

BAHAGIAN PENYELIDIKAN & PEMBANGUNAN  
CANSELORI  
UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

1) Nama Penyelidik: P.M. Dr. H.A. Nadiger

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Nama Penyelidik-Penyelidik  
Lain: (Jika berkaitan) : P.M. Dr. Mazidah Ahmad Mansur

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2) Pusat Pengajian/Pusat/Unit : Sains Perubatan

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3) Tajuk Projek: Kajian Kesan Vitamin E ke Atas Status

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Antioksidans dan Peroksidasi Lipid

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di Kalangan Sukarelawan Manusia

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4) (a) Penemuan Projek/Abstrak

(Perlu disediakan makluman di antara 100 - 200 perkataan di dalam Bahasa Malaysia dan Bahasa Inggeris. Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).

Kesan pengambilan Vit.E (Natopheral, 400 IU/hari) sahaja atau ditambah dengan kompleks Vit.B untuk jangka masa satu tahun ke atas aras Malonaldehyde (MDA) di dalam serum (Indeks peroksidasi lipid), status jumlah antioksidans, jumlah kolesterol dan kolesterol HDL dikaji di kalangan 74 orang sukarelawan lelaki Melayu di dalam lingkungan umur antara 18-55 tahun.

Keputusan kajian ini menunjukkan:

1) Aras MDA di dalam serum meningkat dengan umur tanpa sebarang perubahan di dalam status antioksidans jumlah yang menyokong teori bahawa ketidakseimbangan di antara tekanan oksidatif dan status antioksidans mungkin terbabit di dalam proses ketuaan.

2) Pengambilan Vit.E & Kompleks Vit.B menurunkan aras serum MDA dan meningkatkan status jumlah antioksidans; kombinasi Vit.E&B lebih berkesan daripada Vit.E sahaja.

3) Vit.E sahaja ataupun Vit.E&B, telah dapat merendahkan aras kolesterol jumlah ke tahap yang sama.

4) Pengambilan Vit.E sahaja tidak mempengaruhi aras kolesterol HDL ke tahap yang signifikan tetapi sebaliknya kombinasi Vit.E&B meningkatkan secara signifikan tahap kolesterol HDL.

5) Kedua-dua Vit.E sahaja dan kombinasi Vit.E&B merendahkan tahap kolesterol jumlah: kolesterol HDL, Kerendahan ini lebih signifikan pada kombinasi Vit.E&B daripada Vit.E sahaja.

Sebagai kesimpulan daripada kajian ini, pengambilan kombinasi Vit.E&B

berbanding dengan Vit.E sahaja, memberi perlindungan yang lebih baik kepada gejala yang disebabkan oleh radikal bebas. Pengambilan Vit.E&B juga memberi perlindungan yang lebih efektif kepada penyakit Jantung Kardiovaskular Iskemik (IHD) melalui kesannya terhadap aras kolesterol HDL.

## Summary:

Effect of supplementation of VitaminE ( Natopherol, 400 IU/day ) alone or in combination with B-complex for a period of one year on serum levels of MDA ( index of lipid peroxidation ), total antioxidant status, total cholesterol & HDL cholesterol was studied in 74 male healthy Malay volunteers ranging in age from 18 - 55 years. Results of the study indicated :

(1) serum MDA levels increase with age without any change in the total antioxidant status which supports the theory that imbalance between oxidative stress and antioxidant status may be involved in aging process,

(2) supplementation of Vit. E & B-complex reduced serum MDA levels and increased total antioxidant status, E+B administration being superior to E alone

(3) both E alone and E+B administration reduced total cholesterol levels to the same extent

(4 ) supplementation of E alone did not affect HDL cholesterol levels significantly but E+B administration significantly increased the HDL cholesterol

(5) both E alone and E+B administration reduced the tot. chol.:HDL chol. ratio, the reduction being significantly more with E+B compared to E alone.

It is concluded from the study that administration of E+B as opposed to E alone provides better protection against free radical mediated damage . E+B administration may also be more effective in prevention of IHD through their effect on HDL cholesterol levels.

(b) Senaraikan Kata Kunci yang digunakan di dalam abstrak:

<u>Bahasa Malaysia</u>	<u>Bahasa Inggeris</u>
Peroksidasi Lipid .....	Lipid Peroxidation .....
Vitamin E .....	Vitamin E .....
Penyakit Jantung Iskemik .....	Ischaemic heart-disease .....
Kolesterol serum .....	Serum cholesterol .....
Malonaldehyde .....	Malonaldehyde .....
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5) Output Dan Faedah Projek

(a) Penerbitan (*termasuk laporan/kertas seminar*)  
(*Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan*).

3 penerbitan sedang disediakan.  
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- (b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.  
(Jika ada dan jika perlu, sila gunakan kertas berasingan)

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- (c) Latihan Gunatenaga Manusia

i) Pelajar Siswazah .....

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ii) Pelajar Prasiswazah: .....

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iii) Lain-Lain: Seorang Teknologis Perubatan, .....

En. Basir Abdullah mendapat latihan dan .....

pendedahan di dalam semua kerja-kerja .....

makmal yang dijalankan. ....

