

Title:

Effects of Nigella Sativa on T Cells, Cytokine Profile, and
SLEDAI Score in Pediatric SLE Patients

NCT number:

NCT ID not yet assigned

Document date:

31 July 2024

Objective(s) of the Study:

The aim of this study was to determine the immunomodulating effect of *N. sativa* on T lymphocyte cell activity, various inflammatory cytokines, and SLEDAI Score in paediatric SLE patients.

Study design :

This is a clinical trial study, pre and post test control group design, that has received permission from the ethics committee of Health Research Ethics Commission General Hospital Dr. Saiful Anwar Malang with number (400/089/K3/302/2021). The research design is single blind and uses a placebo group as the control.

Methods:

A total of 32 SLE paediatric patients were randomly divided into two groups, the *N. sativa* oil treatment group and the placebo group. Each patient consumed 1 gram of *N. sativa* oil capsule or 1 gram of starch capsule daily for 8 weeks. *N. sativa* oil capsules were purchased from CV Rizki Abadi, Tuban, East Java, Indonesia, (POM TR.183314171). Standard therapy in all patients was Prednisone, Hydroxychloroquine, and Mycophenolate acid. Every week, the research team monitored the patients by phone to determine the progress of the patients' condition and any possible side effects.

Research Procedures

1. Blood Collection

Blood samples were taken twice, at the beginning of the study and at the end of the treatment at the RSSA Clinical Pathology Laboratory by laboratory staff. The volume of blood taken in each research sample was 8 ml. Furthermore, blood samples were made serum through a centrifugation process at 3000 rpm for 5 minutes, and stored at 4°C for measurement of the patient's immunological profile.

2. Measurement of T lymphocyte cell profile

From the peripheral blood samples obtained earlier, PBMC isolation was carried out. PBMCs were isolated using Ficoll gradient density centrifugation. The PBMC suspension was incubated overnight at 37°C in RPMI 1640 media with 100 U/mL penicillin and streptomycin, 2 mM glutamine, and 10% FBS. The PBMCs were collected, washed, and stained with fluorescein isothiocyanate-conjugated anti-human CD4. After surface labeling, cells were stained with phycoerythrin (PE)-conjugated anti-human IFN- γ to identify Th1. Th2 detection was carried out using PE-conjugated anti-human IL-4. Treg detection was performed using CD25+Foxp3-PE antibodies. Th17 identification was carried out using CD4+IL-17A-PE antibodies. All antibodies were using brands from BD Bioscience (San Jose, US).

Afterwards, the analysis was carried out using a BD FACS Calibur flow cytometer (Becton Dickinson, USA). The cell population percentages were organized and analyzed using the CELL Quest pro software application.

3. Measurement of cytokine levels

Serum levels of IFN- γ , TNF- α , IL-2, IL-4, IL-6, IL-17, and IL-10 were measured at both baseline and endpoint of the trial. They were measured using commercially available cytokine CBA (Cytometric Bead Array) flow cytometry kits, which can measure multiple analytes at the same time. All antibodies were using brands from Becton Dickinson (US) and the software to analyse it was CELL Quest Pro Software from Becton Dickinson (New Jersey, USA).

4. Scoring SLEDAI

SLE disease activity was measured using SLEDAI 2K. The data required are clinical manifestations, including arthritis, fever, elevated dsDNA, and other conditions such as photosensitivity and arthralgia. Each clinical manifestation was given a score, and the scores were then summed to obtain the total SLEDAI 2K score.

Ethical clearance:

Please find attached

Statistical analysis

Data for each patient were collected in the form of a data collection sheet. Numerical data were analysed by Independent T Test if the data were normally distributed or by Mann-Whitney test if the data were not normally distributed. For pre and post treatment, each group (N. sativa treatment and placebo), if normally distributed using Paired T Test, but if not normally distributed using Wilcoxon test. Statistical tests were considered meaningful if the p value was ≤ 0.05 . All calculations were performed with the help of SPSS for Windows 25.0 software.



