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An Investigation on Anti-malarial Effects of Tehranolide Isolated from *Artemisia diffusa* Against Human Malaria Parasite, *Plasmodium falciparum in vitro*

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ABSTRACT

Tehranolide which has been isolated from *Artemisia diffusa* has similar functional group to Artemisinin. More attention has been paid to *Artemisia annua* L., for its anti-plasmodial properties. In the present study, we investigated the anti-malarial effects of Tehranolide against human malaria parasite, *Plasmodium falciparum in vitro*. The chloroquine (CQ)-sensitive strain (3D7) of *P. falciparum* was continuously cultured in PRMI medium with addition of HT serum Albumax, RBC of O^+ blood group, 05% CO_2 at 37°C. The anti-malarial activity was determined by using different concentrations including 10, 30, 50 mg mL^{-1} of Tehranolide were made in drug vehicle including distilled water, methanol, DMSO and applied for therapy. Percentage of parasitaemia was counted after 24, 48 and 72 h after treatment for each concentration. Results indicated no effects of low concentration of Tehranolide on parasitaemia, however the concentrations of 10, 30 and 50 mg mL^{-1} represented their anti-plasmodial activities. The cytotoxic effects of high concentration occurred by destroying both parasites and RBCs in culture medium. Inhibition concentration of 50% (IC_{50}) on plasmodial survival was observed at concentration of 10 mg mL^{-1} after 48-72 h of treatment. It is concluded that, Tehranolide seems to be a promising drug exhibiting good anti-malarial effects in this human malaria *P. falciparum* model *in vitro*. However, more research is required before Tehranolide can be used for malaria treatment in human cases.

Key words: Anti-malarial, *Artemisia diffusa*, *Plasmodium falciparum*, Tehranolide

INTRODUCTION

Artemisia (*A.*) is a large, diverse genus of plants belonging to the daisy family Compositae (Asteraceae), one of the most bulky vegetal groupings, which comprises about 1000 genera and over 20,000 species. The genus Artemisia has always been of great botanical and pharmaceutical interest and is useful in traditional medicines for the treatment of a variety of diseases and complaints. *Artemisia annua* is a traditional medicinal herb which is presently being cultivated on a commercial scale in China and Vietnam for its anti-malarial sesquiterpene lactone artemisinin and its essential oil (Weyerstahl *et al*., 1993a, b).

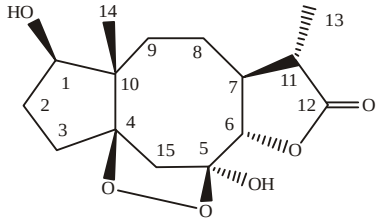

Fig. 1: Chemical structure of tehranolide (Artediffusin) a sesquiterpene lactone isolated from *A. diffusa*

The Iranian Artemisia species has been investigated chemically and presence of monoterpenes (Rustaiyan *et al*., 1987), sesquiterpene especially sesquiterpene lactones (Rustaiyan *et al*., 1989a, b) and essential oils reported (Rustaiyan *et al*., 2000a, b).

The malaria situation is aggravated mainly by the appearance of strains of *Plasmodium falciparum* resistant to anti-malarial drugs. Severe malaria is treated with intravenous or intramuscular injection of quinine or, increasingly, the artemisinin derivative artesunate which is superior to quinine in both children and adults (Dondorp *et al*., 2010). The disease results from the multiplication of malaria parasites within red blood cells, causing symptoms that typically include fever and headache, in severe cases progressing to coma and death. Symptoms of malaria include fever, shivering, arthralgia (joint pain), vomiting, anemia (caused by hemolysis), hemoglobinuria, retinal damage and convulsions (Beare *et al*., 2006).

There can be no doubt that the need for new anti-malarial drugs is being taken very seriously both by the scientific community and the pharmaceutical industry. Artemisinin and their derivatives are currently effective against these drug resistant strains. Tehranolide, is reported to have some antiparasitic properties *in vivo* on murine malaria (Rustaiyan *et al*., 2009b; Taherkhani *et al*., 2013). Previously the anti-malaria activity of Artediffusin (Tehranolide) a sesquiterpene lactone with an endoperoxide group on *P. berghei in vivo* was investigated (Fig. 1) (Rustaiyan *et al*., 2009a, 2011).