6. Peralatan Yang Telah Dibeli:

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UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

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PROF MADYA (DR) MAFAUZY MOHAMED  
Dekan  
Pusat Pengajian Sains Perubatan  
Universiti Sains Malaysia  
16150 Kubang Kerian  
Kelantar

TANDATANGAN PENERUSI  
JAWATANKUASA PENYELIDIKAN  
PUSAT PENYELIDIKAN

**Final report on USM short term grant project No. 331/0500/3086  
( no. Rujukan Kami : FPP 96/022 )**

**Title : “ Studies on effect of Vitamin E supplementation on antioxidant status  
and lipid peroxidation in human volunteers “**

**Investigators :**

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## Introduction :

Free radical ( a group of highly reactive partially reduced species of  $O_2$  ) mediated oxidative damage to PUFA ( poly unsaturated fatty acids ), Proteins, DNA & Cell membranes is being implicated in the pathogenesis of Atherosclerosis, Age related Degenerative disorders, Cancer and Senescence itself.<sup>1</sup> It is now established beyond doubt that free radicals are generated *in vivo* through normal aerobic metabolic activities like mitochondrial electron transport, peroxisomal fatty acid oxidation, Cyt. P-450 reactions, oxidation of monoamines etc.<sup>2</sup> In addition free radicals present in the atmosphere ( the concentration of free radicals in the atmosphere is increasing with increasing atmospheric pollution by industrialisation, automobile exhaust etc. ) and cigarette smoke can enter the human body and are capable of inflicting oxidative damage similar to the free radicals generated *in vivo*.

Free radical mediated oxidation of PUFA, a process called lipid peroxidation, leads to the formation of highly reactive aldehydes like Malonaldehyde ( MDA ).<sup>3</sup> Levels of MDA in tissues and circulating fluids are considered indicative of the extent of free radical mediated oxidative damage. Nature has evolved defense mechanisms against oxidative damage in the form of antioxidant systems like Superoxide dismutase, catalase, Glutathione system etc. In addition dietary antioxidants particularly vitamin E can also function as free radical scavengers to decrease the initiation and propagation of fatty acid oxidation. It is now being realised that an imbalance between the oxidative stress and the antioxidant protective mechanisms may lead to oxidative damage to the various biomolecules and the consequent disease processes.

Among the disease processes thought to be mediated by free radical mediated oxidative damage, Atherosclerosis and the consequent ischaemic heart disease ( IHD ) have attracted the attention of many workers because of their high incidence and associated mortality. Rapidly increasing evidence suggests that oxidation of LDL is important in atherogenesis. Evidences in support of this include : oxidised LDL is preferentially taken up by the macrophages to create foam cells<sup>4</sup>, oxidised LDL appears to be cytotoxic to the arterial endothelial cells<sup>4</sup> and raised levels of lipid peroxides like MDA have been found in sera of men at high risk of IHD like smokers.<sup>5</sup> This has led to a large volume of data both experimental and epidemiological to correlate the serum levels and intake of vitamin E to the risk of IHD. Experimental data from animal studies have shown that : vitamin E is effective in protecting LDL from oxidation<sup>6</sup>, can inhibit smooth muscle cell proliferation<sup>7</sup> and may reduce risk of IHD through its effect on platelet adhesion<sup>8</sup>. Epidemiological data in humans though controversial appears to overwhelmingly suggest that lowered serum levels and dietary intakes of vitamin E are associated with increased risk of IHD<sup>9, 10, 11</sup>. Unlike other fat soluble vitamins, vitamin E has been shown to be nontoxic even at high doses<sup>12</sup>. This led to a large number of clinical trials using pharmacological doses of vitamin E to reduce incidence of IHD and its complications. These clinical trials have again brought out conflicting results. A critical analysis of these clinical trials has shown that the conflicting results could have been due to:

- a) non-uniformity in the dose and duration of vitamin E administered
- b) no attention having been paid to the micronutrient interactions<sup>13</sup>

Results of the Cambridge Heart Antioxidant study group( CHAOS ), one of the largest clinical trials whose results are available till now, have shown that supplementation of a minimum of 400 IU/day of vitamin E for a period of one year is necessary to reduce complications in subjects with angiographical evidence of coronary arteriosclerosis<sup>14</sup>.



Vitamin E exhibits interactions with both *in vivo* & dietary antioxidants as well as other micronutrients. While studies have already been initiated to look into the effect of combined administration of dietary antioxidants, no attempts are being made to evaluate the role of combined administration of vitamin E and other dietary micronutrients in the prevention of IHD. Our earlier studies have shown that vitamin E exhibits interaction with B-complex vitamins<sup>15,16</sup> and combined administration of vitamin E and B-complex is superior to administration of vitamin E alone in terms of membrane stabilisation<sup>17</sup> & clinical efficacy<sup>18</sup> in humans. There are no studies to evaluate the role of combined administration of vitamin E and B-complex in the prevention of IHD.

