palm vitamin E for 6 months. Symptoms were assessed using the Western Ontario and McMaster Universities (WOMAC) osteoarthritis index and visual analogue scale (VAS).

Results: Sixty-four patients completed the trial (vitamin E n=33, glucosamine sulfate n=31). After 6 months of treatment, both groups showed a significant improvement in WOMAC scale and significant reduction in the VAS score during standing and walking. There was no significant difference in WOMAC scale and VAS score between the 2 groups. Except for mild allergic reaction and abdominal discomfort in one patient, there were no other serious adverse effects reported. Serum malondialdehyde was significantly higher in the glucosamine group compared to palm vitamin E treated group at the end of the study. Serum of vitamin E was significantly higher in the palm vitamin E group compared to glucosamine.

Conclusion: The finding of this study suggests that oral palm vitamin E in a dose of 400mg taken daily has a potential role in reducing symptoms of patients with OA of the knee. It may be just as effective as glucosamine sulphate in reducing the symptoms and free from serious side effects. Further study is required to ascertain the mechanism of action beside its antioxidant effect.

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Osteoarthritis (OA) is a disease of advancing age, which can cause significant pain and disability, disrupting patients' daily activity. Reactive oxygen species (ROS), also called free radicals (which could be determined by measuring serum malondialdehyde [MDA]), have been implicated in the pathogenesis of OA. Our body continuously produces ROS, which have been implicated in several chondrocytes activities including cell activation, proliferation, and matrix remodeling. The main ROS is a superoxide ion, which can be converted to hydroxyl radical and hydrogen peroxide.[1,2] In Galleron et al[2] study, exposed synoviocytes to superoxide ions *in vitro* lead to apoptosis of synoviocytes. To counteract its effect, the body in turn also produces anti-oxidants such as metalloproteins, catalase, and gluthathione peroxidase. However, if its production is exceeded by that of ROS, then a state of oxidative stress occurs leading to cartilage damage.[2,3] Ostolowska et al[3] reported that there was increased anti-oxidant enzymes activity in patients with knee OA.[3] They attributed this to potential adaptation to the an increased ROS in the osteoarthritic joint. Hacklar et al[4] examined the association of reactive oxygen species and nitric oxide in the pathogenesis of OA in 40 patients with chondral or meniscal lesions or both. Synovial fluids were collected from the subjects. They found high levels of ROS and nitrogen species in patients with OA compared to control. High concentrations of reactive oxygen, nitrogen species, and polymorphonuclear leukocytes' granular products may interact at the articular surface in inflammatory arthritis resulting in increased oxidative damage within the joint cavity.[5] The deleterious effect of ROS can also be prevented by the presence of free radical scavengers like alpha tocopherol, vitamin C, and beta carotene.[6-9] Vitamin E is a lipid-soluble compound that consists of 4 tocopherols and 4 tocotrienols -alpha, beta, gamma, and delta.[9] It possesses anti-oxidant effects and has been shown to be effective in reducing free radical-induced pathological conditions.[10-13] In addition to its anti-oxidant effects, vitamin E also has anti-inflammatory effects. It acts by blocking the formation of arachidonic acid from phospholipids and inhibits lipo-oxygenase activity.[4] Previous studies have shown mixed results regarding the efficacy of vitamin E in the treatment of OA.[15-20] Vitamin E derived from palm oil is found to contain high levels of tocotrienols compared to other resources, which contain higher levels of tocopherols; palm vitamin E contains 21.9% tocopherol, and 78.1% tocotrienol. Previous studies have mainly used tocopherols.[21,22] Alpha tocotrienol have been shown to have better anti-oxidant effects compared to alpha tocopherol.[11,14,] Our study objective is to assess the efficacy of palm vitamin E in the treatment of OA of the knee.

