Antiplasmodial Activity and Cytotoxicity of Plants Used in Traditional Medicine of Iran for the Treatment of Fever

Somayeh Esmaeili, Azadeh Ghiaee, Farzaneh Naghibi and Mahmoud Mosaddegh

Abstract

Malaria is the most serious parasitic disease and one of the oldest recorded diseases in the world. Because of the resistance of malaria parasites to current drugs, it is necessary to discover new antimalarial drugs. Traditional medicine is one of the important sources of new antimalarial drugs.

In this study, twenty methanolic extracts from different parts of sixteen medicinal plants used in traditional medicine of Iran for the treatment of "Nobeh fever" and/or fever were screened for in-vivo antimalarial activity against Plasmodium berghei and cytotoxic effect on Madin–Darby bovine kidney cells (MDBK).

Eleven species (55%) were found to have antimalarial activity. Methanolic extract from Rosa damascena Mill. reduced parasitemia by 57.7% compared to untreated control mice at intra-peritoneal (i.p.) injection doses of 10 mg/Kg per day for 4 days. This is the first report that mentioned in-vivo antimalarial activity of Rosa damascena Mill.

Keywords: Rosa damascena; Antiplasmodial; Malaria; Fever; Traditional medicine.

Introduction

Malaria is the most serious parasitic disease and one of the oldest recorded diseases in the world. The name «mala» «aria»(meaning «bad air» in Italian) comes from 18 century Italian word (1). It affects 219 million people per year worldwide and is the cause of 660,000 deaths per year (2).

In recent years, the increasing resistance of malaria parasites to drugs or malaria vectors to pesticides, led to discover and develop new antimalarial and new pesticides agents (1, 3).

Traditional medicines have been used to treat malaria for thousands of years and are the source of the two main groups (artemisinin and quinine derivatives) of modern antimalarial drugs (3). The vast majority of people on this planet still rely on their traditional materia medica for their everyday health care needs (4). Besides, herbal medicines are widely believed to be safe and efficacious. Therefore, the potential of plants traditionally used to inhibit parasite growth without host toxicity must be assured (5). One of the methods which are applied for the safety evaluation is in-vitro cytotoxicity assay against normal cells (6).

Tertian, quartana and tropical are three different forms of malaria according to the type
Experimental

Plants selection

Fever attacks are the most common symptoms of malaria. So, the term “fever” was searched in the ancient medicinal books of Iran for selecting the plants.

Preparation of plant samples

Seven plant species out of sixteen plants used for treating nobeh fever and other fevers were collected from different places of Iran. The others were purchased from herbal market (Attari) (Table 1). The plants were identified by the taxonomist and voucher specimens were deposited at Traditional Medicine and Materia Medica Research Center (TMRC) herbarium.

Plant extraction

The selected parts of the collected plants were air-dried in shadow. Plants were crushed into powder using a hammer mill and extracted by maceration of 10 g of powdered dried material.
in methanol at room temperature for 24 h with constant shaking. The filtrates were concentrated to dryness by means of a rotary evaporator and used for antiplasmodial and cytotoxicity tests.

**Biological assays**

**In-vivo antiplasmodial assay**

To carry out the screening of the extracts, the Peters’ 4-day suppressive test against *Plasmodium berghei* infection in mice was employed (18-21). All the procedure was accepted by Shahid Beheshti University Ethics Committee and in accordance with the principles for laboratory animal use and care in the European Community guidelines. On day 1 (D₀), all experimental adult male albino mice weighing 20–25 g were infected by intra-peritoneal (i.p.) injection with 1×10 infected erythrocytes. The mice were randomly divided into groups of five per cage and treated during consecutive days with 10 mg/Kg of the sample by i.p injection for 4 days (on days D₀, D₁, D₂ and D₃). Two control groups were used in this experiment, one treated with chloroquine as a positive control while the other group was kept untreated as a negative control. On day 5 (D₄) of the test, thin blood smears were prepared and blood films were fixed with methanol. The blood films were stained with Giemsa, and then microscopically examined. Percentage of parasitaemia was counted based on infected erythrocytes calculated per 1000 erythrocytes.

**In-vitro cytotoxic assay**

Methanolic extracts of all plants were screened for cytotoxic with the Madin–Darby bovine kidney normal cells (MDBK). Suspension containing 1×10 cell/mL was seeded into 96-well micro plates. After 24 h, cells were washed and maintained with different concentrations of extract for 3 days, at 37 °C, under 5% CO₂ atmosphere. The initial concentration of extracts was 100 µg/mL in DMSO, which was serially diluted in complete culture medium with two fold dilutions to give six concentrations (100 – 3.125 µg/mL). The cytotoxicity of the plant extracts was determined using the colorimetric methylthiazole tetrazolium (MTT) assay (19-23) and scored as a percentage of absorbance reduction at 570 nm of treated cultures versus untreated control cultures. IC₅₀ values on cell growth were obtained from the drug concentration–response curves. 5-Fluorouracil (5-Fu) was examined as a positive control.