Such intervention trials should ideally include clinical events as end points, but will be time consuming, difficult to perform and costly. Before suggesting such expensive clinical trials we have studied in this project the effect of supplementation of vitamin E alone or in combination with B-complex for a period of one year, on certain biochemical markers which could indicate possible beneficial effects in lowering the risk of IHD. We have studied the effect of Vitamin E, and E+B administration on :

1. serum malonaldehyde levels
2. serum total antioxidant status
3. serum levels of total cholesterol, HDL cholesterol and their ratio

#### Materials & Methods :

A total of 84 Malay male volunteers were recruited for the study ranging in age from 18 - 55 years and were divided into three groups according to their age < 30 years ( 30 ), 30 - 45 years( 28 ) and > 45 years ( 26 ). Fasting baseline blood samples were drawn from all the subjects. In each age group volunteers were assigned randomly to one of the treatment groups placebo, Vit.E( 200 Iu twice daily ) and Vit. E+B-complex ( vit.E 200 Iu twice daily + a commercial B-complex tablet twice daily ) keeping the number of volunteers in each treatment group comparable. Supplements were given on a monthly basis and at each visit compliance was ascertained by oral interviews. Followup fasting blood samples were drawn at 3, 6 & 12 months.

2 subjects showed hyperglycaemia in their basal blood sample and 3 showed mild hypertension and were therefore eliminated from the final analysis. In the remaining 79 subjects complete follow up data was available on 72 subjects : <30 yrs.(26), 30 -45 yrs(28)and >45 yrs.(22). Subjects receiving vitamin supplements expressed a general sense of well being and did not report any untoward effects. Generally the followup was poor in the placebo group.

Vitamin E capsules ( Natopherol ) were supplied by Abbot Laboratories Malaysia Sdn.Bhd free of charge.

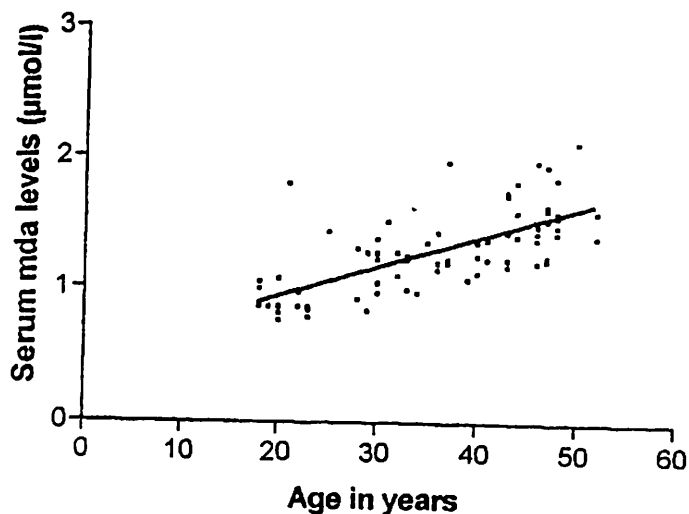
B-complex tablets ( Dumex ) contained : B<sub>1</sub> 10 mg , B<sub>2</sub> 5 mg , B<sub>6</sub> 1 mg, Nicotinamide 50 mg , Calcium Pantothenate 3 mg & B<sub>12</sub> 1 mcg

All blood samples were analysed for :

1. serum MDA levels as Thiobarbituric acid reactive substance as described by Nadiger et al<sup>19</sup>
2. serum total antioxidant status using the kit from Randox laboratories
3. serum tot. Cholesterol & HDL Cholesterol using routine automated methods

## RESULTS & DISCUSSION :

### Correlation between age and serum MDA levels ( Baseline data ) :



#### Results of Linear regression analysis :

Slope  $0.02208 \pm 0.002680$   
Y - intercept  $0.5047 \pm 0.09913$   
X - intercept  $-22.86$   
1/slope  $45.30$

#### 95% Confidence Intervals

Slope  $0.01673$  to  $0.02743$   
Y - intercept  $0.3068$  to  $0.7026$

#### Goodness of Fit

$r^2$   $0.4887$   
 $S_{y.x}$   $0.2342$

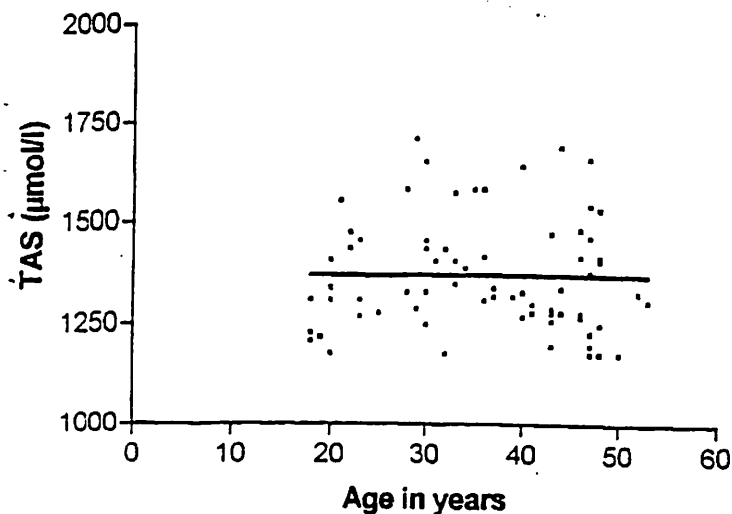
#### Deviation from zero

F  $67.85$   
DFn, Dfd  $1.000, 77$   
P value  $<0.0001$

#### Summary of statistical analysis :

Highly significant positive correlation between serum MDA levels and age. Though the free radical theory of aging has been in vogue for almost 40 years, human data in support of this theory has been very scanty. Results of our study provide human data in support of the free radical theory of aging.