Methods. *Patient selection.* This study was carried out at the Universiti Kebangsaan Malaysia Medical Center, Kuala Lumpur, Malaysia between March 2006 and November 2007. The Research Ethics Committee of University Kebangsaan Malaysia approved the study. All patients aged over 40 years old that were recruited from our Arthroplasty clinic were eligible for the study. There was no upper limit age restriction. The clinical diagnosis of OA of the knee is defined as knee pain (articular and not peri-articular or referred) for most days of the prior month with radiographic osteophytes at the tibio-femoral joint margins. For patients presenting with bilateral knee pain, the worst affected knee joint would be defined as the target joint. Only patients with Kellgren-Lawrence[24] 2 and 3 OA of the knee were included. Patient who had secondary OA and took medications or treatments that could alter the disease process were excluded from the study. This included intra-articular steroids and intra-articular hyaluronic acid, glucosamine sulphate, chondroitin sulphate, non-steroidal anti-inflammatory analgesia, and arthroscopy of the knee joint within the last year. Those with impaired kidney and liver function were also excluded. The identified patients entered a one-week washout period in which all anti-inflammatory and analgesic treatments (except tramadol) were terminated in order to assess baseline values of pain and function. At the end of this period, the patients were randomized to receive either oral palm vitamin E (400mg daily) or glucosamine sulphate (500mg thrice daily). Patients were asked to document any side effects or adverse event and bring all their study medications to each clinic visit. Patients were not allowed to take other forms of analgesia.

Outcome measurements. During each visit, patients were assessed for pain on standing and pain on walking 50m distances in the 24 hours prior to the clinical visit using a 10cm visual analogue scale (VAS). For the VAS, patients were asked regarding the severity of their pain from no pain (0 on the VAS) to the most severe pain they have experienced (10 on the VAS). We used a modification of the WOMAC (Western Ontario and McMaster Universities)[25] score to assess knee pain, stiffness, and function. The original WOMAC score included 22 questions. For each question, the Likert scale was used whereby the score ranged from 0 to 4. In our modified version, patients were assessed using a scale similar to the VAS on a 10cm ruler for each question. Thus, the maximum score was 220 indicating severe symptoms. Serum MDA and tocotrienol levels were also measured before and after treatment. The MDA content in the serum was determined using a method described by Ledwozyw et al[26] with some modifications. A sample of 0.5 ml was acidified with 2.5 ml of 1.22 M

trichloroacetic acid /0.6 M hydrochloric acid and left to stand at room temperature for 15 minutes after which 1.5 ml of 0.67% thiobarbituric acid/0.05 M sodium hydroxide was added. The samples were incubated in a 100°C water bath for 30 minutes. Subsequently, they were left to cool at room temperature before the addition of 4 ml of n-butanol. After thorough mixing, the mixture was centrifuged for 10 minutes at 1500xg. The concentration of vitamin E in serum was determined by high-performance liquid chromatography (HPLC) (Hewlett Packard HP1100, city/country). The HPLC analysis was performed using YMC column 150 x 0.6 mm internal diameter (ID). The mobile phase used was composed of 0.5% iso propanol /hexane, and the flow rate was one ml/min. The injection volume was 20µL. Detection was performed using an excitation wave length of the fluorescence detector (295nm), Em 330?. A standard was analyzed before the samples. The standard concentration was 40ppm for each component. Quantification of vitamin E was calculated by using this formula: Quantification of vitamin E is calculated by multiplying the volume of sample, area of sample, volume of standard and concentration of standard. The value is then divided with the multiplication of weight of sample area, area of standard and volume of sample. X ppm (part/million) = Volume of sample x Area of sample x Volume of standard x Concentration of standard Weight of sample area x Area of standard x Volume of sample

Statistical analysis. Paired t-test was used for comparison of means of outcome measurements (WOMAC scale, VAS score, global status scale) MDA, and Vitamin E before and after treatment and between groups. Confidence interval for difference between pre- and post-medication for both groups was measured using paired samples test. Repeated measure ANOVA was used to assess the progression of outcome measurements for VAS during standing and walking and WOMAC score during the 6 months duration of treatment. A $p<0.05$ was considered significant.

Results. Sixty-four out of 79 patients completed the trial. There were 15 drop outs, which were due to minor side effects, and lost to follow up. The mean age group was 58-59 years old. Most of the patients were female. Body weight ranged from 63.6 kg to 64.55 kg. The race distribution was almost the same for Malay and Chinese (**Table 1**). There was significant improvement in the VAS during standing in both groups (p=0.01, p=0.01). For the palm vitamin E group, the mean standing VAS score at baseline was 3.18 and at 6 months was 1.42. The mean standing VAS score for the glucosamine sulphate group, at baseline was 3.48 and at 6 months was 0.83. The mean improvement was 1.75 (<95% CI:

