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Immunomodulatory effect of vitamin e in Wstar albino rats (*Rattus norvegicus*)

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Abstract
Twenty four Wistar Albino rats an age of five to six month and weighing 100 to 200 g body weight were randomly divided into four experimental groups each group comprising of six rats. The group I given distilled water and group II given Vitamin E 200 µg/kg body weight administered orally were normal non-antigen stimulated groups, in these groups, group I served as control. The group III given antigen 0.4 ml intraperitoneal and distilled water and group IV given antigen 0.4 ml intraperitoneal plus Vitamin E 200 µg/kg body weight administered orally were antigen stimulated groups, in these, Group III which was given only antigen and distilled water, served as antigen control group. All these groups were fed with pelleted feed and given water *ad libitum*. They were maintained hygienically under standard laboratory conditions. The rats were allowed to get accustomed to laboratory environment for a week before the start of the experiment. The blood was collected from all the groups on day zero, i.e., immediately before administering distilled water and drug and then on the 14, 20, 28, 35 and 42 days after treatment. In non-antigen stimulated group II highest immunoglobulin concentration was seen on day 35 and in antigen stimulated group IV the highest immunoglobulin concentration was found on day 28. But There was no significant (P>0.05) difference in the mean total serum immunoglobulin concentration of normal non-antigen and antigen stimulated groups when compared with their respective control groups. But in both normal non-antigen stimulated and antigen stimulated groups which were given Vitamin E alone and Vitamin E plus antigen, did not produce any significant change in the values of TIG, TSP, SA, TSG, A:G ratio and DNCB induced skin thickness when compared with their respective control groups. But There was a significant ($P< 0.05$) increase of TLC values in group II from day 35th to 42nd and in the group IV the TLC values increased on day 20, 28, 35 and 42, compared to their respective non-antigen and antigen control groups (Group I and III). There was a significant ($P< 0.05$) increase of ALC values in both group II and IV compared to their respective control groups (Group I and III), the increase was seen from 35th to 42nd day. Also there was a significant ($P< 0.05$) increase of PI values in group II and IV compared to their respective control groups, the increase was seen from 28th to 42nd day.

Keywords: Vitamin E antigen innate immunity nonspecific defence host mechanism

Introduction
The survival of the animal depends on the successful defence against microbial invasions. A functional immune system is essential to protect living organisms from external injuries like virus, bacteria, fungi, parasites and certain environmental pollutants associated with morbidity, mortality and decrease performance. Thereby immune system plays an important role to sustain host defence mechanisms to maintain homeostasis.
Immune deficiencies following severe thermal injuries, trauma and sepsis can predispose patients to potential infections. The development of antibacterial has revolutionized the treatment of bacterial disease and eventual complete control of bacterial diseases was predicted. But development of drug resistance by the microbes combined with immunodeficiency in the patients results in not only failure of chemotherapy, but also aggravates the situation by increasing the susceptibility of host to infection. Therefore, the necessity of good immune-modulators is being realized in recent times.
Immunomodulation by drugs and chemicals appears to have its rudimentary beginning in the use of herbs and decoctions which were used to cure several maladies in oriental cultures. Ancient Chinese medicine used ginsing tea, which was found to have immunomodulating activity by virtue of its content of trace mineral germanium (Goodman, 1988) [8].
Immunomodulation by pharmacological manipulations as a therapy for revitalizing the suppressed immune system is under extensive trials. A large number of drugs and nutrients,

such as vitamins and trace elements are being evaluated for the immunomodulating effects.

Among the various vitamins and trace minerals studied, the Vitamin E has been reported to have beneficial effects in boosting the immune responses and resistance to various infections. Vitamin E is a fat soluble Vitamin and is the name given to a group of biologically active tocopherols. The principal naturally occurring active compound is d-α tocopherol and its chemical name is 5, 7, 8- trimethyltocol.

Vitamin E form the vital part of biological antioxidant system thereby stabilizing the cell membrane. The Vitamin E is used in clinical practice in treatment of muscular dystrophy, sterility, abortion, poor breeding performance and exudative diathesis. Although the effect of Vitamin E as immunomodulant is known to some extent, but there is paucity of information which needs further research to generate more information on its immunomodulatory effects. Keeping the above facts in view the experiments was designed to study the effect of Vitamin E in rats on,

Natural host defence mechanism
and
Cell mediated immune responses.