## Correlation between age and serum total antioxidant status ( Baseline data ) :



### Results of Linear regression analysis :

Slope	0.07631 ± 1.583
Y - intercept	1368 ± 58.60
X - intercept	-17920
1/slope	13.10

### 95% Confidence Intervals

Slope	-3.083 to 3.236
Y - intercept	1251 to 1485

### Goodness of Fit

$r^2$	0.00003275
Sy.x	138.8

### Deviation from zero

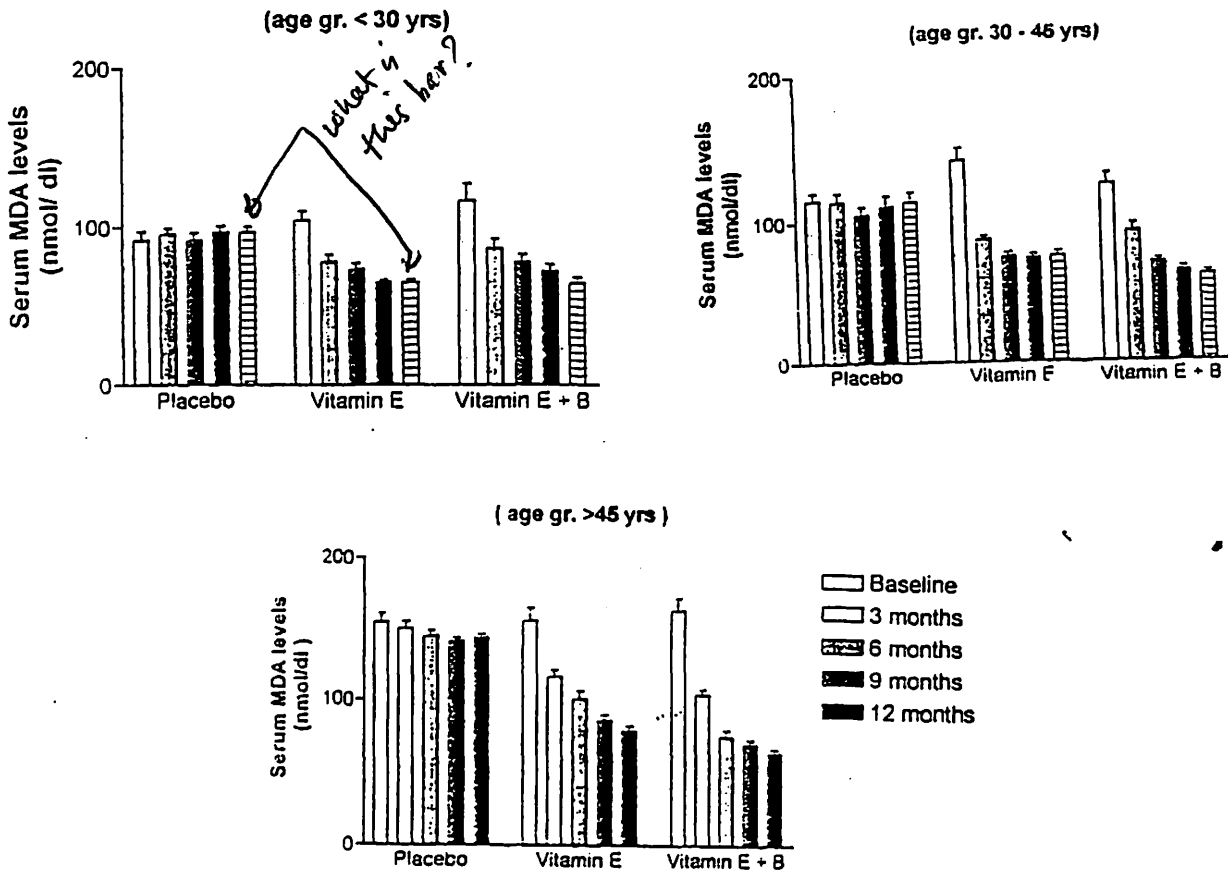
F	0.002325
DFn, Dfd	1.000, 77
P value	0.9617

### Summary of statistical analysis :

No statistically significant correlation between serum total antioxidant status and age. Many workers have attempted to study the changes in individual antioxidant systems like SOD, glutathione system & vitamin E, associated with aging process and the results have been controversial. These conflicting results are thought to be due partly to the existence of close interactions between the different antioxidant systems. It is now suggested that the measurement of total antioxidant status rather than the individual components, reflects the true picture of the antioxidant defense status in an individual.

Taken together our results on serum MDA levels and total antioxidant status in relation to age suggest that there is an imbalance between the oxidative stress and the antioxidant defense systems with advancing age, supporting the free radical theory of aging.