Table 1 - Demographic details of patients in both groups

Demographic details	Treatment	
	Vitamin E n=33	Glucosamine sulphate n=31
Age (years)	58.3	59.5
Gender (M:F)	3:30	7:24
Race (Malays:Chinese:Indians)	14:17:2	11:17:3
Height (cm)	156.06	158.97
Weight (kg)	63.61	64.55
Body mass index (kg/m^2)	26.19	25.42
Occupation (Employed:Others*)	12:21	7:24
Kellgran 2	25	25
Kellgran 3	8	6
Mean VAS standing	3.18	3.48
Mean VAS walking	4.03	4.50
Mean WOMAC	60.05	64.66

* 'Others' refers to those who are either retired or are housewives, VAS - visual analogue scale, WOMAC - Western Ontario and McMaster Universities osteoarthritis index

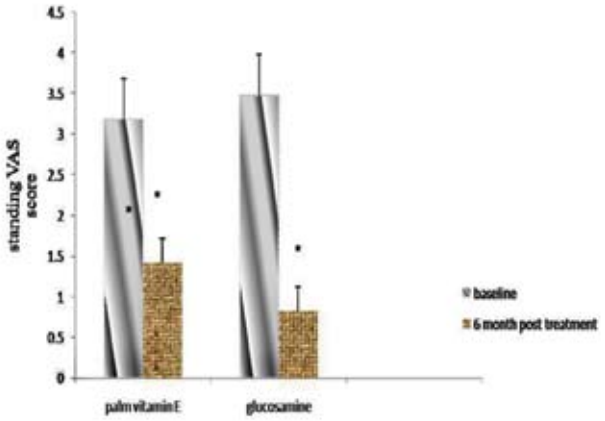

Figure 1 - Effect of palm vitamin E and glucosamine sulphate on standing visual analogue scale (VAS).

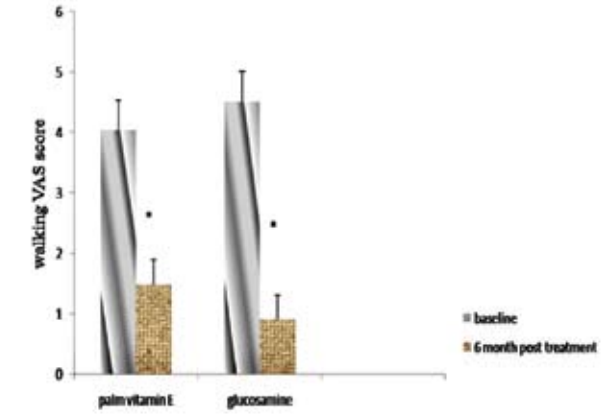

Figure 2 - Effect of palm vitamin E and glucosamine sulphate on walking visual analogue scale (VAS).

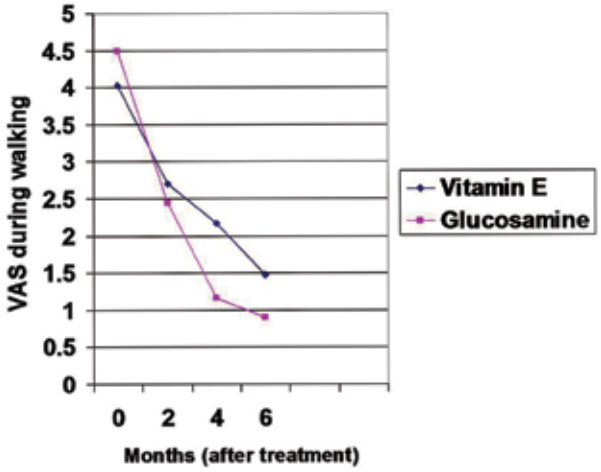

Figure 3 - Effects of palm vitamin E and glucosamine sulphate on the regression rate of walking visual analogue scale (VAS) score.

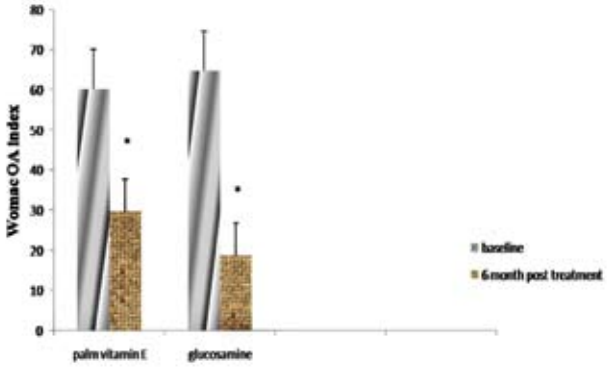

Figure 4 - Effect of palm vitamin E and glucosamine sulphate on modified Western Ontario and McMaster Universities (WOMAC) osteoarthritis index score.