**Results and Discussion**

The results of the cytotoxicity and the in-vivo antiplasmodial of plant extracts were reported in Table 2. Most of them exhibited no significant cytotoxicity. The *Convolvulus scammonia* L. extract was found to be cytotoxic. Twenty extracts were prepared from the selected parts of the sixteen plants species. Eleven extracts (55%) showed in-vivo antiplasmodial activity. *Rosa damascena* Mill. showed significant suppression of parasitemia (57.7%). Three plants, *Carthamus tinctorius* L., *Cotoneaster nummularia* Fisch. & Mey. and *Cassia angustifolia* Vahl. showed moderate antiplasmodial activity. *Rosa damascena* Mill. commonly known as rose having several pharmacological properties including anti-HIV, antibacterial, antioxidant, antitussive, hypnotic, anti-diabetic and relaxant effect on tracheal chains have been reported for this plant. Several components were isolated from flowers, petals and hips (seed-pot) of *R. damascena* including terpenes, glycosides, flavonoids, and anthocyanins. This plant contains carboxylic acid, myrcene, vitamin C, kaempferol and quercetin. Flowers also contain a bitter principle, tanning matter, fatty oil and organic acids. The essential oil of *R. damascena* contains eighteen compounds represented more than 95% of the total oil. The identified compounds were; β-citronellol (14.5-47.5%), nonadecane (10.5-40.5%), geraniol (5.5-18%), andnerol and kaempferol were the major components of the oil. Analyses of rose absolute showed that phenyl ethylalcohol(78.38%), citrenellol (9.91%), nonadecane (4.35%) and geraniol (24-26). In traditional medicine of Iran, *R. damascena* Mill. was used to treat depression, headache, strengthening the heart, skin problems, wounds and quartan fever (16).

The main goal of this work was to investigate the potential antiplasmodial properties of some plants used in traditional medicine of Iran against nobeh fever and/ or fever. Among sixteen plant species, only three of them (*Althaea officinalis*...
Ms. Leila Farivar for her assistance to carry out this work. We would like to express our special gratitude for late Farzaneh Naghibi. She is remembered for her tremendous intellect, integrity, energy and enthusiasm devoted to promotion of scientific research particularly in this work.

Acknowledgments

This paper is a part of PhD thesis (No. 121) conducted by Azadeh Ghiaee at Department of Traditional Pharmacy, school of Traditional Medicine, Shahid Beheshti University of Medical Sciences. This work was supported by a grant (No. 7987) from Shahid Beheshti University of Medical Sciences. The authors would like to thank Dr. Mehdi Nateghpour management of Medical Parasitology and Mycology Department, Tehran University of Medical Science for providing the facilities for in-vivo assays. The authors wish also to thank Ms. Leila Farivar for her assistance to carry out this work.

We would like to express our special gratitude for late Farzaneh Naghibi. She is remembered for her tremendous intellect, integrity, energy and enthusiasm devoted to promotion of scientific research particularly in this work.

References

(1) Kalra BS, Chawla S, Gupta P and Valecha N. Screening of antimalarial drugs: An overview. Indian J. Pharmacol. (2006) 38: 5-12.
(2) WHO fact sheet malaria (2010). Available from: URL: http://www.who.int/mediacentre/factsheets/fs094/en/.
(3) Kazembe T, Munyarari E and Charumbira I. Use of traditional herbal medicines to cure malaria. BEPLS (2012) 1: 63-85.
(4) Gurib-Fakim A. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Mol. Aspects Med. (2006) 27: 1-93.
(5) Rasoanaivo Ph, Deharo E, Ratsimamanga-Urverg S and Frappier F. Guidelines for the Nonclinical
Antiplasmodial Activity of Plants Used in Traditional Medicine of Iran

Evaluation of the Efficacy of Traditional Antimalarials. In: Wilcox M, Bodeker G and Rasonaivo Ph (eds.) Traditional Medicinal Plants and Malaria. 1 ed. CRC Press, London (2004) 255-271.

(6) Husoy T, Syversen T and Jenssen J. Comparisons of four in-vitro cytotoxicity tests: The MTT assay, NR assay, uridine incorporation and protein measurements. Toxicity In-vitro (1993) 7: 149-154.

(7) Adams M, Alther W, Kessler M, Kluge M and Hamburger M. Malaria in the renaissance: Remedies from European herbalbs from the 16th and 17th century. J. Ethnopharmacol. (2011) 133: 278-288.

(8) Edrissian GhH. Malaria in Iran: Past and Present Situation. Iran. J. Parasitol. (2006) 1: 1-14.

(9) Ghiace A, Naghibi F, Esmaeili S and Mosaddegh M. A brief review of herbal remedies connected to malaria like fever in Iranian ancient medicinal books. Iran. J. Parasitol. (2014) 9: 553-559.