**Effect of Vit. E & E + B-complex supplementation on serum MDA levels ( nmol/dl, Mean  $\pm$  SEM )**



**Summary of statistical analysis :** Supplementation of Vit. E alone and in combination with B-complex resulted in a highly significant reduction in serum MDA levels, in all the three age groups. While there was no difference in the extent of reduction in MDA levels by E alone or in combination with B-complex in the lowest age group ( < 30 yrs ), in the higher age groups ( 30 - 45 & > 45 yrs. ) reduction in serum MDA levels by combined administration of Vit. E and B-complex were significantly higher compared to administration of Vit. E alone. It is interesting to note that the extent of reduction in MDA levels depended on the initial levels, higher the initial levels greater the reduction. However in all the age groups the serum MDA levels appeared to stabilise around a value of 65 nmol/dl. It would be interesting to study the level to which the serum MDA levels can be brought down or is there a critical level of lipid peroxides below which they can not be reduced to maintain normal aerobic metabolism ?

( refer the table and details of statistical analysis on the next page )

**Effect of Vit. E & E + B-complex supplementation on serum MDA levels ( nmoles/dl, Mean  $\pm$  SEM )**

Group ( <30yrs )	Duration of treatment				
	Baseline	3 months	6 months	9 months	12 months
<b>PLACEBO</b> ( 7 )	91.9 $\pm 5.58$	95.9 $\pm 3.7$	91.9 $\pm 4.76$	97.07 $\pm 4.01$	97.45 $\pm 3.47$
<b>Vit. E</b> ( 10 )	104.4 $\pm 6.25$	78.7 $\pm 4.31$ <sup>3, a</sup>	73.8 $\pm 4.02$ <sup>3, b</sup>	66.3 $\pm 1.63$ <sup>3, b</sup>	66.3 $\pm 2.00$ <sup>3, c</sup>
<b>Vit. E + B</b> ( 9 )	106.9 $\pm 11.07$	86.9 $\pm 6.31$ <sup>3, a</sup>	78.7 $\pm 4.66$ <sup>3, b</sup>	73.07 $\pm 4.16$ <sup>3, b</sup>	65.70 $\pm 3.53$ <sup>3, c</sup>

Group ( 30 - 45 yrs )	Duration of treatment				
	Baseline	3 months	6 months	9 months	12 months
<b>PLACEBO</b> ( 6 )	115.8 $\pm 5.51$	114.9 $\pm 6.05$	105.5 $\pm 5.83$	111.5 $\pm 7.37$	114.5 $\pm 7.09$
<b>Vit. E</b> ( 10 )	142.8 $\pm 8.23$	87.7 $\pm 2.53$ <sup>3, c</sup>	75.4 $\pm 3.27$ <sup>3, c</sup>	74.4 $\pm 3.08$ <sup>3, c</sup>	75.9 $\pm 3.52$ <sup>3, c</sup>
<b>Vit. E + B</b> ( 8 )	125.9 $\pm 7.46$	93.2 $\pm 5.60$ <sup>3, c</sup>	71.2 $\pm 2.48$ <sup>3, c</sup>	64.9 $\pm 3.02$ <sup>3, c, x</sup>	62.4 $\pm 2.49$ <sup>3, c, y</sup>

Group ( > 45 yrs )	Duration of treatment				
	Baseline	3 months	6 months	9 months	12 months
<b>PLACEBO</b> ( 6 )	154.7 $\pm 6.54$	150.6 $\pm 5.01$	145.6 $\pm 4.32$	142.1 $\pm 2.54$	144.5 $\pm 2.35$
<b>Vit. E</b> ( 9 )	156.4 $\pm 9.37$	116.8 $\pm 5.31$ <sup>3, c</sup>	101.4 $\pm 5.85$ <sup>3, c</sup>	86.7 $\pm 3.90$ <sup>3, c</sup>	79.2 $\pm 3.74$ <sup>3, c</sup>
<b>Vit. E + B</b> ( 7 )	163.9 $\pm 8.96$	104.4 $\pm 3.98$ <sup>3, c</sup>	75.5 $\pm 4.63$ <sup>3, c, y</sup>	70.3 $\pm 3.28$ <sup>3, c, y</sup>	64.10 $\pm 2.65$ <sup>3, c, y</sup>

Figures in parentheses indicate number of subjects

“ 1, 2, 3 ” - comparisons with baseline values within the groups, ( Paired 't' test),

1  $p < 0.05$ , 2  $p < 0.01$ , 3  $p < 0.001$

“ a, b, c ” - comparisons with placebo values at corresponding time ( unpaired 't' test)

a  $p < 0.05$ , b  $p < 0.01$ , c  $p < 0.001$

“ x, y, z ” - comparisons with Vit.E values at corresponding time ( unpaired 't' test )

x  $p < 0.05$ , Y  $p < 0.01$ , z  $p < 0.001$

**Effect of Vit. E & E + B-complex supplementation on serum Total Antioxidant Status ( TAS ) (  $\mu\text{moles/l}$ , Mean  $\pm$  SEM )**

