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Synergistic anti-malarial effects of *Ocimum sanctum* leaf extract and artemisinin

Efectos antipalúdicos sinérgicos del extracto de hoja de Ocimum sanctum *y artemisinina*

Malaria remains a major health problem in Indonesia. It has been reported that the prevalence of parasitemia in Timika, Papua was 16.3%, and almost 50% of cases were caused by *Plasmodium falciparum* (*P. falciparum*).[1] Papua province has not only the highest prevalence of malaria in Indonesia but also the highest prevalence of multidrug-resistance to both *P. vivax* and *P. falciparum*.[2] Although artemisinin-based combination therapy (ACT) was adopted as the first-line anti-malarial treatment, studies have demonstrated the failure of ACT towards malaria elimination in several Southeast Asian countries.[3] Hence, an alternative combination therapy against malaria is needed.

Ocimum sanctum (*O. sanctum*, Indonesian name Kemangi) belongs to the Lamiaceae family and is widely distributed throughout Indonesia. *O. sanctum* is used by Indonesian to treat several diseases, including malaria; however, the underlying mechanism remains elusive. A study revealed that the ethanolic leaf extract of *O. sanctum* displays more potent antiplasmodial activity than other Ocimum species *in vitro*.[4] Therefore, this study aims to evaluate the synergistic anti-malarial properties of *O. sanctum* and artemisinin (ART) against the *Plasmodium* infection *in vivo*. In addition, the level of transforming growth factor-beta (TGF-β) was examined.

The leaves of *O. sanctum* were collected from Malang, East Java, Indonesia. The species was identified and confirmed by a plant taxonomist of the herbarium unit, UPT Materia Medica, Batu, East Java, Indonesia. The ethanolic extract preparation was conducted as previously described.[5] For *in vivo* experiments, female Balb/c mice between 8 and 12 weeks of age were used. Sixty-three mice were randomly assigned into seven groups (9 mice per group), namely negative control, positive control, infected mice treated with ART (0.036 mg/g/day); two different doses of *O. Sanctum* extract (0.25 and 0.5 mg/g/day); and combinations of artemisinin and *O. Sanctum* extract. The malaria model was performed by i.p. injection of *Plasmodium berghei* adjusted to 10^6 parasites in 0.2 mL blood per mouse. Infected mice were then treated on the sixth day of infection with parasitemia approximately around 10% for seven consecutive days. To examine TGF-β levels, mice peritoneal macrophages on day seven post-treatment were cultured as described previously,[6] and the supernatants were used to quantify the concentration of TGF-β (BioLegend) by ELISA.[7–10] The study was approved by the medical ethics committee of Brawijaya University with Reference No. 27-KE. The reduction of parasitemia and TGF-β levels were analyzed by two and one-way ANOVA, respectively, followed by the Fisher LSD *post hoc* test using StatPlus. Significant differences were accepted when $p < 0.05$.