Materials and Methods

The experiment was designed to evaluate the effects of Vitamin E on the immune status in rats i.e., natural host defence mechanisms in normal non-antigen stimulated rats and the immune response in antigen stimulated rats.

Animals

Twenty four Wistar Albino rats an age of five to six month and weighing 100 to 200 g body weight were obtained from the Experimental Animal House of the University of Agricultural Sciences, Bangalore. They were randomly divided into four experimental groups each group comprising of six rats. They were fed with pelleted feed and given water *ad libitum*. They were maintained hygienically under standard laboratory conditions (Alastrain, 1989) [1]. The rats were allowed to get accustomed to laboratory environment for a week before the start of the experiment.

Drugs

Vitamin E (Vitamin E in the powder form each gram containing Vitamin E 100 mg)supplied by M/s. Vetcare, Division of Tetragon Chemie Ltd., Bangalore was used in the experiment.

Antigen (sheep RBC suspension)

Sheep red blood cells (SRBC) were used as the antigen. Sheep blood was collected in Alsever's solution and stored at 4ºC for one week. SRBC's were washed three times in large volume of pyrogen free sterile normal saline and two per cent SRBC suspension was prepared at the time of administration (Shah and Gupta, 1998) [15]. The sheep blood was collected from the Sheep Farm of the University of Agricultural Sciences, Bangalore.

Experimental Methods

The animals were divided into four experimental groups
The details of the treatment given were as follows
Group I distilled water administered orally (control)
Group II Vitamin E 200 µg/kg body weight administered orally
Group III Antigen 0.4 ml intraperitoneal and distilled water administered orally (antigen control)
Group IV Antigen 0.4 ml intraperitoneal and Vitamin E 200 µg/kg body weight administered orally

Groups I and II were normal non-antigen stimulated groups, in these groups, Group I which received distilled water, served as control.

The Groups III and IV were antigen stimulated groups, Among them, Group III which was given only antigen and distilled water, served as antigen control.

Collections of blood samples

The blood samples were collected in heparinised vials for estimation of phagocytic index and in Na_2 EDTA vials for estimation of cellular parameters. The blood was also collected in the sterile test tube for separation of serum which was stored at 4ºC in a refrigerator. The rats were anaesthetized with diethyl ether and blood was collected from orbital plexus in both normal, non –antigen stimulated and antigen stimulated groups. The blood was collected on Day zero, i.e., immediately before administering distilled water and drug and then on the 14, 20, 28, 35 and 42 days after treatment.

The serum was used to study the following humoral parameters

Total serum immunoglobulin concentration (TIG)
Total serum protein (TSP)
Serum albumin concentration (SA)
Total serum globulin (TSG)
Serum albumin: globulin ration (A:G)

Heparinised blood was used to study

Phagocytic index (PI)

The whole blood was used to study the following cellular parameters

Total leucocyte count (TLC)
Absolute lymphocyte count (ALC).

The delayed type of hypersensitivity was measured by using

Dinitro-chlorobenzene (DNCB) skin sensitivity (DTH)

Total serum immunoglobulin concentration (TIG)

Total serum immunoglobulin concentration was estimated by following the procedure as described by Mullen (1975) [16]. The procedure in brief is described below.

Solutions

Zinc sulphate solution

This solution was prepared freshly by dissolving 208 mg of zinc sulphate ($ZnSO_4 7H_2O$) in one litre of distilled water, from which carbon dioxide was excluded by boiling it for 10 to 15 minutes and then stored the solution in aspirator bottle containing soda lime in the stopper. This solution was prepared freshly every time.

Barium chloride solution

A solution of barium chloride ($BaCl_2.H_2O$) was prepared by dissolving 1.15 g of barium chloride in 100 ml of distilled water with 0.2 N sulphuric acid. This solution was considered to give a standard turbidity reading of 20 units under standard conditions.