Komisi Etik Penelitian Kesehatan
Rumah Sakit Umum Daerah Dr. Saiful Anwar Malang
Health Research Ethics Commission
General Hospital Dr. Saiful Anwar Malang

Accredited Snars Edillon 1



18 Februari 2018 s.d 18 Februari 2021

Jl. Jaks Agung Suprpto No 2 Malang 65111 Telp 0341-362101, Fax 0341-369384



KETERANGAN LOLOS KAJI ETIK

ETHICAL APPROVAL

Nomor : 400/089/K.3/302/2021

Komisi Etik Penelitian Kesehatan Rumah Sakit Umum Daerah Dr. Saiful Anwar Malang dalam upaya melindungi hak asasi dan kesejahteraan subyek penelitian kedokteran, telah mengkaji dengan teliti protokol berjudul :

The Ethics Commission of General Hospital Dr. Saiful Anwar Malang, with regards of the Protection of human rights and welfare in medical research, has carefully reviewed the research protocol entitled :

"Uji Klinis Pemberian Minyak Jinten Hitam (*Nigella Sativa* L.) Sebagai Terapi Adjuvan Lupus Eritematosus Sistemik Pada Anak"

Peneliti Utama : Dr. dr. Wisnu Barlianto, Sp.A (K), Msi, Med
Principal Investigator

Peneliti Anggota : dr. Desy Wulandari, Sp.A, M.Biomed
Member Of Investigator dr. Ni Luh Putu Herli Mastuti, Sp.A, M.Biomed
Dr. dr. Zahrah Hikma, Sp.A (K)
dr. Rayi Ijqi Asasain
dr. Rafika Rodhiah Rachmaningrum

Nama Institusi : Fakultas Kedokteran Universitas Brawijaya Malang
Name of the Institution / Rumah Sakit Umum Dr. Saiful Anwar Malang
Faculty OF Medicine Brawijaya University Malang /
General Hospital Dr. Saiful Anwar Malang

dan telah menyetujui protokol tersebut di atas.
and approved the above-mentioned protocol.

Malang, 12 April 2021
Ketua
Chairman


dr. Hidayat Sujuti, Sp.M, Ph.D

*Ethical approval berlaku satu tahun dari tanggal persetujuan

** Peneliti berkewajiban

1. Menjaga kerahasiaan identitas subyek penelitian
2. Memberitahukan status penelitian apabila
 - a. Setelah masa berlakunya keterangan lolos kaji etik penelitian masih belum selesai dalam hal ini *ethical clearance* harus diperpanjang
 - b. Penelitian berhenti di tengah jalan
3. Melaporkan kejadian serius yang tidak diinginkan (*serious adverse events*)
4. Penelitian tidak boleh melakukan tindakan apapun pada subyek sebelum penelitian lolos kaji etik dan *informed consent*

RUMAH SAKIT UMUM DAERAH Dr. SAIFUL ANWAR
BIDANG PENDIDIKAN DAN PENELITIAN

NOTA DINAS

Kepada : Kepala Instalasi Rawat Jalan
Kepala Instalasi Rawat Inap IV
Dari : Kepala Bidang Diklit
Tanggal : 19 APR 2021
Nomor : 070/190 /1.20/302/2021
Sifat : Biasa
Perihal : **Penghadapan Izin Penelitian bagi Fakultas Kedokteran Universitas Brawijaya Malang a.n. Dr. dr. Wisnu Barlianto, Sp.A(K), Msi. Med, dkk**

Menindaklanjuti surat dari Wadir Pendidikan dan Pengembangan Profesi RSUD Dr. Saiful Anwar Malang No. 070/0745 /302/2021 tanggal 16 April 2021 perihal izin penelitian, bersama ini kami hadapkan Peneliti tersebut untuk melaksanakan penelitian di satuan kerja yang Saudara pimpin sesuai dengan judul proposal, atas nama :

No	Nama	Judul
1.	Dr. dr. Wisnu Barlianto, Sp.A(K), Msi. Med	Uji Klinis Pemberian Minyak Jinten Hitam (<i>Nigella Sativa L.</i>) Sebagai Terapi Adjuvan Lupus Eritematosus Sistemik pada Anak

Setelah yang bersangkutan selesai melaksanakan penelitian, maka satuan kerja Saudara **wajib** memberikan informasi tertulis kepada kami bahwa yang bersangkutan telah selesai melaksanakan penelitian di satuan kerja yang Saudara pimpin, sebagai dasar kami membuat Surat Keterangan Selesai Penelitian bagi yang bersangkutan.