MATERIALS AND METHODS

Plant material: The aerial parts of *A. diffusa* collected in April 2012 from Ahmad Abad and Zaman Abad, North East of Iran, Khorassan Province, Iran. Voucher specimens have been deposited at the Herbarium of the Research Institute of Forests and Rangelands (HRIFR), Tehran, Iran.

Extraction and isolation: Ground aerial parts of *A. diffusa* (900 g) were extracted with Et_2O/MeOH/petroleum ether (1:1:1) (2×6 L) at room temperature for 72 h. Evaporation at reduced pressure furnished (35 g) of crude extract which was suspended in EtOH (120 mL), diluted with H_2O (300 mL) and extracted successively with hexane (2×250 mL) and $CHCl_3$ (3×250 mL). Evaporation of the $CHCl_3$ extract at reduced pressure furnished (13.5 g) of residue which was column chromatographed over silica gel (520 mg, 70-230 mesh) using hexane and increasing amounts of EtOAc (0-100%) and EtOAc/MeOH (9:1) to afford 32 fractions (All materials from Sigma Chemical Co.). Fractions that were similar were added together and the fractions were reduced to 20. The ^{13}C-NMR spectrums were taken from all fractions. Fractions 12 showed signals related to Tehranolide. Fractions 12 (120 mg) were reunited and rechromatographed on silica gel

