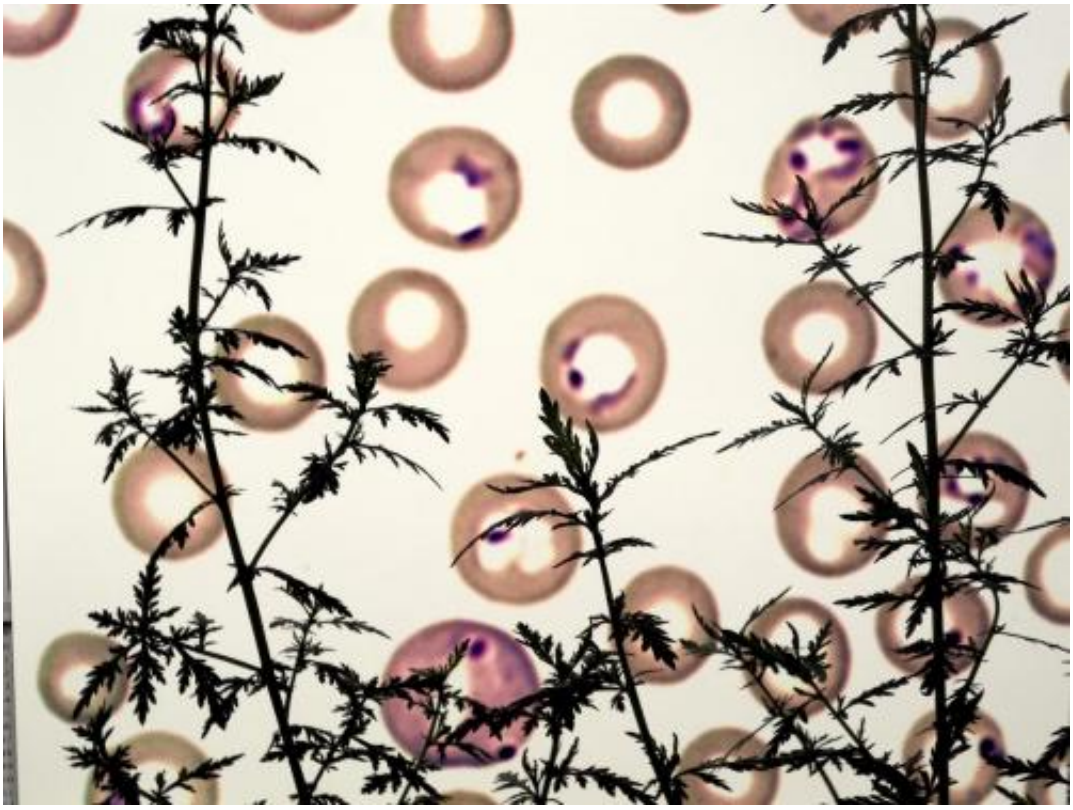
Whole plant therapy shows promise to beat malaria parasites' drug resistance

January 5 2015



Artemisia annua growing in front of a photomicrograph of a thin blood smear stained with Giemsa. The round balls are healthy red blood cells while those with magenta stain are malaria-infected. Credit: University of Massachusetts Amherst

For decades, physicians and public health officials worldwide have been thwarted by the malaria parasite's ability to evolve resistance to the succession of drugs developed to treat it. But now University of

Massachusetts Amherst microbiologist Stephen Rich and his research team report an effective and sustainable malaria intervention that shows great promise in laboratory models.

Details appear this week in an early online edition of the *Proceedings of the National Academy of Sciences*. The new treatment is based on a use of the whole plant (WP) *Artemesia annua*, from which the current pharmaceutical drug artemisinin (AN) is extracted. Rich and colleagues found that the whole plant treatment withstands the evolution of resistance and remains effective for up to three times longer than the pure drug. They also found the whole plant therapy effective in killing rodent parasites that have previously evolved resistance to pure AN.

Together the results suggest that medical researchers should more aggressively explore this inexpensive, non-pharmaceutical treatment for the millions of people who suffer from malaria each year, the authors say.

Artemisinin is the drug most commonly used in treatment of human malaria worldwide, though resistance to it is now established in some areas. Rich, with his entomology doctoral student Mostafa Elfawal, biostatistician Nicholas Reich of the UMass Amherst School of Public Health and Health Sciences and plant biochemist Pamela Weathers and her postdoctoral fellow Melissa Towler of Worcester Polytechnic Institute, previously demonstrated the WP approach is more effective at killing rodent malaria than a comparable purified AN drug approach.

In the present study, Rich and his team conducted a series of experiments to determine rates at which parasites become resistant to their new WP treatment compared to the rate with pure AN, and if WP can overcome resistance to pharmaceutical AN.

They chose two rodent malaria species for particular characteristics:

Plasmodium yoelii because an artemisinin-resistant strain exists and can test whether the whole plant can overcome that resistance. And a second strain, *P. chabaudi*, because among several species of rodent malaria, it most closely biologically resembles the deadliest of the five human malaria parasites, *P. falciparum*. "Conducting these experiments in different rodent malaria species also provides a robust test of the therapy," Rich adds.

To determine the respective evolutionary rates of resistance to WP and AN, Rich and colleagues conducted artificial evolution experiments to compare rates at which resistance to these two treatments arises in serial passage among wild-type parasite lines. In this technique, parasite proliferation rates determine resistance. Parasites that are resistant are expected to reach a certain target level at the same time whether treatment is present or absent. Sensitive, that is non-resistant parasite strains, will grow more slowly in presence of treatment and reach the target later than untreated.

In experiments designed to select for resistance, AN-treated parasites achieved stable resistance to low dose (100 mg/kg) on passage 16. Those parasites were then treated with a doubled AN dose (200 mg/kg) to which they became resistant after an additional 24 passages. By comparison, even after 49 passages parasites never became resistant to even the low dose of WP (100 mg/kg). From this the researchers conclude that the WP treatment lasts at least three times longer than its AN counterpart, and at least twice as long as the doubled dose of pure AN.

Rich says, "This is especially important given the recent reports of resistance to artemisinin in malaria-endemic regions of the world." Clinicians for years have been combining drugs, particularly AN with others, to try to outmaneuver the parasites' evolution, but they still adapt alarmingly fast. Drug longevity is crucial since new drugs are costly to

develop, not only in dollars but in the cost of lives lost, he adds.

The researchers also tested whether dried WP can overcome existing resistance to pharmaceutical AN. Groups of mice infected with AN-resistant malaria were fed either WP or AN mixed with water. To test dosage, single treatments were given in low (40 mg) and high (200 mg) dose. Control groups received a mouse chow placebo.

The researchers measured the parasite levels in the rodents' bloodstream at nine points after treatment began. Rich and colleagues report that mice given either low or high dose of WP showed significantly greater reduction in parasitemia than those in their respective AN group. As expected for these resistant parasites, parasitemia in mice in the low-dose AN did not differ from controls.

The authors point out that consuming the whole plant may be more effective than the single purified drug because the whole plant "may constitute a naturally occurring combination therapy that augments artemisinin delivery and synergizes the drug's activity."

While Rich is quick to point out that the exact mechanisms of WP's effectiveness still need to be identified, he also notes that the demonstrated anti-malarial activity of WP Artemisia against artemisinin-resistant parasites provides "compelling reasons to further explore the role of non-pharmaceutical forms of artemisinin to treat human malaria ."

More information: Dried whole-plant Artemisia annua slows evolution of malaria drug resistance and overcomes resistance to artemisinin, *PNAS*, www.pnas.org/cgi/doi/10.1073/pnas.1413127112

Provided by University of Massachusetts Amherst