**Nobel Prizes 2015:**
**Physiology or Medicine**

**Mao, Malaria and the Discovery of Artemisinin**

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**Project 523**

The story begins in the context of the war in Vietnam. North Vietnam soldiers were fighting the US Army in the jungles of Southern Vietnam in support of the unification of the country. Soon they realised that their main enemy was not the US army, but a teeny-weeny mosquito.

More than two-thirds of the VietCong soldiers who reached the mosquito-infested battlefields were down with malaria and were unfit to fight. By that time every frontline antimalarial drug, chloroquine, sulfadoxine, pyrimethamine, had become obsolete due to development of drug-resistant strains. Chloroquine-resistant *Plasmodium falciparum* was ravaging the countryside of Indochina, debilitating soldiers and at times even causing death.

Ho Chi Minh, the legendary leader of Vietnam, is reported to have said, “we can beat the US army, but we cannot fight malaria”. Ho Chi Min approached Mao Zedong the leader of China, his ally in the fight against US imperialism in Indochina for help. The Chinese themselves were reeling under severe malaria infection. Mao decided to launch a decisive battle against malaria.

Zhou Enlai, the premier of China, called for a meeting of experts at Beijing on 23 May 1967 to discuss the problem of drug-resistant malaria parasites. This led to the establishment of the secret programme called ‘Project 523’, involving over 500 scientists drawn from around 60 different laboratories and institutes across China.

The project team was given a short-term goal to develop antimalarial drugs that could immediately be used in the battlefield. However, the project’s long-term goal was to search for new antimalarial drugs by screening synthetic chemicals and by searching recipes and practices of age-old Chinese herbal medicine.

Although unaware of the role of mosquitoes or the parasite, healers of the bygone era, the world over, had noticed the classic cyclic symptoms and had recommended specific cures. Tu Youyou and her colleagues were looking for cures mentioned in folklore and ancient texts for intermittent fever that is the most visible cyclic symptom of malarial infection.
The wonder herb was Qinghao, and used by farmers to treat intermittent fever, characteristic of malaria, as a folk remedy. Qinghao was recommended as a remedy for hemorrhoids in the ancient Chinese.

Tu Youyou
The memoir compiled by Zhang Jianfang, who was the deputy director of Project 523 [A Detailed Chronological Record of Project 523 and the Discovery and Development of Qinghaosu (Artemisinin)] reveals the trials and tribulations the 500 odd scientists had to undergo in that tumultuous era. While some researchers tried to find an effective chemical compound from known drugs by trying modifications in its chemical structure, others focused on developing effective mosquito repellents in the hope of preventing the mosquito-borne disease.

However based upon Mao’s ideas of ‘Modern medicine looks at Traditional Chinese medicine’ policy quite a few researchers were engaged in exploring the traditional Chinese medicine for possible cures. One such researcher was then thirty-seven-year-old Tu Youyou.

Tu Youyou was born in Ningbo, Zhejiang province of China on 30 December 1930. She studied medicine at the Peking University Medical School during 1955, and also underwent a two and half year training in traditional Chinese medicine as per the regulations in China. After graduation, she joined the Academy of Chinese Medicine in Beijing as a researcher. With her training in modern medicine and traditional Chinese medicine, she was seen as a valuable asset to the project and was transferred to Project 523.

The Search
Soon after joining Project 523, Tu was sent to the malaria-infested tropical islands of Hainan close to Indochina to observe the effects of the disease firsthand. Recalling her days at the faraway malaria-infested Hainan province, Tu Youyou said that she had to leave her three-year-old daughter behind at Beijing in the care of her days at the faraway malaria-infested disease.

The ordeal of malaria-infested young children, many badly suffering children died within few days of the infection, made a big impression on her. She recalled, “I saw a lot of children who were in the last stages of malaria...Those kids died very quickly.” Moved by the plight of the children she was determined to find a solution to the menace.

The malarial parasite was discovered by India-born English medical researcher Ronald Ross in 1897. In their life cycle, the malaria parasites develop, reproduce, and are released from the red blood cells and liver cells in the human body in a cyclic manner resulting in a cyclic pattern of malaria symptoms. If one is infected with P. vivax or P. ovale usually fever and other symptoms recur every 48 hours while if infected with P. malariae the periodicity is 72 hours.

