In vivo ameliorative effects of vitamin E against hydralazine-induced lupus

Fiona Muthoni Githaiga, George Isanda Omwenga, Mathew Piero Ngugi

ABSTRACT

Objective In this study, we investigated the in vivo ameliorative effects of vitamin E in a hydralazine-induced lupus model, which closely resembles SLE in humans. We aim to shed light on its potential as a therapeutic agent for managing SLE.

Methods Forty BALB/c mice were used in this study. Hydralazine hydrochloride was orally administered in a concentration of 25 mg/kg to the five mice groups once weekly for a period of 5 weeks to induce a lupus-like condition. The untreated group was the normal control group. To confirm the development of lupus, an ANA test was conducted. After the mice tested positive for ANA, drug treatments commenced. The negative control group did not receive any drug treatment. The treatments included prednisolone, methotrexate and vitamin E, all administered at a concentration of 25 mg/kg, with a higher dose of vitamin E (50 mg/kg) also administered.

Results Notably, on day 35, after drug treatment, we observed that mice that received vitamin E at a dosage of 50 mg/kg (3.01±0.100) had a slight decrease in lymphocyte hydrogen peroxide radicals when compared with the group receiving 25 mg/kg of vitamin E (3.30±0.100) (p<0.05). This finding suggests that the scavenging potential of vitamin E is dose dependent.

Conclusion This study suggests that vitamin E supplementation, especially at a higher dose (50 mg/kg), holds promise in ameliorating lupus-like conditions. These findings warrant further exploration and may offer a potential avenue for improving the disease status of patients experiencing SLE.

INTRODUCTION

SLE is a chronic autoimmune disease where the immune system attacks the body’s cells and tissues resulting in inflammation.1 It is treated with high to low doses of corticosteroids usually followed by the use of steroid-sparing measures in the form of immunosuppressives such as methotrexate.2 Long-term use of glucocorticoid can increase the susceptibility to infections, osteoporosis, cardiovascular problems, dermatological issues and neuropsychiatric symptoms.3 Immunosuppressive drugs such as methotrexate expose the patient to infections, lowered blood counts and liver problems.4 To address the need for safer lupus management, the study explores the potential of hydralazine, a hypertension medication known to induce a lupus-like syndrome in some patients.5 This phenomenon offers insights into potential treatments of SLE. While antioxidants have been studied in autoimmune diseases,6–9 their effectiveness against reactive oxygen species is limited.10 Hydrogen peroxide is a potent oxidising agent that is a byproduct of the mitochondrial electron transport chain.11 Excess hydrogen peroxide levels in the cell trigger apoptosis. This facilitates autoantigenic exposure and contributes to the development of lupus.12 Vitamin E is recognised for its potent antioxidant properties and anti-inflammatory effects and it has shown promise in mitigating certain health conditions.9 13 The study’s findings support its potential for

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ SLE poses significant challenges due to its incurable nature and the adverse effects associated with standard treatments such as corticosteroids and immunosuppressants. Additionally, drug-induced lupus-like syndrome, such as that induced by hydralazine, represents a distinct entity, although with some similarities to classic SLE. Vitamin E has been long recognised for its antioxidant and anti-inflammatory properties.

WHAT THIS STUDY ADDS

⇒ This study delves into the potential of vitamin E as an ameliorative agent for lupus-like conditions induced by hydralazine, providing an alternative approach to managing the complex nature of SLE. The research unveils a dose-dependent reduction in hydrogen peroxide radicals with a higher dose of vitamin E, highlighting its potential therapeutic impact.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings of this study offer a promising avenue for addressing lupus. Specifically, the higher dosage of vitamin E shows potential in mitigating the condition. While further investigation is essential, these results could eventually translate into improved management strategies for individuals experiencing SLE, potentially reducing reliance on corticosteroids and immunosuppressants.
MANAGING A LUPUS-LIKE CONDITION, OFFERING A PROMISING AVENUE FOR FURTHER INVESTIGATION.