Test procedure
The measurements were made in a colorimeter with Cuvettes inserted at 498 nanometre. To a control tube containing 6.0 ml of distilled water and a test tube containing 6.0 ml of zinc sulphate solution 0.1 ml of serum was added and vortexed. Then they were left to stand for one hour at room temperature (20ºC) before measurement of the turbidity developed.
First, colorimeter was set to zero, using a tube with distilled water, the turbidity reading of the test samples were found by subtracting the reading obtained from the control tube and then multiplying the difference by 10. The readings were related to that of the 20 units given by barium chloride standard solution similarly treated.

X = Zst unit
R = coefficient of correlation IgG (a) mg/ml
a = 0.97× - 1.95, γ = 0.959, IgM (b) mg/ml
b = 0.1× - 0.24, γ = 0.780, IgG + IgM (c), mg/ml
c = 1.07× - 2.17, γ = 0.962

Total serum protein concentration (TSP)
Total serum protein concentration was estimated using colorimetric method of Doumas (1981) [5].

Test procedure
Mixed 20 µl total serum protein standard and 1000 µl total protein reagent in a Cuvettes and incubated for five minutes. The absorbance was read at 520 to 560 nanometre and the standard gave a total serum protein concentration of four g/dl. Then the test samples were run similarly, by mixing 20 µl of serum samples to be tested and 1000 µl of total protein reagent in a cuvette. It was incubated for five minutes and then cuvette was introduced into a colorimeter and the readings were taken.

Serum albumin concentration
Total serum albumin concentration was estimated using colorimetric method (Doumas and Watson, 1971) [4].

Test procedure
Mixed 10 µl of albumin standard and 1000 µl of albumin reagent in a cuvette and incubated for five minutes. The absorbance was read at 630 nanometre and the standard gave a total serum albumin concentration of four g/dl. Then the test samples were similarly by mixing 10 µl of serum sample to be tested and 1000 µl of albumin reagent in a cuvette. It was incubated for five minutes and then the cuvettes was introduced into a colorimeter and readings were taken.

Serum albumin - globulin ration (A: G)
Total serum protein and albumin values were calculated by the method described above from serum protein values and serum albumin values; serum globulin values were derived. Then albumin globulin ratio (A: G) was calculated.

Total leucocyte count (TLC) and absolute lymphocyte count (ALC)
Total leucocyte count and differential leucocyte count were estimated by the procedure described by Jain (1990) [12]. From the values, the absolute lymphocyte count was calculated.

Phagocytic index (PI)
The PI was assessed following the procedure outlined by Vanfurth *et al.* (1979) [20]. using *staphylococcus* as antigen.

Procedure
The blood samples were collected individually in sterile heparinized vials. Then to each tube containing 1 ml of heparinized blood sample, 0.1 ml killed whole cell suspension of *staphylococcus* antigen was added. The vials were incubated at 37ºc for one hour. Then smears were prepared and stained with Giemsa's stain. The mean number of bacteria ingested per 100 phagocytes were calculated.

Dinitrochlorobenzene (DNCB) skin sensitivity test
This test was performed on the lines of Brummerstedt and Basse (1973) [2]. An area of three cm in diameter was marked on the left flank region. The site was clipped off the hair close to the skin. A metallic ring of three centimetre diameter was used to mark the area for primary sensitization. Two per cent solution of 2, 4- dinitrochlorobenzene (DNCB) in acetone was used and 0.4 ml of this solution was applied slowly drop by drop to the marked area keeping the metallic ring at the site. The solution was allowed to evaporate quickly by blowing gently during application. After 14 days of primary sensitization, a challenge dose of 0.25 ml was applied similarly.
Skin thickness was measured using slide callipers before challenge dosing i.e., at zero hour, 24 and 48 hours intervals after challenge dosing.

Statistical analysis
Mean values and standard error of mean (× ± SE) were calculated. *Student's "t"* test was applied to assess the significance (Snedecor and Cocharan, 1976) [17].