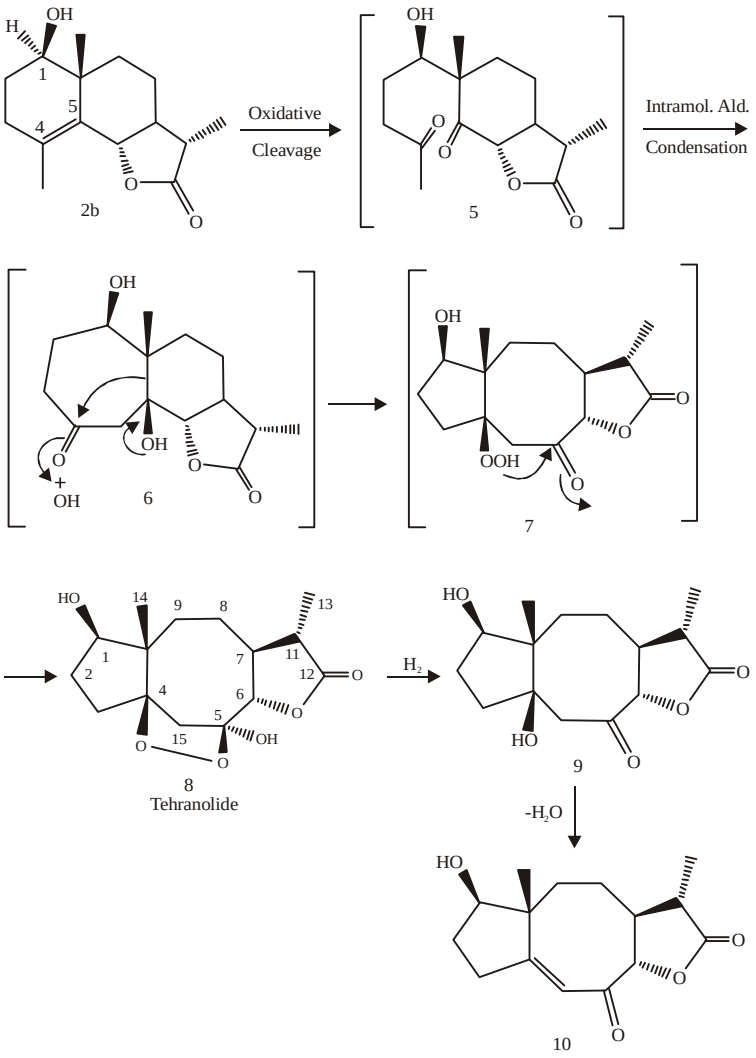

Fig. 2: Biosynthesis of tehranolide

(230-400 mesh) to give 26 fractions. The ^{13}C-NMR spectrums were taken from all achieved fractions. Fractions 14 afforded Tehranolide. The structure of Tehranolide was elucidated by the 500 MHz ^{13}C-NMR and ^{1}H-NMR Techniques. The most obvious characteristic of Tehranolide is 105 signals that related to C-5 attached to two oxygens in ^{13}C-NMR (Table 1).

Tehranolide: Colourless crystals, Mp 99ºC, IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3600 (OH), 1775 (γ-Lactone); MS m/z (rel. int.) 280[M-H_2O]+(2), 264[M-H_2O_2]$^+$(5), 246[264-H_2O]+(14), 135(31), 107(56), 84(96), 55(100) (Fig. 2) (Rustaiyan *et al*., 2011).

***Plasmodium falciparum* and its cultivation:** The chloroquine (CQ)-sensitive strain (3D7) of *P. falciparum* was continuously cultured according to the method described by Trager and Jensen (1976). Briefly, *P. falciparum* 3D7 parasites were cultured in a complete RPMI medium at 1% haematocrit, serum Albumax at 37°C in a 5% CO_2/3% O_2/balanced N_2 gas mixture as described

Table 1: NMR spectroscopic data (400 MHz, CD_3OD) for tehranolide

H	Tehranolide (CD_3OD)	C	Tehranolide
1	3.40^{dd}	1	80.9^{d}
2	1.78^{m}	2	24.6^{t}
2′	1.63^{m}	3	33.8^{t}
3	2.09^{m}	4	93.8^{s}
3′	1.80^{m}	5	105.6^{s}
6	4.11^{d}	6	84.7^{d}
7	1.80^{dq}	7	50.5^{d}
8	1.85^{m}	8	29.6^{t}
8′	1.53^{m}	9	37.7^{t}
9	$1.96^{br\ dd}$	10	50.1^{s}
9′	$1.36^{br\ dd}$	11	43.1^{d}
11	2.31^{dq}	12	175.9^{s}
13	1.21^{d}	13	12.2^{q}
14	0.91^{s}	14	12.6^{q}
15	2.98^{d}	15	50.1^{t}
15′	2.30^{d}		

(J[Hz]: 1, 2 = 6, 1, 2′ = 11, 7, 8 = 11, 7, 8′ = 1.5, 8, 9 = 8, 8′, 9′ = 12, 9, 9′ = 15, 15, 15′ = 14, 6, 7 = 11, 7, 11 = 11, 11, 13 = 7)

previously (All materials from Sigma Chemical Co.) (Miao and Cui, 2011). Human red blood cells (RBCs) were washed three times, stored at 4-8°C and used within three weeks. The haematocrit was measured from a packed RBC volume that was centrifuged in a swinging bucket rotor at 2,000x g for 5 min at room temperature. Schizonts were isolated on Percoll cushions centrifuged at room temperature in a swinging bucket rotor at 2,000x g for 5 min. For RS parasite analysis, aliquots were layered on top of 70% Percoll in 1.6 mL tubes and centrifuged at 4,000x g for 5 min. Pellets were washed once with RPMI 1640 medium and immediately frozen at -20°C until used (Childs *et al*., 2013; Sadeghi Tafreshi *et al*., 2009; Sangian *et al*., 2013).

Effects of tehranolide on *P. falciparum in vitro*: The anti-malarial activity of Tehranolide against human malaria parasite *P. falciparum* chloroquine-sensitive 3D7 strain was determined according to method of Sadeghi Tafreshi *et al*. (2009). Different concentrations including 10, 30, 50 mg mL^{-1} of Tehranolide were made in drug vehicle including distilled water, methanol, DMSO (3:1; 1; all from Sigma) and applied for therapy. Percentage of parasitaemia was counted after 24, 48 and 72 h after treatment for each concentration.