MATERIAL AND METHODS

Experimental animals

Thirty-six healthy BALB/c mice aged 3–4 weeks were acquired from the animal breeding unit at the Ministry of Agriculture, Fisheries, and Livestock, Kabete, Kenya, for this study. The remaining four were used to produce reference ranges for both the haematological and serological tests. The study adhered to the ARRIVE checklist for reporting animal research. The mice were acclimatised for 7 days in shoebox cages in rooms that were kept between 20°C and 25°C before the experiments began. The mice were supplied with pellets and wood shavings as bedding and access to clean drinking water throughout the study. Mice were identified by sex and cage cards.

Experimental design

BALB/c mice were randomly allocated to six groups (table 1), with three female and three male mice in each group. Group A (normal control) received 1.0 mL of sterile water. Groups B–F were administered hydralazine hydrochloride at a concentration of 25 mg/kg every 7 days for 5 weeks. The lupus-like condition was confirmed with an antibody nuclear test on the 35th day during hydralazine treatment.

Subsequent drug treatments were as follows:

- Group B (negative control) received no drug treatment.
- Group C was treated with prednisolone at 25 mg/kg every 7 days for 5 weeks.
- Group D received methotrexate at 25 mg/kg every 7 days for 5 weeks.
- Group E was treated with vitamin E at 25 mg/kg every 7 days for 5 weeks.
- Group F received vitamin E at 50 mg/kg every 7 days for 5 weeks.

Lupus-like condition detection

Following a 1-week acclimation period, mice aged 5–6 weeks, from groups B to F, received oral doses of hydralazine hydrochloride blood pressure 25 mg/kg every 7 days for a period of 35 days. The untreated group served as normal control group. A sandwich ELISA was performed using the Biorbyt ANA Mouse ANA ELISA Kit (catalogue number, orb566261) once weekly to confirm the development of autoantibodies specific to lupus. Mice were closely monitored weekly for weight changes.

Drug treatments

On the 35th day post-hydralazine treatment, all treated groups that had received hydralazine had confirmed the presence of ANA antibodies. Drug treatments were initiated in all lupus-induced mice every 7 days for 5 weeks. The negative control group did not receive any drug treatments. Oral administration was performed using a gastric oral gavage.

Table 1 Experimental design

<table>
<thead>
<tr>
<th>Groupings</th>
<th>Treatments</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Normal control</td>
<td>Sterile water</td>
</tr>
<tr>
<td>Group B</td>
<td>Negative control</td>
<td>25 mg/kg hydralazine</td>
</tr>
<tr>
<td>Group C</td>
<td>1st positive control</td>
<td>25 mg/kg hydralazine+25 mg/kg prednisolone</td>
</tr>
<tr>
<td>Group D</td>
<td>2nd positive control</td>
<td>25 mg/kg hydralazine+5 mg/kg methotrexate</td>
</tr>
<tr>
<td>Group E</td>
<td>Experimental group 1</td>
<td>25 mg/kg hydralazine+25 mg/kg vitamin E</td>
</tr>
<tr>
<td>Group F</td>
<td>Experimental group 2</td>
<td>25 mg/kg hydralazine+50 mg/kg vitamin E</td>
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Table 2 Scavenging potential of vitamin E

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>H₂O₂ concentration (mM) (interpolated)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Normal control</td>
<td>2.065±0.064&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.95±0.105&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative control</td>
<td>7.91±0.080&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.63±0.040&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Prednisolone 25 mg/bdw</td>
<td>7.23±0.006&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.72±0.139&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Methotrexate 25 mg/bdw</td>
<td>7.64±0.184&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.68±0.200&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Vitamin E 25 mg/bdw</td>
<td>7.26±0.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.52±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Vitamin E 50 mg/bdw</td>
<td>7.41±0.116&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.12±0.017&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values expressed as mean±SD of the three replicates. Column values with different superscript letters are significantly different (p<0.05). bdw, body weight.
Sample collection