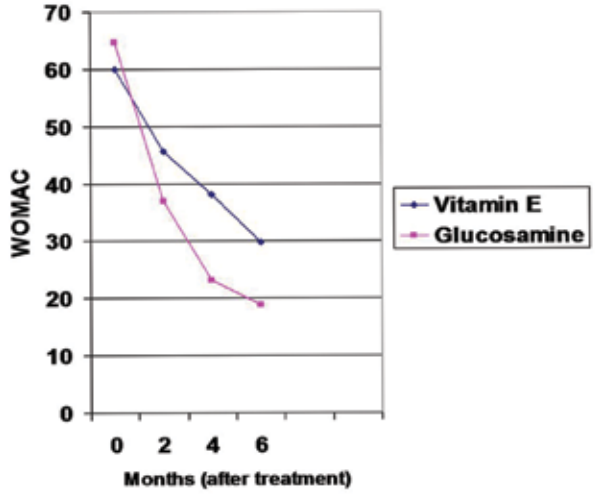

Figure 5 - Effects of palm vitamin E and glucosamine sulphate on the regression rate of modified Western Ontario and McMaster Universities (WOMAC) osteoarthritis index score.

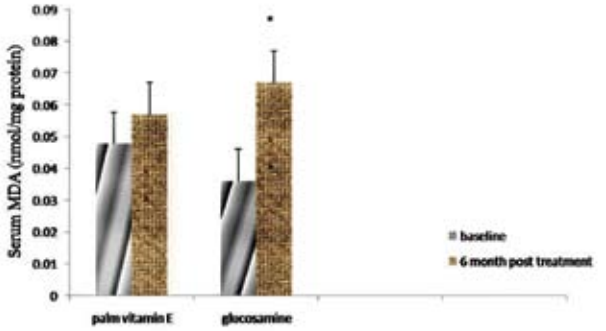

Figure 6 - Effect of palm vitamin E and glucosamine sulphate on serum malondialdehyde (MDA).

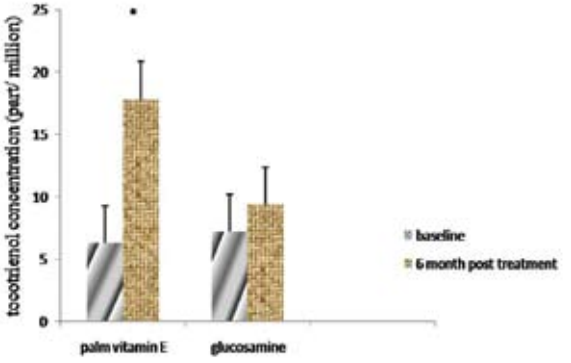

Figure 7 - Effect of palm vitamin E and glucosamine sulphate on plasma tocotrienol.

<2.69, 0.81) in the vitamin E group and 2.64 (<95% CI: <3.55, 1.73) in the glucosamine group respectively. However, there was no significant difference in VAS score between the 2 treatment groups (**Figure 1**). There was a significant improvement in walking VAS Score in both groups (p=0.006, p=0.000). The mean walking VAS score for palm vitamin E at baseline was 4.03, and at the end of the study period was 1.48. Whereas, the mean walking VAS score for glucosamine treated group at baseline was 4.5, and at the end of the study period was 0.90. The mean improvement was 2.54 (<95% CI: <3.49, 1.59) in the vitamin E group, and 3.59 (<95% CI: <4.38, 2.81) in the glucosamine group. However, there was no significant difference in waiting VAS score between the groups (**Figure 2**). There was a significant regression rate in VAS score in both groups after 6 months of treatment (p=0.01, p=0.01) (**Figure 3**). There was a significant improvement in WOMAC score in both groups (p=0.01, p=0.01). The mean WOMAC score for palm vitamin E at baseline was 60.05, and at the end of the study period was 29.77. Whereas, the mean WOMAC score for glucosamine treated group at baseline was 64.66, and at the end of the study period was 18.83. The mean improvement was 30.27