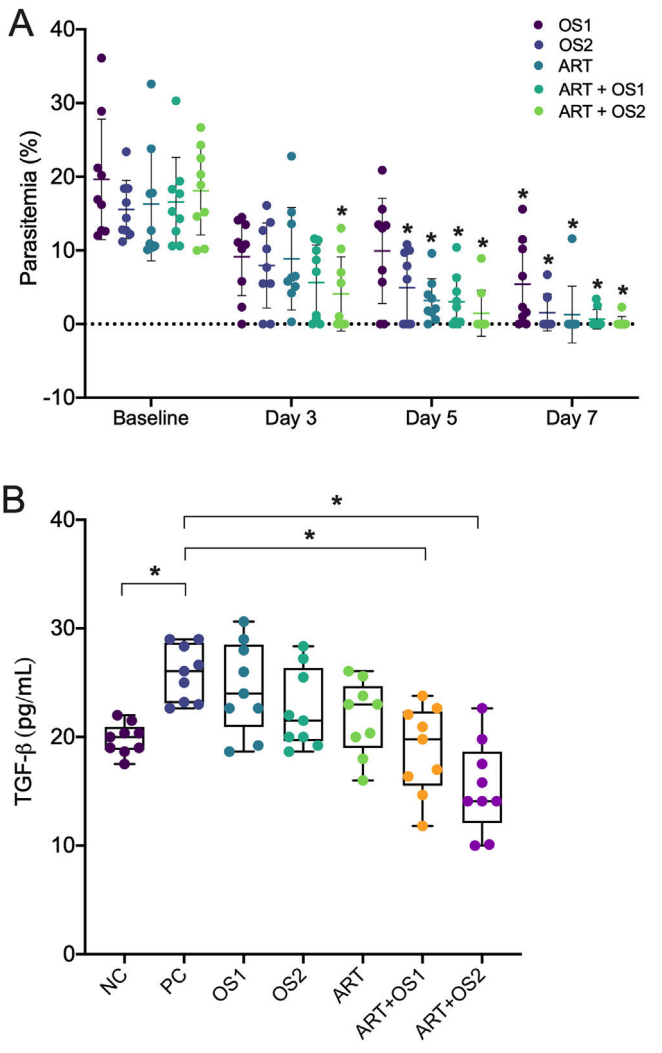

Fig. 1. Synergistic anti-malarial effects of *Ocimum sanctum* leaf extract and artemisinin. (A) Reduction of parasitemia. (B) TGF-β level by peritoneal macrophages during mice malaria infections measured by ELISA after 24 h *in vitro* culture. Data are expressed as a mean ± standard deviation (SD). (*) Indicates a significant difference compared to the baseline level or between two groups indicated in the graphs ($p < 0.05$). OS1 = *O. Sanctum* 0.25 mg/g/day; OS2 = *O. Sanctum* 0.5 mg/g/day; ART = artemisinin 0.036 mg/g/day; ART + OS1 = artemisinin 0.036 mg/g/day + *O. Sanctum* 0.25 mg/g/day; ART + OS2 = artemisinin 0.036 mg/g/day + *O. Sanctum* 0.5 mg/g/day; NC = negative control; PC, positive control.

The reduction of parasitemia was observed in all groups started on day five of the treatment. Notably, the administration of ART and *O. Sanctum* extract at a dose of 0.5 mg/g/day demonstrated a higher effectivity to speed up *Plasmodium* clearance than other groups (at day 3 compared to the baseline level, Fig. 1A). Moreover, the suppression of TGF-β was only observed in mice treated with combination therapy but not with monotherapy (Fig. 1B), thereby implying that combination therapy exhibits synergistic anti-malarial effects towards *Plasmodium* elimination. Various active constituents have been identified in the ethanolic extract of *O. Sanctum,* such as alkaloids, glycosides, flavonoids, phenols, saponins, tannins, steroids, and triterpenoids.[4] The possible mechanisms of *O. Sanctum* extract in eliminating *P. berghei* may have occurred through the inhibition of hemozoin biocrystallization, protein synthesis, or β-haematin formation, stimulation of DNA fragmentation, and cytoplasmic acidification.[4]

In line with previous findings, this study showed that the upregulation of TGF-β levels was observed in *P. berghei* infected mice. Furthermore, the upregulation of TGF-β is known to be associated with the risk of complicated malaria.[11] These results imply that TGF-β is linked to the disease severity of malaria. Therefore, a treatment that modulates the suppression of TGF-β would be beneficial to minimize malaria progression. In summary, the combination of ART and the ethanolic extract of *O. Sanctum* displays synergistic anti-plasmodial activity *in vivo*. Further studies are warranted to investigate the potential of *O. Sanctum* as an alternative ACT regimen in clinical settings.

Authors' contribution

Z.S.U. conceived, performed, analyzed, and wrote the article.

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Conflict of interest

None to declare.

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Elevadas tasas de colonización por *Staphylococcus aureus* en los estudiantes de medicina antes de realizar sus prácticas clínicas

High rates of colonization by Staphylococcus aureus *in medical students before doing their clinical practices*

Las infecciones por *Staphylococcus aureus* resistente a meticilina (SARM) suponen un problema importante de salud pública. Se recomienda la detección activa de portadores entre el personal sanitario cuando existen brotes nosocomiales o en situación de elevada endemia en determinadas áreas de riesgo[1]. Los estudiantes de medicina, como futuros profesionales del ámbito sanitario, deben conocer su papel como fuentes potenciales de transmisión de *S. aureus* a los pacientes. Nuestro trabajo tuvo los siguientes objetivos: a) determinar la tasa de portadores (nasales y/o faríngeos) de *S. aureus* sensible a la meticilina (SASM) y SARM entre los estudiantes de tercer curso de medicina de la Universidad Complutense de Madrid que aún no habían iniciado sus prácticas en el Hospital Clínico San Carlos, y b) analizar los posibles factores de riesgo.

Durante seis cursos académicos consecutivos (del 2014 al 2022), a todos los estudiantes, de forma voluntaria, se les realizó una toma de muestra faríngea y otra nasal, excepto en el curso 2021/2022, en el que solo se realizó una autotoma de muestra nasal, debido a las medidas de prevención y de seguridad frente a SARS-CoV-2. Inmediatamente se procedió a la siembra en dos medios de cultivo para el aislamiento y la identificación de *S. aureus*: el agar chromID SAID (bioMérieux *S. aureus*) y el agar chromID MRSA (bioMérieux *S. aureus*). Los alumnos también cumplimentaron, de forma anónima, un cuestionario en el cual se evaluaban los posibles factores asociados al estado de portador, como son el sexo, la sinusitis crónica, la sinusitis aguda, la toma de antibióticos en los 10 días previos a la muestra, los ingresos o intervenciones hospitalarias y las cirugías locales relacionadas. La sinusitis crónica se definió como la inflamación de los senos con síntomas y signos que duran 12 semanas como mínimo.

El análisis estadístico de los resultados se llevó a cabo con el programa Epi Info 7.2.5.0, considerándose un resultado estadísticamente significativo si $p < 0,05$.