Two hundred microlitres of blood samples were collected from the mice in the various treatment groups every 7 days, during the drug treatment period of 5 weeks. The lymphocyte isolation procedure used was originally described by Sigma Aldrich. Hydrogen peroxide was used as a positive control. One hundred microlitres of lymphocyte cell suspension isolated from blood was divided into two equal portions. The first portion was used to assay for the presence of cellular hydrogen peroxide using 5-carboxy-6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCFDA) (Invitrogen), while the second portion was used to obtain microscopic images of the fluorescence intensity of the cells. Fifty microlitres of lymphocyte cell suspension was added to the wells of a clear v-bottomed 96 multi-well plate together with carboxy-H2DCFDA. The cells were covered and incubated for 30 min in the dark, in a conventional incubator at 37°C and 5% CO₂ concentration. Carboxy-H2DCFDA containing medium was removed and the cells were washed by adding 200 µl phosphate buffer saline. Fifty microlitres of cell buffer was added to the multi-well plate. The plate was then be placed in a microscope reader and was read at 480 nm excitation and 520 nm emission. Fifty microlitres of lymphocyte cell suspension was pelleted by at 250×g for 5 min. A P1000 pipette was used to plate the cells in a 35 mm dish with a cell density of 1×10⁶ cells/mL. The culture plate was then left stationary for 30 min at room temperature to allow for sedimentation and adhesion of cells onto the cover slip or well bottom. A drop of sensor was added to the cell culture and mixed gently. Cells were analysed using the EVOS M5000 fluorescence microscope at excitation 488 nm and emission at 515 nm. Digital images were captured under the same acquisition parameters.

Haematological, biochemical and immunological analysis

On the 35th day post-drug treatments, 2 mL of blood was collected through the renal-orbital sinus. One millilitre was placed into EDTA tubes for a full, while the other 1 mL was placed in serum separator tubes to be used for biochemical analysis. One millilitre of blood was divided into two equal portions to determine a complete blood count and erythrocyte sedimentation rates (ESRs). Complete blood count was performed using the veterinary haematology auto-analyser (Sysmex Roche DM9600 VET). The serum was divided into two portions to determine biochemical parameters and to measure ANA levels. The collected (0.5 mL) serum was then transferred into a polypropylene tube, and 80 µL of the serum was loaded into the biochemistry auto-analyser VB1.

Investigating ESRs

The Westergren method was used to measure the ESR. Whole blood (0.5 mL) was diluted with 0.5 mL of 0.85% NaCl. The tube was carefully filled to the zero mark without adding bubbles. The tubes were then placed on a vertical stand at level position for 60 min at room temperature. The ESR was measured using the Westergren method. After 60 min, the tube was placed on a vertical stand at level position and the time was noted. The ESR was calculated using the formula: ESR = time (in seconds) × 0.05 × dilution factor. The dilution factor was used to adjust the ESR based on the dilution factor. The ESR was expressed as mm/h.

Figure 1  Fluorescence microscopy images of lymphocyte cells captured under ×400 magnification stained using the roGFP sensor in both control and experimental mice groups. (A) normal control, (B) negative control, (C) prednisolone (Pred.), (D) methotrexate (MTX), (E) vitamin E (Vit.E) 25 mg/kg bdw, (F) Vit.E 50 mg/kg bdw. The scale bar on each image is 50 µm. bdw, body weight.
temperature. ESR was calculated as the distance from the zero mark to the top of the red blood cell (RBC) column.

**Determining ANA concentrations**

ANAs were analysed to track the development of lupus induced in mice. One hundred microlitres of standard solution was added into five tubes from the zero tube. One hundred microlitres of diluted sample diluted at one-half with sample dilution buffer and added into test wells. The plate was sealed and incubated at 37°C for 90 min. The seal was removed, and its contents were disposed. Wash buffer was used to rinse the plate twice. One hundred (100) µL of biotin-labelled antigen working solution was added into standard, test sample and blank wells. The plate was sealed and incubated at 37°C for 60 min. The plate was washed again. Ninety (90) µL TMB (3,3',5,5'-Tetramethylbenzidine) substrate was added into each well and incubated at 37°C in the dark room for 20 min. Fifty (50) µL of stop solution was then added into each well. Optical density was recorded after adding the stop solution using the GO ESW V.1.01.12. The target concentration of the samples can be interpolated from the standard curve.