Group	Duration of treatment				
	Baseline	3 months	6 months	9 months	12 months
<b>PLACEBO</b> ( 19 )	1376 $\pm 39.24$	1407 $\pm 30.07$	1441 $\pm 29.48$	1451 $\pm 22.72$	1441 $\pm 30.15$
<b>Vit. E</b> ( 29 )	1347 $\pm 22.91$	1772 $\pm 44.99$ <sup>3, c</sup>	1694 $\pm 25.61$ <sup>3, c</sup>	1697 $\pm 27.60$ <sup>3, c</sup>	1685 $\pm 26.48$ <sup>3, c</sup>
<b>Vit. E + B</b> ( 24 )	1391 $\pm 26.17$	1754 $\pm 51.44$ <sup>3, c</sup>	1799 $\pm 44.43$ <sup>3, c, x</sup>	1806 $\pm 40.11$ <sup>3, c, x</sup>	1891 $\pm 35.12$ <sup>3, c, z</sup>

Figures in parentheses indicate number of subjects

“ 1, 2, 3 ” - comparisons with baseline values within the groups, ( Paired 't' test ),

1  $p < 0.05$ , 2  $p < 0.01$ , 3  $p < 0.001$

“ a, b, c ” - comparisons with placebo values at corresponding time ( unpaired 't' test )

a  $p < 0.05$ , b  $p < 0.01$ , c  $p < 0.001$

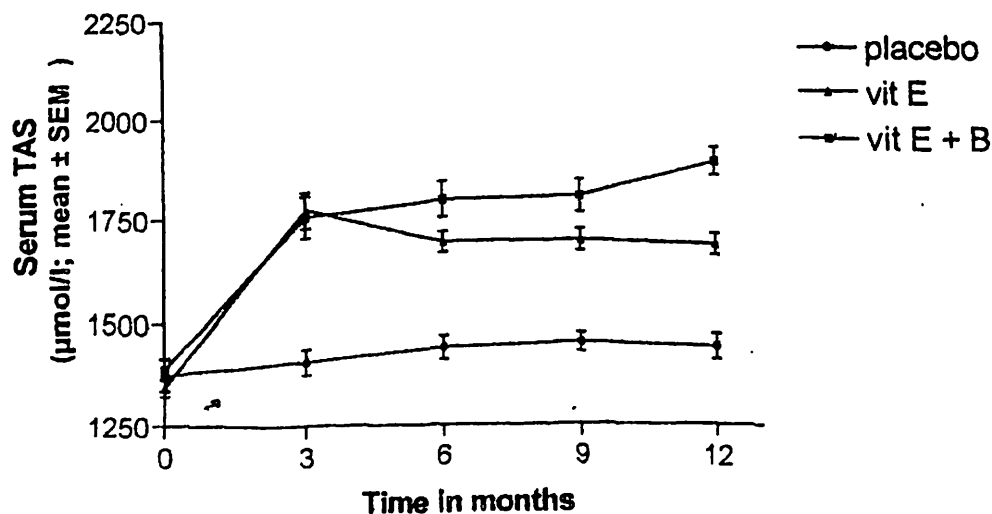
“ x, y, z ” - comparisons with Vit.E values at corresponding time ( unpaired 't' test )

x  $p < 0.05$ , y  $p < 0.01$ , z  $p < 0.001$

**Summary of statistical analysis :** Supplementation of Vitamin E alone for a period of 3 months increased the serum TAS significantly. Continued administration of Vitamin E alone did not bring about any further increase in serum TAS .

Supplementation of Vitamin E + B-complex for a period of 3 months increased serum TAS to the same extent as Vitamin E alone. Continued administration of Vit. E + B-complex resulted in further increase in serum TAS in contrast to Vitamin E alone. The serum TAS values at 6,9 & 12 months were significantly higher in the group supplemented with Vit.E + B-complex compared to the group supplemented with Vit. E alone.

**Effect of vitamin E and E + B-complex supplementations on serum TAS**



**Effect of Vitamin E & E + B-complex administration on serum total Cholesterol levels ( mmol/l, Mean  $\pm$  SEM )**

Group	Duration of treatment				
	Baseline	3 months	6 months	9 months	12 months
<b>PLACEBO</b> ( 19 )	5.9 $\pm 0.21$	5.9 $\pm 0.17$	5.9 $\pm 0.19$	5.8 $\pm 0.17$	5.8 $\pm 0.18$
<b>Vit. E</b> ( 29 )	5.6 $\pm 0.24$	5.3 $\pm 0.29$ <sup>2, a</sup>	5.2 $\pm 0.12$ <sup>2, b</sup>	5.0 $\pm 0.16$ <sup>2, c</sup>	4.9 $\pm 0.17$ <sup>3, c</sup>
<b>Vit. E + B</b> ( 24 )	5.5 $\pm 0.19$	5.2 $\pm 0.19$ <sup>3, c,</sup>	5.1 $\pm 0.20$ <sup>3, c,</sup>	4.9 $\pm 0.16$ <sup>2, c,</sup>	4.7 $\pm 0.13$ <sup>3, c,</sup>

Figures in parentheses indicate number of subjecte

“ 1, 2, 3 ” - comparisons with baseline values within the groups,( Paired 't' test),

