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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 $p < 0.05$.

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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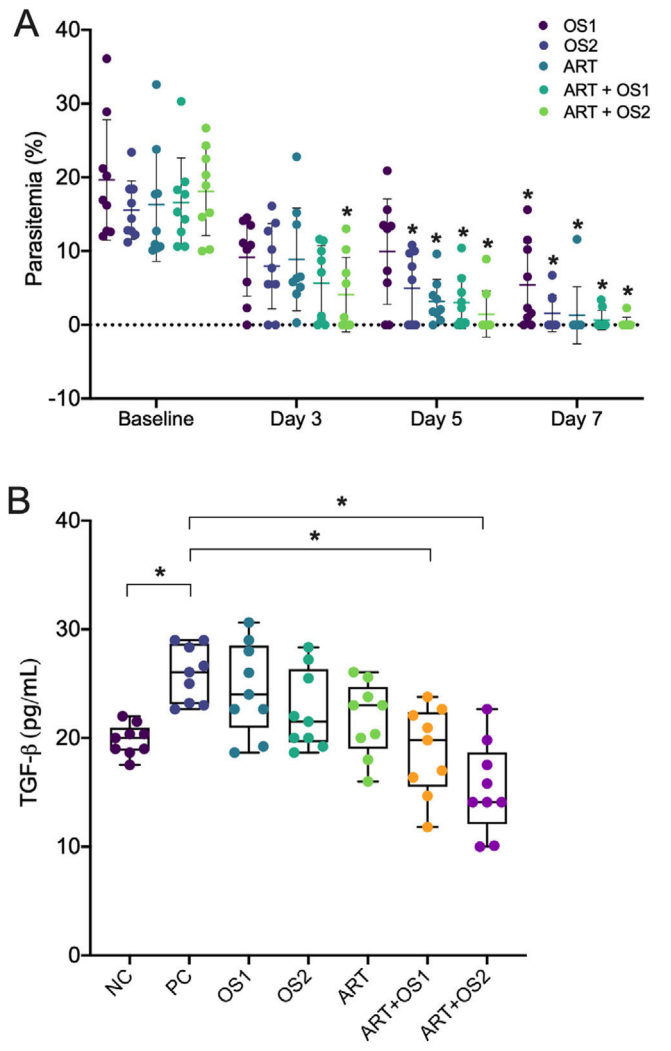

Fig. 1. Synergistic anti-malarial effects of *Ocimum sanctum* leaf extract and artemisinin. (A) Reduction of parasitemia. (B) TGF-β level by peritoneal macrophages during mice malaria infections measured by ELISA after 24 h *in vitro* culture. Data are expressed as a mean ± standard deviation (SD). (*) 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.

Conflict of interest

None to declare.

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

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

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are a major public health problem. Active carrier detection is recommended among healthcare staff when there are nosocomial outbreaks or in highly endemic situations in certain risk areas.[1] As future healthcare professionals, medical students must be aware of their role as potential sources of *S. aureus* transmission to patients. Our study had the following objectives: (a) to determine the carrier rate (nasal and/or pharyngeal) of methicillin-sensitive *S. aureus* (MSSA) and MRSA among third-year medical students at Universidad Complutense de Madrid, in Madrid, Spain, who had not yet started their practical placements at Hospital Clínico San Carlos; and (b) analyse possible risk factors.

Over six consecutive academic years (from 2014 to 2022), all students voluntarily had a pharyngeal and nasal sample taken, except in the 2021/2022 academic year when, due to the SARS-CoV-2 prevention and safety measures, the student's themselves