**Determination of body and relative organ weights**

Weekly body weights for each animal were measured and recorded during drug treatments. Following anaesthesia, mice were sacrificed by decapitation 24 hours after the last dose of drug treatments. Kidney, liver, brain, spleen and heart were rapidly harvested.

![Figure 2](http://lupus.bmj.com/)  
Red blood cell (RBC) indices in mice with hydralazine-induced lupus. *Significant differences were observed at p<0.05. (A) Haematocrit (HCT), (B) RBCs, (C) Haemoglobin (HGB), (D) mean corpuscular haemoglobin (MCH), (E) mean corpuscular volume (MCV) and (F) mean corpuscular haemoglobin concentration (MCHC).

![Figure 3](http://lupus.bmj.com/)  
Effect of vitamin E (Vit.E) on erythrocyte sedimentation rate (ESR). *Significant differences were observed at p<0.05. MTX, methotrexate; Pred., prednisolone.
Figure 4  Effect of vitamin E on differential white blood cell (WBC) count (A), lymphocytes (B), neutrophils (C), monocytes (D), basophils (E) and eosinophils (F). Data analysis was done using one-way analysis of variance with Tukey’s test for comparison of group means. Statistical significance key: *p<0.05. bdw, body weight.

Figure 5  Effect of vitamin E on platelet (PLT) count (A), platelet distribution width (PDW) (B) and mean platelet volume (MPV) (C). Data analysis was done using one-way analysis of variance with Tukey’s test for comparison of group means. Statistical significance key: *p<0.05. bdw, body weight.
The relative organ weights were measured and recorded.

**Histopathological examinations of organs**

Standard examinations were performed on the liver, kidney, spleen, heart and brain tissues. Tissues were excised and fixed in a solution of 10% neutral buffered formalin followed by gradual dehydration in ascending concentrations of alcohol at 50%, 60%, 70%, 80%, 90% and 100%. Samples were cleared with xylene and embedded in liquid paraffin to prevent their destruction. The tissues were then processed routinely for examination by slicing them into thin sections of 5 µm using a rotary and transferred onto a microscope slide.

**Statistical analysis**

Statistical analysis was performed using GraphPad Prism V.8.0 software package. Data were compared using one-way analysis of variance followed by Tukey’s post-hoc test. Simple linear regression was used to determine hydrogen peroxide concentrations. The level of significance was set at p<0.05 (95% CI).

**RESULTS**

**Evaluating the impact of vitamin E on hydrogen peroxide radical levels**

Day 0 data were recorded on the 35th day post-hydralazine treatment. Vitamin E 50 mg/kg had significantly (p<0.05) depleted levels of lymphocyte hydrogen peroxide radical levels compared with the lower dose of vitamin E on the 35th day post-drug treatments. The total hydrogen peroxide concentrations were quantified using a standard curve (Y=0.007740x+0.01703; R²=0.9298; table 2).

**Figure 6** Evaluating the effect of vitamin E on liver function in lupus-induced mice. *Significant differences were observed at p<0.05. (A) Alanine transaminase (ALT), (B) aspartate aminotransferase (AST), (C) gamma-glutamyl transferase (GGT), (D) alkaline phosphatase (ALP), (E) albumin and (F) total bilirubin.

**Table 3** ANA titre concentrations

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>ANA titre concentration (ng/mL)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.58±0.0944^a</td>
<td>1.64±0.1499^a</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>30.48±0.9395^b</td>
<td>32.30±3.274^b</td>
<td></td>
</tr>
<tr>
<td>Prednisolone 25 mg/kg</td>
<td>28.04±0.4760^b</td>
<td>28.70±0.8509^b</td>
<td></td>
</tr>
<tr>
<td>Methotrexate 25 mg/kg</td>
<td>26.23±0.6452^b</td>
<td>26.95±1.4430^b</td>
<td></td>
</tr>
<tr>
<td>Vitamin E 25 mg/kg</td>
<td>2.33±0.5686^a</td>
<td>3.71±1.5690^a</td>
<td></td>
</tr>
<tr>
<td>Vitamin E 50 mg/kg</td>
<td>1.50±0.1950^a</td>
<td>1.71±0.4031^a</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD for three animals per group. Means that do not share a letter are significantly different (p≤0.05).
Animal models