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She and her colleagues collated the traditional recipes and cures advocated by the traditional healers. They combed through ancient medicine books and folklore recipes and compiled a notebook, A Collection of Single Practical Prescriptions for Anti-Malaria consisting of 2,000 candidate recipes for treating fever. Of these, 640 recipes were suggested for an illness that resembled malaria. After careful examination they came up with about 808 potential herbs. More than 380 extracts from the herbs, including pepper, were tested on the rodent model of malaria. Many have them had some effect, but were too feeble to make any mark.

However, one of the herbs, surprisingly, inhibited the malarial parasite growth by 68%. They knew they had got something.

Qinghao
The wonder herb was Qinghao, recorded in ancient tomb carvings as early as 168 B.C. and used by farmers to treat intermittent fever, characteristic of malaria, as a folk remedy. Many Chinese medical scrolls including the Book of Seasonal Fevers written in 1798 hailed the plant.

Qinghao was recommended as a remedy for hemorrhoids in the ancient Chinese. The recipes for 52 kinds of diseases dated to the West Han Dynasty (168BCE) and as an anti-inflammatory herb in another work dated to 200 CE. A handbook written by Ge Hong in 341 CE noted the anti-febrile properties.

Including the oral traditions many Chinese medical works such as Compendium of Materia Medica, 1596
Twist in the Tale

While the early results were seemingly promising the subsequent laboratory studies, however, only showed 12% to 40% inhibition of the malarial parasites in mice, far below the expectation levels. The researchers were in a fix.

Tu Youyou realised that the low inhibition rate is possibly due to a low concentration of the active ingredient in the preparations being tested. She recalled the precise procedure prescribed by the medical texts. *The Handbook of Prescriptions for Emergency Treatments* was particular: “take one bunch of Qinghao, soak in two sheng (0.4 liters) of water, wring it out to obtain the juice and ingest it in its entirety.”

However, the research teams had been preparing the infusion of the herb at boiling temperature. When the juice was extracted using the method prescribed by the ancient text, the results once again looked bright. Noting that high temperature somehow destroyed the chemical structure of the active ingredient in the herb Tu Youyou tried extraction using ether solvent, which boils at 35 °C. The resultant extract worked wonders in malaria-infected laboratory mice. With an improvement in the extraction technique without damaging active ingredients, Tu Youyou and her team achieved reproducible inhibition rates for parasites in mice and monkey.

Although the extract tested fine on rodents and three of the group members, it was found to be not very effective in clinical trials and had toxic side effects. Tu Youyou then further removed from the extract an acidic portion and found that the resultant neutral extract was not only much less toxic but also exhibited improved anti-malarial activity. By 1971, Tu Youyou could establish that the neutral extract, termed extract number 191, resulted in almost 100% inhibition of *Plasmodium berghei* in lab mouse tests. But would it work in humans, and was it safe? In the tradition of great medical doctors, Tu Youyou and her colleagues volunteered to test the extraction on themselves.

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Wonder Molecule

Tu Youyou’s findings helped another chemist to obtain pure artemisinin crystals and dihydroartemisinin. Teams led by Zeyuan Luo of Yunnan Institute of Drug Research and Zhangxing Wei of the Shandong Institute of Chinese Traditional Medicine extracted the pure crystals of the active ingredient, artemisinin. The pure crystals proved to be highly active against rodent malaria parasites. Subsequently researchers from China have developed more potent derivatives of artemisinin including artemether by Li Ying and artemunate by Liu Xu.

In 1978, Project 523 finally announced the new drug ‘qinghaosu’. Subsequently, artesunate, arteether and artelinic acid have been derived and all these grouped together as ‘artemisinins’ are the backbone of the current anti-malarial treatment.

At the end of 1975, Chinese chemists found the unique chemical structure of artemisinin to be sesquiterpene lactone bearing a peroxide bridge. The structure was surprisingly distinct from all other known anti-malarial drugs. Further research led to the discovery of more active derivatives, including artesunate.
researchers have established that the endoperoxide bridge is necessary for its anti-malarial efficacy. Studies have shown that many analogues that share this structural feature are active while artemisinin derivatives lacking the endoperoxide bridge are inactive.