1  $p < 0.05$ , 2  $p < 0.01$ , 3  $p < 0.001$

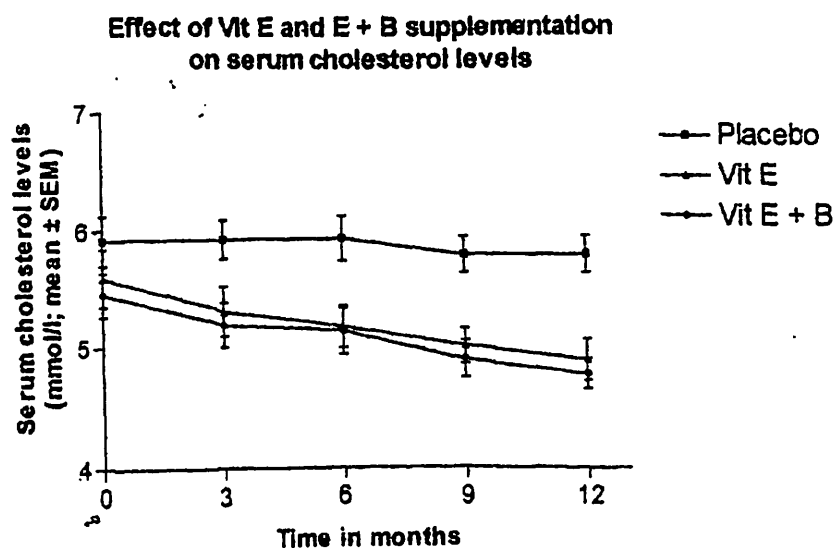
“ a, b, c ” - comparisons with placebo values at corresponding time ( unpaired 't' test)

a  $p < 0.05$ , b  $p < 0.01$ , c  $p < 0.001$

“ x,y, z ” - comparisons with Vit.E values at corresponding time ( unpaired 't' test )

x  $p < 0.05$ , y  $p < 0.01$ , z  $p < 0.001$

**Summary of statistical analysis :** Supplementation of Vit. E alone or in combination with B-complex reduces serum total cholesterol levels. There were no statistically significant differences in the extent of reduction between the two groups.



**Effect of Vitamin E & E + B-complex supplementation on serum HDL Cholesterol levels ( mmol/l , Mean  $\pm$  SEM )**

Group	Duration of treatment				
	Baseline	3 months	6 months	9 months	12 months
<b>PLACEBO</b> ( 19 )	1.21 $\pm 0.076$	1.21 $\pm 0.063$	1.12 $\pm 0.055$	1.13 $\pm 0.059$	1.14 $\pm 0.061$
<b>Vit. E</b> ( 29 )	1.07 $\pm 0.044$	1.13 $\pm 0.042$	1.18 $\pm 0.052$	1.21 $\pm 0.062$	1.21 $\pm 0.067$
<b>Vit E + B</b> ( 24 )	1.10 $\pm 0.063$	1.36 $\pm 0.072$	1.48 $\pm 0.089$ <sup>2, a, y</sup>	1.69 $\pm 0.084$ <sup>3, b, y</sup>	1.80 $\pm 0.086$ <sup>2, b, z</sup>

Figures in parentheses indicate number of subjects

“ 1, 2, 3 ” - comparisons with baseline values within the groups, ( Paired 't' test),

1  $p < 0.05$ , 2  $p < 0.01$ , 3  $p < 0.001$

“ a, b, c ” - comparisons with placebo values at corresponding time ( unpaired 't' test)

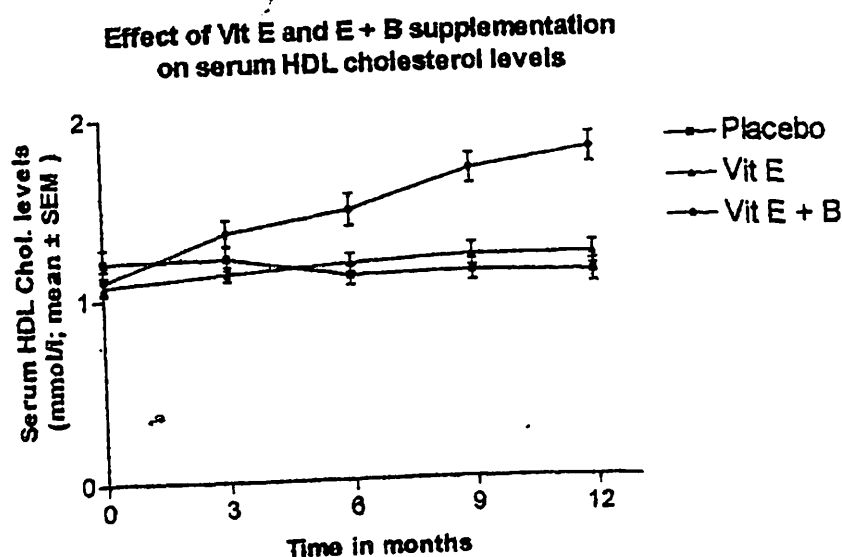
a  $p < 0.05$ , b  $p < 0.01$ , c  $p < 0.001$

“ x, y, z ” - comparisons with Vit.E values at corresponding time ( unpaired 't' test )

x  $p < 0.05$ , y  $p < 0.01$ , z  $p < 0.001$

**Summary of statistical analysis:** Supplementation of vitamin E alone showed a trend towards elevation of serum HDL cholesterol levels, however these elevations were not statistically significant

Supplementation of Vitamin E in combination with B-complex brought about a statistically significant elevation in serum HDL cholesterol levels after 6 months of supplementation and increased further with continued supplementation for one year.