(10) Avicenna. Canon in Medicine (in Arabic). 1 ed. Dar Ihyaal-Turath al-Arabi, Beirut (2005) 4: 5-90.

(11) Muhammad ibn Zakariya Razi. Al-Mansouri (in Arabic). 1 ed. Arab League Education, Culture and Science Organization, Kuwait (1987).

(12) Ahmad Akhawayni Bukhari. Hedayat al-Motaallemin fi-Tebb (in Persian). 2 ed. Mashhad University, Mashhad (1992).

(13) Ismaeil Jurjani. Zakhireyi Kharazmshahi (in Persian). 1 ed. Academy of Medical Sciences, Islamic Republic of Iran, Tehran (2001).

(14) Bahaaaddin Nurbakhsh. Khulasat al-Tajarub (in Persian). 1 ed. Tehran University of Medical Sciences, Tehran (2008).

(15) Hakim Mohammad Akbar Arzani. Tebbe Akbari (in Persian). 1 ed. Institute of Natural Medicine, Qom (2008).

(16) Seyyid Mohammad Hossein. Makhzan al-Adwiyyah (in Persian). 1 ed. Tehran University of Medical Sciences, Tehran (2011).

(17) Muhammad ibn Zakariya Razi. Al-Heavi (in Arabic). 1 ed. Dar Ihyaal-Turath al-Arabi, Beirut (2001) 21.

(18) Peters W. The chemotherapy of rodent malaria. XXII. The value of drug resistantstrains of P. berghei in screening for blood schizonticidal activity. Ann. Trop. Med. Parasitol. (1975) 69: 155-171.

(19) Esmaeili S, Naghibi F, Mosaddegh M, Sahranavard Sh, Ghafari S and Abdullah NR. Screening of antiplasmodial properties among some traditionally used Iranian plants. J. Ethnopharmacol. (2009) 121: 400-404.

(20) Rocha e Silva LF, Lima ES, de Vasconcellos MC, Aranha ES, Costa DS, Mustafa EV, de Morais SK, Alecrim Md, Nunomura SM, Struve L, de Andrade-Neto VF and Pohlit AM. In-vitro and in-vivo antimalarial activity and cytotoxicity of extracts, fractions and a substance isolated from the Amazonian plant Tachia grandiflora (Gentianaceae). Mem. Inst. Oswaldo Cruz. (2013) 108: 501-507.

(21) Naghibi F, Esmaeili S, Abdullah NR, Nateghpour M, Taghvai M, Kamkar S and Mosaddegh M. In-vitro and in-vivo antimalarial evaluations of myrtle extract, a plant traditionally used for treatment of parasitic disorders. Biomed. Res. Int. (2013) 2013: 1-5.

(22) Mossman T. Rapid colorimetric assay for cellular growth and survival: applicationto proliferation and cytotoxicity assay. J. Immunol. Methods (1983) 65: 55-63.

(23) Behzad S, Pirani A and Mosaddegh M. Cytotoxic activity of some medicinal plants from Hamedan district of Iran. Iran. J. Pharm. Res. (2014) 13: 199-205.

(24) Boskabady MH, Shafei MN, Saberi Z and Amini S. Pharmacological Effects of Rosa Damascena. Iran. J. Basic. Med. Sci. (2011) 14: 295-307.

(25) Himesh S, Nanda S, Singhai AK and Jitender M. Radical scavenging activities and naturalindicator activity of aqueous and ethanolic extract of Rosa damascena. Int. J. Pharm. Pharm. Sci. (2012) 4: 581-586.

(26) Abdassi Maleki N, Abdassi Maleki S and Bekhradi R. Suppressive effects of rosa damacena essential oil on naloxone precipitated morphine withdrawal signs in male mice. Iran. J. Pharm. Res. (2013) 12: 357-361.

(27) Sanon S, Gansane AP, Ouattara L, Traore AN, Ouedraogo I, Tiono A, Taramelli D, Basilico NB and Sirima S. In-vitro antiplasmodial and cytotoxic properties of some medicinal plants from western Burkina Faso. AJLM (2013) 2: 1-7.

(28) Mobaj F. Antimalarial natural products: a review. AJVP (2012) 2: 52-62.

(29) Bischoff TA, Kelley ChJ, Karchesy Y, Laurantsos M, Nguyen-Dinh P and Arefi AG. Antimalarial activity of Lactucin and Lactucopicrin: sesquiterpene lactones isolated from Cichorium intybus L. J. Ethnopharmacol. (2004) 95: 455-457.

(30) Sangian H, Faramarzi H, Yazdinezhad AR, Mousavi SJ, Zamani Z, Nourbarani M and Ramazani A. Antiplasmodial activity of ethanolic extracts of some selected medicinal plants from the northwest of Iran. Parasitol. Res. (2013) 112: 3697-3701.

This article is available online at http://www.ijpr.ir
Back issues?
Visit http://www.ijpr.ir
or
http://ijpr.sbm.ac.ir