To validate the results of this assay, the fluorescence intensity of lymphocytes collected from the mice in all experimental groups was analysed using fluorescence microscopy. Live cell imaging of lymphocyte cells from the negative control group, methotrexate and prednisolone groups showed elevated fluorescence intensity. Lymphocytes collected from the vitamin E treatment groups had minimal fluorescence (figure 1).

**Vitamin E prevented haemolytic anaemia associated with lupus**

After observing vitamin E’s positive impact on haematocrit levels in lupus-induced mice, we examined its effects on RBCs and haemoglobin. In the prednisolone and methotrexate treatment groups, RBC count and haemoglobin levels notably decreased. However, both vitamin E doses significantly improved RBC count and haemoglobin levels. We also investigated vitamin E’s influence on specific RBC indices, finding that both doses provided significant protection against the reduction in mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values. In contrast, mice in the prednisolone and methotrexate treatment groups exhibited significantly lower RBC indices (figure 2).

**Vitamin E prevented inflammation associated with lupus**

ESRs were used to assess the impact of vitamin E on inflammation associated with lupus. Mice groups that received prednisolone and both doses of vitamin E recorded low ESRs (figure 3).

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**Figure 7** Creatinine levels in lupus-induced mice. *Significant differences were observed at p<0.05. MTX, methotrexate; pred., prednisolone; Vit.E, vitamin E.

**Figure 8** Effect of drug treatments on electrolytes and lipids in lupus-induced mice. *Significant differences were observed at p<0.05. (A) Sodium, (B) calcium, (C) phosphorus, (D) triglycerides (TRIGL), (E) high-density lipoprotein (HDL) and (F) low-density lipoprotein (LDL). MTX, methotrexate; Pred., prednisolone; Vit.E, vitamin E.
Vitamin E prevented alterations in white cell count and its differential counts
Mice treated with vitamin E showed no significant differences in white cell counts (k/µL) compared with the normal control group. In contrast, prednisolone-treated mice had higher white cell counts, lower lymphocyte counts and significantly elevated eosinophil counts. Meanwhile, methotrexate-treated mice exhibited significantly lower counts across all types of white blood cells (figure 4).

Vitamin E prevented thrombocytopenia associated with lupus
No significant differences were observed between platelet count values (k/µL) between mice treated with both doses of vitamin E compared with the normal control group. Mice treated with prednisolone recorded significantly elevated platelet count, while methotrexate-treated mice had significantly low platelet count (figure 5).

ANA titre concentrations in drug treatment groups
Positive ANA titre concentration values determined using the positive index method showed that mean ANA titres from prednisolone, methotrexate and negative control groups were above the positive index number and considered to be positive for the presence of ANA. Mice treated with vitamin E had significantly low ANA titres (table 3).

Vitamin E prevented a rise in liver and kidney biomarkers
Vitamin E-treated mice had no significant change in alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase, gamma-glutamyl transferase (GGT), albumin and total bilirubin levels. Prednisolone and methotrexate treatment groups recorded significantly high levels of ALT, GGT and ALP. Prednisolone-treated mice showed significantly reduced total bilirubin levels. Albumin was significantly reduced in the methotrexate group (figure 6). Kidney function was assessed by evaluating serum creatinine and electrolyte levels. Vitamin E effectively prevented an increase in serum creatinine levels, while methotrexate-treated mice had significantly higher serum creatinine levels (figure 7).

Effect of drug treatments on body and relative organ weights
All treatment groups did not show any significant differences in relative organ weights (table 4). Exposure of mice to prednisolone resulted in a significant increase in body weight compared with the normal control group (figure 9).

Effect of drug treatments on serum glucose levels
This study assessed blood glucose levels of lupus-induced mice. Mice exposed to prednisolone recorded significantly higher glucose levels compared with healthy mice (figure 10).