(<95% CI: <41.30, 19.23) in the vitamin E group, and 45.82 (<95% CI: <57.61, 34.03) in the glucosamine group. However, there was no significant difference in WOMAC score between the groups (**Figure 4**). There was a significant regression rate in WOMAC score before and after treatment in the glucosamine group (p=0.01), but not in the palm vitamin E group (p=0.64) (**Figure 5**). The serum MDA was measured in all the patients before and after 6 months of treatment. There was no significant difference in serum MDA level in the palm vitamin E group before and after 6 months of treatment. The mean serum MDA levels before and after treatment for the palm vitamin E group were 0.477 ± 0.07/mg protein and 0.057 ± 0.03/mg protein. However, there was a significant increase in serum MDA levels after 6 months treatment in the glucosamine sulphate group. The serum MDA levels for the glucosamine sulphate group before and after treatment was 0.036 ± 0.09/ mg protein and 0.067 ± 0.04/ mg protein (**Figure 6**). The serum alpha tocotrienol levels were measured in all patients before and after treatment. There was a significant increase in serum palm vitamin E level after 6 months treatment in the palm vitamin E group compared to baseline. The serum alpha tocotrienol levels in the palm vitamin E group before and after treatment were 6.24 ± 2.60 part/million and 17.81 ± 11.16 part/ million. However, there was no significant change in serum alpha tocotrienol level before and after treatment in the glucosamine sulphate group. The levels before and after treatment in this group were 7.19 ± 1.75 part/ million and 9.34 ± 1.54 part/million (**Figure 7**). One patient had abdominal discomfort with glucosamine sulphate. Another in the glucosamine group complained of general ill health whilst on the medication. No abnormality was detected on physical examination and relevant blood investigations were normal. No organic cause was found that could explain her symptoms. In the vitamin E group, one patient developed itchiness that resolved once the medications were discontinued. All these 3 patients were discontinued from the study.

Discussion. Osteoarthritis is a common condition in the older age group. Although it is not potentially a life-threatening condition, it can cause severe pain affecting a person's daily function. The end point in the treatment of this condition is arthroplasty of the affected joint. Over the years, several medications have been used to alleviate pain and also to delay the progression of the disease. The literature reports the results of various studies that have used vitamin E in patients with OA. In an early study, Macthey et al[15] reported that tocopherol in a dose of 600mg daily for 10 days had good analgesic effect in 52% of their patients compared to 4% in the placebo group. Blakenhorn[16] carried out a randomized, double blind, controlled trial comparing vitamin E in the form of 400mg alpha tocopherol with placebo over a period 6 weeks. Vitamin E was shown to produce greater pain relief at rest and on movement when compared to placebo. There was also a reduction in the use of analgesia in the vitamin E group. Brand et al[17] found that vitamin E had no effect on OA knee compared to placebo. Scherak et al[18] found that 400mg vitamin E and 50mg diclofenac 3 times daily had a similar effect on OA of the hip and knee. In a double-blind placebo controlled trial, Wluka et al[19] conducted a double blind placebo controlled trial and found no difference between vitamin E and placebo in terms of symptoms or cartilage volume loss. The Framingham Osteoarthritis Cohort Study[20] explored the association of dietary intakes and progression of OA of the knee. The study found that there was protective effect of vitamin E in OA progression, but not its incidence.[22] These studies used vitamin E, which consisted mainly of alpha tocopherol, and they have yielded mixed results. Our study assessed the efficacy of palm oil-derived vitamin E (palm vitamin E), which consisted mainly of alpha tocotrienol, in the treatment of OA of the knee in humans. Alpha tocotrienol has been reported as a better anti-oxidant compared with alpha tocopherol.[10,13,23] In our study, comparison was made between vitamin E and glucosamine sulphate, as glucosamine sulphate has been shown to be effective in the treatment of OA.[27-29] We discovered that palm vitamin E in a dose of 400mg daily was effective in controlling symptoms of OA as reflected by the significant decrease in WOMAC scale as well as standing and walking VAS scores. However, there was no difference in these parameters between the patients treated with palm vitamin E and those treated with glucosamine sulphate. This may suggest that palm vitamin E is equally effective as glucosamine sulphate in reducing symptoms in patients with OA of the knee. The effect of palm vitamin E in this study is comparable to the findings of Machtey et al,[15] Blankenhorn et al,[16] and the Framingham Osteoarthritis Cohort.[20] However, the reports of this study were not in accordance with the findings of Scherak et al,[18] and Wluka et al,[19] who reported that vitamin E did not give significant improvement in patients with OA. The discrepancies in these results may be attributed to various factors including dose and type of vitamin E used, duration of the studies, and sample size. Our study evaluated the medium-term effect of palm vitamin E in patients with OA of the knee. Despite the small sample size, we found that palm vitamin E gave significant reduction of symptoms in the treatment of patients with OA of the knee. In this study, we found out that there was a significant increase in serum MDA in the glucosamine treated group compared to the palm vitamin E group