**Effect of Vitamin E & E + B-complex supplementation on serum Tot. Chol. : HDL Chol. ratio ( Mean  $\pm$  SEM )**

Group	Duration of treatment				
	Baseline	3 months	6 months	9 months	12 months
PLACEBO ( 19 )	5.4 $\pm 0.41$	5.1 $\pm 0.32$	5.5 $\pm 0.36$	5.6 $\pm 0.40$	5.5 $\pm 0.34$
Vit. E ( 29 )	5.4 $\pm 0.28$	4.8 $\pm 0.22$	4.7 $\pm 0.23$	4.5 $\pm 0.25$ <sup>1, a</sup>	4.4 $\pm 0.26$ <sup>2, b</sup>
Vit. E + B ( 24 )	5.4 $\pm 0.40$	4.1 $\pm 0.29$ <sup>2, b</sup>	3.8 $\pm 0.29$ <sup>3, c, x</sup>	3.1 $\pm 0.23$ <sup>3, c, z</sup>	2.8 $\pm 0.16$ <sup>3, c, z</sup>

Figures in parentheses indicate number of subjects

“ 1, 2, 3 ” - comparisons with baseline values within the groups,( Paired 't' test),

1  $p < 0.05$ , 2  $p < 0.01$ , 3  $p < 0.001$

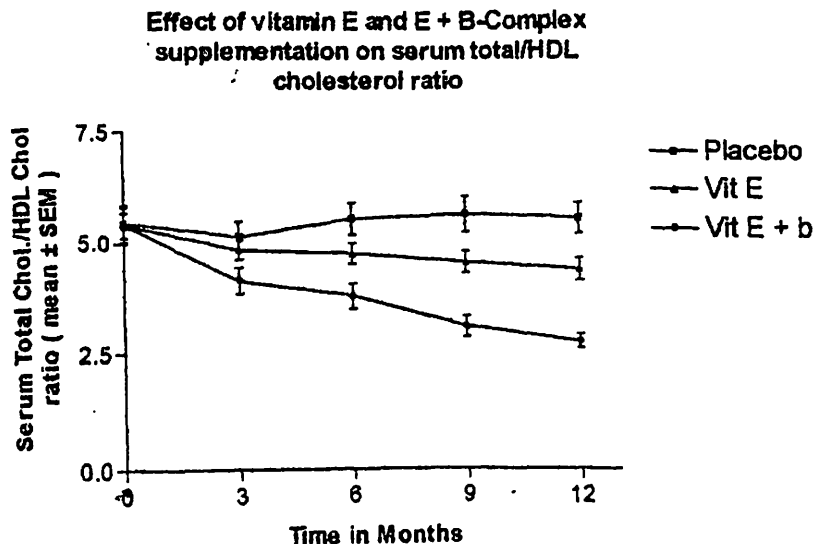
“ a, b, c ” - comparisons with placebo values at corresponding time ( unpaired 't' test)

a  $p < 0.05$ , b  $p < 0.01$ , c  $p < 0.001$

“ x, y, z ” - comparisons with Vit.E values at corresponding time ( unpaired 't' test )

x  $p < 0.05$ , y  $p < 0.01$ , z  $p < 0.001$

**Summary of statistical analysis :** Supplementation of Vitamin E alone or in combination with B-complex significantly reduced the serum total Chol : HDL Chol. ratio. However Vitamin E alone was able to reduce the ratio after 9 months of supplementation while combination of Vit. E and B-complex reduced the ratio after 3 months of supplementation. Cobined supplementation of Vit. E and B-complex brought about a progressive reduction of the ratio and the reductions were significantly higher compared to Vit. E alone at all time points, suggesting that combined supplementation of Vit. E and B-complex was more effective in reducing the Tot. Chol : HDL Chol. ratio. This may imply that combined supplementation of Vit. E and B-complex may afford better protection against atherosclerosis compared to supplementation of Vit. E alone.



## Summary of Results & Conclusions:

Results of the study indicated :

(1) serum MDA levels increase with age without any change in the total antioxidant status which supports the theory that imbalance between oxidative stress and antioxidant status may be involved in aging process,

(2) supplementation of Vit. E & B-complex reduced serum MDA levels and increased total antioxidant status, E+B administration being superior to E alone

(3) both E alone and E+B administration reduced total cholesterol levels to the same extent

(4) supplementation of E alone did not affect HDL cholesterol levels significantly but E+B administration significantly increased the HDL cholesterol,

(5) both E alone and E+B administration reduced the tot. chol.:HDL chol. ratio, the reduction being significantly more with E+B compared to E alone.

## Conclusions:

It is concluded from the study that administration of E+B as opposed to E alone provides better protection against free radical mediated damage . E+B administration may also be more effective in prevention of IHD through their effect on HDL cholesterol levels.

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