Results and Discussion
All the experimental rats appeared clinically normal during the experimental period. The group I (non-antigen control group) which received distilled water, group II which received Vitamin E were described as normal non-antigen stimulated groups. The group III (antigen control group) which received antigen plus distilled water, group IV which received antigen plus Vitamin E were described as antigen-stimulated groups.
The mean TIG concentrations of normal non- antigen stimulated and antigen stimulated groups were presented in table 1. The mean TIG concentration in the group I ranged from 25.20±0.59 to 29.08±0.39, in group II varied from 26.04±0.75 to 29.16±0.74, in the group III varied from 25.49±0.53 to 30.20±0.75 and in the group IV ranged from 26.07±0.52 to 30.83±0.52.
In non-antigen stimulated group II highest immunoglobulin concentration was seen on day 35 and in antigen stimulated group IV the highest immunoglobulin concentration was found on day 28. But There was no significant ($P>0.05$) difference in the mean total serum immunoglobulin concentration of normal non-antigen and antigen stimulated groups when compared with their respective control groups.
These observations are in accordance with Franklin *et al.* (1998) [7]. who noted that serum immunoglobulin IgM and IgG proportions were not affected in a calves supplemented with Vitamin A (0, 15000 and 30000 IU/day) up to six weeks of age, Tamara *et al.* (1999) [19]. found that there was no variation in the serum levels of immunoglobulins in humans treated with Vitamin B_{12}.
The mean TSP concentrations of normal non -antigen and antigen stimulated rats were presented in table 2. The mean TSP concentration in the group I ranged from 5.540±0.153 to

6.243±0.048, in the group II varied from 5.578±0.123 to 6.283±0.058, in the group III varied from 5.538±0.167 to 6.240±0.040 and in the group IV ranged from 5.558±0.091 to 6.266±0.071.

There was no significant (P>0.05) difference in the mean TSP concentration of normal non-antigen and antigen treated groups when compared with their respective control groups.

Similar result was reported by Franklin *et al.* (1998) [7]. in calves given Vitamin A at a dose rate of 0, 15000 and 30000 IU/kg up to six week of age which did not show any variation in total serum protein levels.

The mean SA concentrations of normal non- antigen stimulated and antigen stimulated groups were presented in table 3. The mean SA concentration in the Group I ranged from 3.585±0.068 to 3.685±0.068, in the Group II varied from 3.600±0.079 to 3.701±0.053. in the Group III varied from 3.596±0.065 to 3.713±0.054 and in the group IV ranged from 3.598±0.036 to 3.721±0.053.

There was no significant (P>0.05) difference in the mean total SA concentration of normal non-antigen stimulated and antigen stimulated groups when compared with their respective control groups. This is in accordance with the calves given trace minerals, zinc (5mg/kg body weight) for a period of 30 days and vaccinated with a live avirulent vaccine against trichophytosis showed lower albumin levels when compared with those given vaccine alone (Paulik *et al.* 1990) [14].

The mean TSG concentrations of normal non- antigen stimulated and antigen stimulated groups were presented in table 4. The mean TSG concentration in the group I ranged from 1.955±0.099 to 2.558±0.098, in the Group II varied from 1.978±0.177 to 2.600±0.103, in the Group III varied from 1.941±0.122 to 2.546±0.093 and in the Group IV ranged from 1.960±0.085 to 2.558±0.095.

But there was no significant (P>0.05) difference in the mean total TSG concentration of normal non-antigen stimulated and antigen stimulated groups when compared with their respective control groups. On contrary there was increased gamma globulin levels against sheep erythrocytes in Vitamin A deficient chickens (Hall, 1976) [10].

The mean A: G ratio of normal non- antigen stimulated and antigen stimulated groups were presented in table 5. The mean A:G ratio in the Group I ranged from 1.45±0.04 to 1.84±0.07, in the Group II varied from 1.44±0.06 to 1.89±0.18, in the group III varied from 1.45±0.07 to 1.88±0.11 and in the group IV ranged from 1.45±0.03 to 1.84±0.08.

There was no significant (P>0.05) difference in the mean A:G ration of normal non- antigen stimulated and antigen stimulated groups when compared with their respective control groups. No reports seem to be available on the effect of vitamins and minerals on albumin –globulin ratio.