### Table 4: Relative organ weight in mice with hydralazine-induced lupus

<table>
<thead>
<tr>
<th>Organs</th>
<th>Normal control</th>
<th>Negative control</th>
<th>Prednisolone 25 mg/kg</th>
<th>Methotrexate 25 mg/kg</th>
<th>Vitamin E 25 mg/kg</th>
<th>Vitamin E 50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>1.532±0.05 a</td>
<td>1.533±0.047 a</td>
<td>1.518±0.032 a</td>
<td>1.538±0.044 a</td>
<td>1.543±0.055 a</td>
<td>1.520±0.043 a</td>
</tr>
<tr>
<td>Liver</td>
<td>4.450±0.393 a</td>
<td>4.500±0.261 a</td>
<td>4.317±0.313 a</td>
<td>4.217±0.371 a</td>
<td>4.517±0.223 a</td>
<td>4.400±0.323 a</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.310±0.019 a</td>
<td>0.3150±0.035 a</td>
<td>0.3217±0.048 a</td>
<td>0.323±0.042 a</td>
<td>0.310±0.023 a</td>
<td>0.307±0.018 a</td>
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<tr>
<td>Brain</td>
<td>0.067±0.005 a</td>
<td>0.066±0.006 a</td>
<td>0.069±0.006 a</td>
<td>0.066±0.007 a</td>
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<tr>
<td>Heart</td>
<td>0.165±0.005 a</td>
<td>0.164±0.007 a</td>
<td>0.163±0.006 a</td>
<td>0.164±0.007 a</td>
<td>0.165±0.007 a</td>
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</tbody>
</table>

No significant differences were observed at p<0.05. Means that do not share a letter are significantly different. Data were analysed using one-way analysis of variance and Tukey’s post-hoc test for comparisons.
Animal models

Determining the impact of vitamin E on the kidney in lupus-induced mice

Prior experiments had demonstrated that administration of vitamin E had prevented the elevation of serum creatinine and ESR in lupus-induced mice. To validate these results, histological examinations were performed on the kidney to determine organ involvement in lupus-induced mice. Microscopic examinations of the kidney sections in the normal control group in both male and female mice showed renal corpuscles comprising glomerulus and intact Bowman’s capsule with adjacent distal and proximal convoluted tubules.

Histopathological analysis of mice from the negative control group showed increased hypercellularity in the glomeruli and inflammatory cell infiltration around the Bowman’s capsule. Notably, there was also degeneration in the renal tubules, tubular necrosis, cytoplasmic vacuolation and tubular dilation. Mice that received prednisolone exhibited moderate tubular degeneration, haemorrhage and dilation of tubules. Kidney tissues from the methotrexate group had the same histological changes as prednisolone only more severe. Kidney sections from lupus-induced female mice treated with vitamin E 25 mg/kg showed mild tubular degeneration. Mice administered with vitamin E 50 mg/kg showed a glomerulus with an intact Bowman’s capsule with mild tubular degeneration being observed in males (figure 11).

Vitamin E ameliorated liver injury

The healthy control group had a visible central vein in their liver sections, while lupus-induced mice showed pathological changes in their liver sections. Prednisolone treatment resulted in mild steatosis in males and severe steatosis in females. Methotrexate-treated mice had severe vacuolation in females and mild steatosis and severe necrosis in males. Vitamin E treatment showed no signs of injury in lupus-induced mice (figure 12).

Effect of drug treatments on spleen sections

Light micrographs from the normal control groups displayed a clear spleen section with distinct white and red pulp. The negative control groups experienced splenic rupture with a more prominent red pulp than white pulp. Prednisolone-treated groups exhibited giant

Figure 10 Elevated glucose levels in prednisolone-treated mice. *Significant differences were observed at p<0.05. MTX, methotrexate; Pred., prednisolone; Vit.E, vitamin E.