and baseline levels. This finding may suggest the increase in MDA level in the glucosamine group might be due to disease progression, which did not happen in the palm vitamin E group. There was no significant difference in MDA level before and after treatment in the palm vitamin E treated group. This is an unexpected finding since serum MDA levels in the group treated with palm vitamin E should be lower after treatment if it acts as an anti-oxidant in relieving the symptoms of OA. There is a possibility that the mechanism of action of palm vitamin E in this study is not mediated through anti-oxidant effect. There is also a possibility that the dose of 400mg daily was inadequate to prevent stress oxidation in patients with OA. However, palm vitamin E in a dose of 400mg was able to prevent a rise of MDA in patients with OA of the knee since levels of MDA in patients treated with glucosamine sulphate were higher compared to the palm vitamin E treated group. In our study, we looked specifically at the knee joint as opposed to studies in which samples included hip and knee OA. Both joints were subjected to different amounts and patterns of stress. Thus, we cannot assume that the effects of analgesia are the same in these 2 joints. Apart from the small sample size, there are other limitations in our study. The WOMAC score and VAS are subject to bias. The outcome measurement was based on the assumption that patients will fully comprehend the questions and VAS. Due to the ethnicity of our population with multiple languages, it was occasionally difficult to convey questions to patient. We also did not include serial radiographic assessment. This would give a more objective form of measurement. However, changes in radiographs may not necessarily correlate with changes in symptoms. Another limitation in our study is the fact that we did not have a placebo group. This is because our ethical committee deemed it unethical to compare palm vitamin E with a placebo. Finally, our study is an open study. Statistically, we did not use intention to treat analysis due to the small number of patients. Palm vitamin E in a dose of 400mg daily gave no significant adverse reactions; only one patient reported mild side effects in the form of itchiness. At low levels prescribed for OA, vitamin E poses no health risk to an individual. However, at higher doses, above 1000mg, patients may experience depression, fatigue, nausea, diarrhea, and blurred vision.[30] In addition, vitamin E can also enhance the effects of anti-coagulants since it also has anti-coagulant properties. In our study, the level of vitamin E prescribed was way below the level of toxicity. Nevertheless, one of our patients did complain of itchiness, which may or may not be due to an allergic reaction.

There have been recent studies in Malaysia that show the protective effect of palm vitamin E against cellular ageing[31,32] as well as prevention of pre-ecclampsia.[33] Palm vitamin E was also shown to have favorable effects on the plasma lipid profile in rabbits fed with a cholesterol-rich diet.[34] In addition to its effects on articular cartilage, it also has effects on bone metabolism. Noorazlina et al,[35] in a study involving ovariectomized rats; found that supplementation of these rats with palm vitamin E or tocopherol maintained the bone mineral density. The tartrate-resistant acid phosphatase levels were elevated, consistent with reduction in osteoclastic activity and alkaline phosphatase levels were elevated. The exact mechanism of palm vitamin E in improving symptoms in OA of the knee is not known. It is possible that palm vitamin E, which consists mainly of alpha tocotrienol, does not act via an anti-oxidant effect as it did not interfere with the serum MDA levels. The possibility that palm vitamin E may act via an anti-inflammatory or analgesic effect cannot be disputed. Further study is required to ascertain the mechanism of action of palm vitamin E. More reliable methods of assessment would be beneficial.

In conclusion, palm vitamin E in a dose of 400mg daily for 6 months appears to be effective in improving symptoms of OA of the knee. However, further cross over studies with a larger size are needed to consolidate our findings. There was no difference in the MDA levels before and after treatment in patients taking palm vitamin E; it is possible that the mechanism of action of palm vitamin E in this study is not mediated through anti-oxidant effects. Palm vitamin E in a dose of 400mg daily is safe in the treatment of patients with OA of the knee.

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