Neutrophil is a phagocytic cell. The function of phagocytic cell is phagocytosis. If there is change in phagocytic index after treatment with a drug it indicates that the drug had altered the phagocytic function of phagocytic cell. The mean PI values of normal non- antigen stimulated and antigen stimulated groups were presented in table 6. The mean PI values in the Group I ranged from 1.72±0.04 to 1.88±0.02, Group II varied from 1.67±0.06 to 2.00±0.05, in the Group III varied from 1.71±0.03 to 1.94±0.05 and in the group IV ranged from 1.73±0.04 to 2.11±0.04.

There was a significant ($P< 0.05$) increase of PI values in group II and IV compared to their respective control groups. the increase was seen from 28th to 42nd day. In contrary Sommer *et al.* (1975) [18]. and Hogon *et al.* (1991) [11]. reported that neither phagocytic index nor percentage of neutrophils phagocytizing differed in cow injected with Vitamin E.

This finding is in accordance with De la Fuente (1998) [6]. who reported that there was a significant increase in phagocytic functions of the neutrophils in women administered 1 g of Vitamin C and 200 mg of Vitamin E daily for 16 weeks.

The mean TLC values of normal non- antigen stimulated and antigen stimulated groups were presented in table 7. The mean TLC values in the group I ranged from 5008.33±96.10 to 5308.33±39.61, in the group II varied from 5016.66±51.09 to 5483.33±52.70, in the Group III varied from 5033.33±72.64 to 8033.33±187.82 and in the group IV ranged from 5000.00±38.72 to 8925.00±218.99.

There was a significant ($P< 0.05$) increase of TLC values in group II from day 35th to 42nd and in the group IV the TLC values increased on day 20, 28, 35 and 42, compared to their respective non-antigen and antigen control groups (Group I and III).

Lymphocytes are the important cells associated with immunological reaction, and their presence in large number will enhance the immune status of the animal. The mean ALC values of normal non- antigen stimulated and antigen stimulated groups were presented in table 8. The mean ALC values in the group I ranged from 75.33±1.80 to 81.50±1.52, in the group II varied from 75.66±2.90 to 85.16±1.35, in the Group III varied from 75.66±2.31 to 85.66±0.80 and in the group IV ranged from 76.00±2.38 to 87.33±0.66.

There was a significant ($P< 0.05$) increase of ALC values in both group II and IV compared to their respective control groups (Group I and III). The increase was seen from 35th to 42nd day.

Similar results were observed in calves supplemented with Vitamin E at the dose rate of 1 g orally it showed increase in the mean lymphocyte stimulation indices. Cipriano *et al.* (1982) [3]. Noted that increased in lymphocyte count in patients supplemented with Vitamin B_{12}. On the contrary, there was significantly low lymphocyte numbers in a patients supplemented with Vitamin C 200 mg/day for 90 days (Jayachandran and Pannerselvam, 1998) [13].

The cutaneous sensitization with DNCB is considered as one of the simple *in-vivo* tests for measuring the cell mediated immune status of the individual. The mean skin thickness of normal non-antigen and antigen stimulated rats are presented in table 9. The mean skin thickness in the group I ranged from 1.250±0.158 to 2.333±0.083, in the group II varied from 1.208± 0.108 to 2.125±0.085, in the group III varied from 1.291±0.135 to 2.250±0.091 and in the group IV ranged from 1.666±0.083 to 2.208±0.163.

There was no significant (P>0.05) difference in the mean skin thickness of normal non- antigen stimulated and antigen stimulated groups when compared with their respective control groups. This indicates that both non-specific as well as specific cell mediated immune responses were not altered.

Similar observation was recorded by Girodon *et al.* (1999) [9] in a long term daily supplementation for a period of 6 months of trace minerals (zinc sulphate and selenium sulphide) or Vitamins (ascorbic acid and Vitamin E) in institutionalized elderly people which did not cause any effect on delayed type of hypersensitivity skin response. On the contrary in aged persons supplemented with 50 and 100 mg of Vitamin E there was significant increase in the cumulative diameter of the skin induration resulting from the delayed type-hypersensitivity test.