Demikian atas perhatian dan kerjasama Saudara, diucapkan terima kasih.

Kepala Bidang Pendidikan & Penelitian


SIGIT TRI CAHYONO, SKM, M.Kes
Pembina
NIP. 19660424 198902 1 003



PEMERINTAH PROVINSI JAWA TIMUR
RUMAH SAKIT UMUM DAERAH Dr. SAIFUL ANWAR
TERAKREDITASI SNARS ED.1 INTERNASIONAL



18 Februari 2018 s.d. 31 Desember 2021
Jl. Jaks Agung Suprpto No.2 MALANG 65111
Telp. (0341) 362101, Fax. (0341) 369384
E-mail : staf-rsu-drsaifulanwar@jatimprov.go.id
Website : www.rsusaifulanwar.jatimprov.go.id



Malang, 16 APR 2021

Nomor : 070 / 0745 / 302 / 2021
Sifat : Biasa
Perihal : Izin Penelitian a.n.
Dr. dr. Wisnu Barlianto, Sp.A(K), Msi. Med,
dkk

Kepada
Yth. Dekan Fakultas Kedokteran
Universitas Brawijaya
Jl. Veteran
di
MALANG

Menindaklanjuti surat Saudara nomor : - tanggal 15 Maret 2021, perihal sebagaimana tersebut pada pokok surat, dengan ini kami sampaikan bahwa pada prinsipnya kami dapat menyetujui permohonan dimaksud. Selain itu ada beberapa hal yang perlu kami informasikan sebagai berikut :

1. Peneliti wajib menaati peraturan dan tata tertib yang berlaku di RSUD Dr. Saiful Anwar Malang yaitu :
 - Mengenakan pakaian yang sopan dan layak pakai;
 - Memakai jas almamater;
 - Tidak mengenakan pakaian dari bahan jeans dan kaos;
 - Kartu Tanda Pengenal harus selalu dipakai selama kegiatan di RSSA;
2. Penelitian bisa dilakukan pada bulan April s/d Mei 2021 di Instalasi Rawat Jalan dan Instalasi Rawat Inap IV RSUD Dr. Saiful Anwar Malang.
3. Besaran biaya:
 - Peneliti Utama : Rp. 100.000,-/orang/minggu/satker;
 - Peneliti Kedua, dst : Rp. 58.000,-/orang/minggu/satker;
 - Surat Keterangan Selesai Penelitian : Rp. 10.000,-/judul;
 - Biaya penelitian disetorkan ke kas umum RSUD Dr. Saiful Anwar Malang melalui loket pembayaran dengan No. Rek. Bank Jatim 0047302960
4. *Wajib* menyerahkan laporan hasil penelitian ke Bidang Diklit sebanyak 2 (dua) exemplar atau sesuai dengan banyaknya tempat penelitian & soft copy dikirim melalui email bidangdiklitrsa@gmail.com di tahun berjalan. Jika tidak mengumpulkan laporan hasil penelitian, maka institusi Saudara tidak diperkenankan melaksanakan penelitian di RSUD Dr. Saiful Anwar Malang sampai dengan kewajiban pengumpulan laporan diselesaikan.

Adapun untuk pelaksanaan selanjutnya, mohon peneliti yang bersangkutan berkoordinasi dengan Bidang Diklit RSUD Dr. Saiful Anwar Malang.

Demikian untuk menjadikan maklum, atas perhatian dan kerjasama Saudara diucapkan terima kasih.

a.n. Direktur RSUD Dr. Saiful Anwar Malang
Wadir. Pendidikan & Pengembangan Profesi



Dr.dr. M. BACHTIAR BUDIANTO, Sp.B (K) Onk, FINACS, FICS

Pembina Tingkat I

NIP. 19670725 199603 1 003