RESULTS

In addition to previous study indicating preventive efficacy of Tehranolide against murine malaria parasites *in vivo*, the anti-malarial effects of Tehranolide were confirmed here in this study against human malaria parasite; *P. falciparum in vitro*. The results of *in vivo* trial in mice indicated no significant toxicity after treatment with Tehranolide extract (Rustaiyan *et al*., 2009a, 2011). Results indicated no effects of low concentration of Tehranolide on parasitaemia (2 mg mL^{-1}), therefore, in this study, the concentrations of 10, 30 and 50 mg mL^{-1} were considered for comparison of antiplasmodial activities. Increasing the incubation time for treatment is leading to decrease the percentage of parasitaemia (Fig. 3).

In addition, the cytotoxic effects of 50 mg mL^{-1} as high concentration occurred by destroying both parasites and RBCs in culture medium. Inhibition concentration of 50% (IC_{50}) on plasmodial survival was observed at concentration of 10 mg mL^{-1} after 48-72 h of treatment (Fig. 3).

DISCUSSION

In the absence of a fully protective antimalarial vaccine, the control of malaria relies principally on the use of drugs for treatment or prophylaxis. Much of the increasing burden of malaria is due

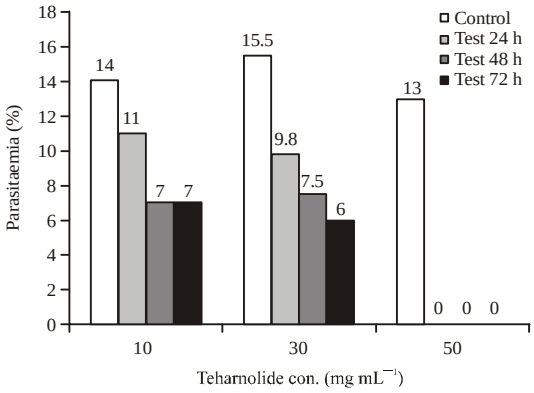

Fig. 3: Comparison of the anti-malarial effects of Tehranolide against human malaria parasite, *Plasmodium falciparum in vitro*

to the spread of the resistance of the major human malaria pathogen, *P. falciparum*, to most drugs presently available. The initial success of any adopted anti-infective strategy to malaria is followed by a descent due to the emergence of resistance to it. The search for new drugs and drug targets is a consistent demand in respect of this disease. Traditional medicinal plants and Artediffusin (Tehranolide) is reported as a new candidate of anti-malarial agent (Rustaiyan *et al*., 2009a; Rustaiyan and Vahedi, 2012). Tehranolide, is reported to have some antiparasitic properties *in vivo* on chloroquine-sensitive *P. berghei* murine malaria (Rustaiyan *et al*., 2009b; Taherkhani *et al*., 2013). As a complementary study, the anti-malarial effects of Tehranolide were investigated here against human malaria parasite; *P. falciparum in vitro*. In this study, aiming to search for new effective anti-malarial agent, we found, for the first time, *in vitro* anti-plasmodial activities of the Tehranolide. In addition to anti-malarial effects of Tehranolide on murine malaria parasites, which were published earlier by this group of authors (Rustaiyan *et al*., 2009a, 2011; Taherkhani *et al*., 2013), it may have similar ability to apply for human malaria therapy. Alternatively, new therapies can be performed based on the use of combination therapy such as artediffusin combination therapy (Rustaiyan *et al*., 2011).

CONCLUSION

Sesquiterpene lactones are found in *A. diffusa* with the peroxide functional group that probably has the same effect of artemisinin as an antimalarial agent. The artemisinin, as a sesquiterpene lactone endoperoxide, is becoming an important plant-derived compound in the treatment of the resistant malaria . It is concluded that, Tehranolide seems to be a promising drug exhibiting good anti-malarial effects in this human malaria *P. falciparum* model *in vitro*. However, more research will have to be carried out before Tehranolide can be used for malaria treatment in human cases.

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