Table 1: Total serum immunoglobulin concentration (mg/ml) in normal non-antigen stimulated and antigen stimulated rats

	Non- antigen stimulated groups		Antigen stimulated group	
Time interval in days	Group I	Group II	Group III	Group IV
0	25.20±0.59	26.04±0.75	25.49±0.53	26.07±0.52
14	26.11±0.51	26.22±0.47	26.41±0.37	26.69±0.42
20	26.94±0.17	26.06±0.28	27.20±0.27	27.28±0.32
28	28.08±0.39	28.20±0.41	30.20±0.75	30.29±0.69
35	29.04±0.58	29.16±0.74	30.08±0.58	30.83±0.52
42	28.29±0.38	28.75±0.79	29.33±0.70	29.45±0.48

Antigen was administered on days 14 and 20
Values: Mean ± S.E.
N = 6
P >0.05

Table 2: Total serum protein concentration (g/dl) in normal non-antigen stimulated and antigen stimulated rats

	Non- antigen stimulated groups		Antigen stimulated group	
Time interval in days	Group I	Group II	Group III	Group IV
0	5.540±0.153	5.578±0.123	5.538±0.167	5.558±0.091
14	5.823±0.022	5.863±0.017	5.843±0.180	5.898±0.070
20	6.123±0.115	6.033±0.090	6.156±0.103	6.148±0.069
28	6.243±0.048	6.283±0.058	6.238±0.050	6.258±0.063
35	6.220±0.050	6.275±0.066	6.240±0.040	6.220±0.050
42	6.211±0.056	6.266±0.076	6.201±0.047	6.250±0.068

Antigen was administered on days 14 and 20
Values: Mean ± S.E.
N = 6
P >0.05

Table 3: Total serum albumin concentration (g/dl) in normal non-antigen stimulated and antigen stimulated rats

	Non- antigen stimulated groups		Antigen stimulated group	
Time interval in days	Group I	Group II	Group III	Group IV
0	3.585±0.068	3.600±0.079	3.596±0.065	3.598±0.036
14	3.615±0.058	3.605±0.021	3.623±0.059	3.615±0.031
20	3.630±0.061	3.635±0.027	3.660±0.060	3.645±0.074
28	3.685±0.068	3.683±0.049	3.691±0.045	3.700±0.041
35	3.681±0.043	3.701±0.053	3.713±0.054	3.721±0.053
42	3.678±0.047	3.698±0.048	3.693±0.044	3.701±0.053

Antigen was administered on days 14 and 20
Values: Mean ± S.E.
N = 6
P >0.05

Table 4: Total serum globulin concentration (g/dl) in normal non-antigen stimulated and antigen stimulated rats

	Non- antigen stimulated groups		Antigen stimulated group	
Time interval in days	Group I	Group II	Group III	Group IV
0	1.955±0.099	1.978±0.177	1.941±0.122	1.960±0.085
14	2.208±0.215	2.258±0.162	2.220±0.196	2.283±0.074
20	2.326±0.199	2.391±0.096	2.496±0.137	2.503±0.068
28	2.558±0.098	2.600±0.103	2.546±0.093	2.558±0.095
35	2.538±0.085	2.573±0.105	2.526±0.081	2.545±0.078
42	2.533±0.058	2.568±0.083	2.508±0.058	2.548±0.043

Antigen was administered on days 14 and 20
Values: Mean ± S.E
N = 6
P >0.05

Table 5: Total serum albumin: globulin ration in normal non-antigen stimulated and antigen stimulated rats

	Non- antigen stimulated groups		Antigen stimulated group	
Time interval in days	Group I	Group II	Group III	Group IV
0	1.84±0.07	1.89±0.18	1.88±0.11	1.84±0.08
14	1.73±0.19	1.63±0.11	1.71±0.19	1.59±0.05
20	1.61±0.13	1.52±0.06	1.45±0.07	1.45±0.07
28	1.45±0.08	1.42±0.07	1.48±0.09	1.45±0.06
35	1.45±0.06	1.45±0.08	1.47±0.06	1.46±0.05
42	1.45±0.04	1.44±0.06	1.47±0.04	1.45±0.03