Figure 11 Representative mice kidney cortex sections stained with H&E (×100 magnification). (A) Normal control male mice. (B) Negative control male mice. (C) Prednisolone-treated male mice. (D) Methotrexate-treated male mice. (E) Vitamin E 25mg/kg treated male mice. (F) Vitamin E 50mg/kg treated male mice. (G) Normal control female mice. (H) Negative control female mice. (I) Prednisolone-treated female mice. (J) Methotrexate-treated female mice. (K) Vitamin E 25mg/kg treated female mice. (L) Vitamin E 50mg/kg treated female mice. Scale bar 50µm. CV, cytoplasmic vacuolation; DCT, distal convoluted tubule; DT, dilated tubules; G, glomerulus; HE, haemorrhage; IC, inflammatory cells; Meth, methotrexate; PCT, proximal convoluted tubule; TD, tubular degeneration; TN, tubular necrosis; Vit.E, vitamin E.
macrophages with a higher presence of white pulp than red pulp. Microscopic examinations from the methotrexate groups showed distorted lymphoid architecture with more defined white pulp than red pulp, accompanied by giant macrophages and granular leukocytes. No visible pathology signs were observed following administration of vitamin E, and the images displayed well-defined spleens with lymphoid follicles (figure 13).

**Myocardial morphology in lupus-induced mice**
Gross examination of the heart tissue showed no signs of pathology in all groups except prednisolone treatment groups. Prednisolone-treated mice exhibited increased interstitial spaces (figure 14).

**No pathology observed in brain cortex sections**
Brain sections from all experimental groups did not demonstrate any histological changes.

**DISCUSSION**
This study demonstrated that the scavenging potential of vitamin E was dose dependent. Mice treated with vitamin E had significantly low ANA titres compared with other treatment groups. ANA positivity is the most common serological finding in hydralazine-induced lupus. Prednisolone-treated mice had significantly lowered levels of RBCs compared with the normal control group. Prednisolone has been associated with complement-mediated...
Corticosteroids such as prednisolone and the antimalarial drug, hydroxychloroquine, have been used in the treatment of lupus.

**Figure 14** Representative mice sections of myocardial morphology. Heart sections stained with H&E (×400). (A) Normal control male mice. (B) Negative control male mice. (C) Prednisolone-treated male mice. (D) Methotrexate-treated male mice. (E) Vitamin E 25mg/kg treated male mice. (F) Vitamin E 50mg/kg treated male mice. (G) Normal control female mice. (H) Negative control male mice. (I) Prednisolone-treated female mice. (J) Methotrexate-treated female mice. (K) Vitamin E 25mg/kg treated female mice. (L) Vitamin E 50mg/kg treated female mice. Scale bar 100 µm. Arrows point to enlarged interstitial spaces. Vit E, vitamin E.


Limitations

This study highlights vitamin E’s potential benefits in a hydralazine-induced lupus model but presents limitations:

- This model may not precisely replicate human SLE, making direct application to humans challenging due to species-specific differences.
- Dosages and treatment duration might not align with clinical practices, necessitating further investigations for optimal therapeutic approaches.
- Focusing on single treatments may not fully represent the complexity of real-world SLE management.
- The relatively small sample size may limit broader applicability, requiring larger cohorts for more reliable results.

Given these constraints, it is vital to interpret the findings cautiously. Further research, including clinical trials and mechanistic studies, is necessary to evaluate the clinical relevance and safety of these treatments for people with SLE.

**CONCLUSION**

In this mouse model, vitamin E demonstrated a significant attenuation of the induced autoimmune lupus-like disease. These findings suggest potential immunomodulatory effects of vitamin E in lupus-like conditions. However, it is essential to acknowledge the limitations of this study, and further research is warranted to elucidate the precise mechanisms involved and to assess the applicability of these findings to human autoimmune diseases, including SLE. The researchers suggest examining the ideal dose of vitamin E to eliminate hydrogen peroxide radicals and other free radicals that cause SLE.

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**Contributors**

FMG designed and performed the experiments. FMG wrote the manuscript with support from MPN and GIO. Prior to submission of this manuscript, all the authors read and approved its final draft. FMG is responsible for the overall content as the guarantor.
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