The direct parasiticidal action of artemisinin against *P. falciparum* in the erythrocytic stage both in vitro and in vivo were first reported in 1979 by Chinese researchers. While the mode of action of artemisinin is still debated, its activity against chloroquine-resistant strains of *Plasmodium* clearly indicates that its operation is distinctly different from quinine-type molecules. Activation mechanisms and ultimate targets of artemisinins are still unclear. However, pathological studies show artemisinins result in rapid damage to the membrane system of the parasites.

Artemisinins are the most vigorous anti-malarial compounds that can reduce the fever twice as fast and reduce the parasite in the blood stream thousand times more efficiently than any other anti-malarial drugs today. It is also one of the few anti-malarial compounds that acts on *Plasmodium* gametocytes, and can drastically reduce parasite transmission.

However, it has poor solubility in water or oil. Further, artemisinins are metabolised rapidly, and have a short half-life, of the order of 2–5 hours, compared to multiday half-lives of
Campbell showed that a component from one of the cultures was remarkably efficient against parasites in domestic and farm animals. Equipped with extraordinary skills, Ōmura isolated new strains of Streptomyces from soil samples and successfully cultured them in the laboratory.

Lumefantrine at nearly cost price.

Malaria researcher associated with the development of mefloquine, got wind of the mysterious Qinghaosu developed from the Chinese herb. Qinghaosu was already widely used in China and Vietnam when the first English description appeared in the Chinese Medical Journal in 1979.

Suspicious about Chinese journals and traditional medicine, Qinghaosu was initially treated with scepticism. However, Keith Arnold’s head-to-head trials in China clearly showed the superiority of artemisinin as compared to mefloquine and the world took notice.

Soon, the World Health Organization sought articles from China’s medical journals, and Tu Youyou made a presentation on behalf of Project 523 to the WHO’s visiting study group on chemotherapy of malaria at Beijing in October 1981. In 1982, the Lancet, prestigious medical journal, published an article by Chinese researchers, which even won an award.

The efficacy of artemisinin was clear as crystal, yet the drug languished and the WHO did not endorse it until 2000. In a curious twist to the tale, no major drug manufacturer came forward to produce the drug, although it was more potent in curing even severe malaria. The communist China during the 1970s eschewed patents and hence there was no way a major drug company could get a monopoly and make big profits.

Finally, when the WHO approved the drug for large-scale use in its malaria control efforts in Africa and Asia, a major drug company came forward to produce and sell a mix of artesunate and lumefantrine at nearly cost price.

Deeper Lesson

The Nobel Prize awarded to this humble Chinese medicine was opened in Shanghai, Guangzhou, Chengdu and Beijing in 1956. These new academies of Chinese medicine were anything but ‘traditional’, striving valiantly to “scientify” the teachings of classics. Classical Chinese medicine books were translated into modern language, with proper annotations and explanations and principles of Chinese medicine were examined in the light of modern science. Qualified professionals in modern medicine were involved in research and standardisation of traditional practices while the famous practitioners of Chinese medicine from across the nation were invited to teach in the medical schools.

The ‘modernisation’ drive meant that firstly obvious ‘superstitious’ elements, such as astrology and phrenology, were stripped off. With a view to harness both the old and new medicines to provide a consolidated form of medicine, ‘improvement schools’ of medicine were set up nationwide. Modern anatomy, physiology, bacteriology, pathology and physiology were taught to the practitioners. Every practitioner of Chinese medicine had to study in these schools in order to obtain a licence and thus be authorised to continue practising. Tu Youyou was a product of this movement.

In India also, an earnest national effort to “diligently explore and improve upon” various Indian systems of medicine and healing practices is necessary, rather than merely deifying them as ‘traditional’ and ‘revealed’ knowledge. Subjecting them to the scrutiny of modern science may produce success stories. Until then, largely, Ayurveda and such other traditional practices may fail to live up to the modern world.