Antigen was administered on days 14 and 20
Values: Mean ± S.E
N = 6
P >0.05

Table 6: Phagocytic index in normal non-antigen stimulated and antigen stimulated Rats

	Non- antigen stimulated groups		Antigen stimulated group	
Time interval in days	Group I	Group II	Group III	Group IV
0	1.72±0.04	1.67±0.06	1.71±0.03	1.73±0.04
14	1.82±0.02	1.85±0.02	1.83±0.02	1.84±0.01
20	1.83±0.01	1.86±0.03	1.87±0.12	1.90±0.16
28	1.85±0.01	1.97±0.03*	1.92±0.03	2.04±0.03*
35	1.88±0.02	1.98±0.03*	1.94±0.05	2.11±0.04*
42	1.87±0.02	2.00±0.05*	1.93±0.04	2.06±0.04*

Antigen was administered on days 14 and 20
Values: Mean ± S.E.
N = 6
P >0.05

Table 7: Total leucocyte count (per cu.mm) in normal non-antigen stimulated and antigen stimulated rats

	Non- antigen stimulated groups		Antigen stimulated group	
Time interval in days	Group I (Distilled water Control)	Group II (vitamin E)	Group III (Antigen and distilled water antigen control)	Group IV Antigen and vitamin E
0	5008.33±96.10	5016.66±51.09	5033.33±72.64	5000.00±38.72
14	5066.66±57.24	5075.00±79.31	5075.00±54.38	5108.33±50.68
20	5041.66±47.28	5141.66±43.61	7900.00±263.31	8758.33±243.72*
28	5141.66±61.12	5233.33±24.71	8033.33±187.82	8925.00±218.99*
35	5308.33±39.61	5483.33±52.70*	7983.33±190.02	8891.66±245.76*
42	5158.33±63.79	5391.66±39.61*	7933.33±200.27	8833.33±218.19*

Antigen was administered on days 14 and 20
Values: Mean ± S.E.
N = 6
P >0.05

Table 8: Absolute lymphocyte count (%) in normal non-antigen stimulated and antigen stimulated rats

	Normal non- antigen stimulated groups		Antigen stimulated group	
Time interval in days	Group I (Distilled water Control)	Group II (vitamin E)	Group III (Antigen and distilled water antigen control)	Group IV Antigen and vitamin E
0	75.33±1.80	75.66±2.90	75.66±2.31	76.00±2.38
14	78.33±2.13	79.16±0.90	79.33±2.15	80.83±1.24
20	80.16±2.02	83.66±0.84	84.33±1.30	86.00±0.73
28	81.50±1.52	84.50±0.99	85.66±0.80	86.83±0.16
35	81.00±0.57	85.16±1.35*	84.83±0.87	87.33±0.66*
42	80.83±1.13	84.50±1.17*	84.00±0.85	87.00±0.81*

Antigen was administered on days 14 and 20
Values: Mean ± S.E.
N = 6
P >0.05

Table 9: Dinitrochlorobenzene skin sensitivity test (mm) in normal non-antigen stimulated and antigen stimulated rats

	Non- antigen stimulated groups		Antigen stimulated group	
Time interval in days	Group I	Group II	Group III	Group IV
0	1.250±0.158	1.208±0.100	1.291±0.135	1.666±0.083
24	2.333±0.083	2.125±0.085	2.250±0.091	2.208±0.163
48	1.666±0.139	1.708±0.076	1.750±0.018	1.791±0.119

Antigen was administered on days 14 and 20
Values: Mean ± S.E.
N = 6
P >0.05

Conclusion
In both normal non-antigen stimulated and antigen stimulated groups which were given Vitamin E alone and Vitamin E plus antigen, did not produce any significant change in the values of TIG, TSP, SA, TSG, A:G ratio and DNCB induced skin thickness when compared with their respective control groups. But there was significant increase in TLC , ALC and PI values in Vitamin E alone and Vitamin E plus antigen treated groups. The present study indicates that Vitamin E alone and Vitamin E plus antigen could stimulate natural nonspecific host defence mechanism, i.e., innate immunity and also cellular immune response, in vaccinated animals even if they were administered before or after the vaccination.

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