

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Assessment of *in vitro* activity of *Peganum harmala* Extract on *Plasmodium falciparum* Growth Compared with Chloroquine

¹M. Nateghpour, ¹M. Sharbatkhori, ¹G.H. Edrissian, ²E. Souri, ¹M. Mohebbali,
³K. Akbarzadeh, ¹A. Motevalli Haghi ¹M. Satvat and ⁴A. Rahimi

¹Department of Parasitology and Mycology,
School of Public Health and Institute of Public Health Research,
Tehran University of Medical Science

²Pharmacy Faculty, Tehran University of Medical Science

³Department of Medical Entomology,
School of Public Health and Institute of Public Health Research,
Tehran University of Medical Science

⁴Department of Epidemiology and Biostatistics,
School of Public Health and Institute of Public Health Research,
Tehran University of Medical Science, Tehran, Iran

Abstract: In this study antiplasmodial activity of *Peganum harmala* was assessed against isolates of Chloroquine -sensitive and Chloroquine-resistant *plasmodium falciparum*, collected from southeast of Iran, in comparison with Chloroquine. *Peganum harmala* extract in four serial dilutions including 12.5, 25, 50 and 100 $\mu\text{g mL}^{-1}$ and chloroquine with 8 picomol/well in the test well were evaluated using *in vitro* method on 23 blood samples from patients infected with *Plasmodium falciparum*. All of four serial dilutions of *Peganum harmala* extract could inhibit parasite growth ($p < 0.05$). In comparison with Chloroquine at doses of 12.5 and 25 $\mu\text{g mL}^{-1}$, it was less effective than Chloroquine, while 50 and 100 $\mu\text{g mL}^{-1}$ had almost similar results to Chloroquine. *P. harmala* in doses of 50 and 100 $\mu\text{g mL}^{-1}$ could decrease significantly schizont formation of those two strains of *P. falciparum* parasites which were resistant to chloroquine.

Key words: *Peganum harmala*, *Plasmodium falciparum*, *in vitro*, chloroquine, Iran

INTRODUCTION

Despite extensive efforts for removing malaria from many countries it is estimated that there are still 300-500 million people affected with malaria parasites annually in the world (WHO, 2001 a, b). The resistance of *Plasmodium* species to some valuable chemotherapeutic agents such as Chloroquine means that malaria must be considered as the most important parasitic disease in tropical and sub-tropical areas. Hence, the necessity of new antimalarial drugs with novel actions are escalating and in this context investigating about some indigenous plants for the treatment of malaria has engaged a special place in the field of malaria (O'Neill *et al.*, 1985; Simonsen *et al.*, 2001). *Peganum harmala* an indigenous plant in Iran, is used as a fumigating plant in some parts of the country. Extract of the plant has been considered as antibacterial

and antiparasitic agent by some investigators (Mahdavi and Masood, 2002; Zafar *et al.*, 1990; Motevalli *et al.*, 2003).

MATERIALS AND METHODS

This study was conducted in the Iranshahr and Sarbaz districts (Sistan and Balouchestan province) located in south-east of Iran, from November 2003 to October 2004.

Plant material: *Peganum harmala* was collected from different parts of Gorgan district located in the Northeast of Iran. Seeds of the collected plant were used for extraction.

Preparation of plant-extract: Dried powdered seeds of *P. harmala* (300 g) was macerated and mixed with 96%

Corresponding Author: M. Sharbatkhori, Department of Parasitology and Mycology,
School of Public Health and Institute of Public Health Research,
Tehran University of Medical Science, Tehran, Iran
Tel/Fax: 98 218891392

ethanol (1200 mL) for 2 h and then the mixture was kept overnight in ambient room temperature.

The process was repeated five times and at each time somewhat extract was collected.

The total ethanolic extracts concentrated to dryness under reduced pressure and the amount of extracted material was registered. The crude extract was dark brown in colour.

Preparation of dilutions: The crude extract of *P. harmala* was dissolved in 5% aqueous solution of dimethylsulfoxide (DMSO)(sigma) as to yield final concentrations of 12.5, 25, 50 and 100 µg mL⁻¹ at the test wells.

Chloroquine-phosphate, as a positive control, was dissolved in distilled water so that the final concentration reached 8 Pico mol/well at the test wells. All of dilutions and Chloroquine-phosphate and DMSO were sterilized by pumping through a 0.22 µm filter (Millipore).

Preparation of culture medium: The culture medium was prepared in a one liter volume by dissolving 10.4 g of RPMI 1640 powdered medium (GibcoBRL), 2.2 g sodium bicarbonate (Merck), 50 mg hypozanthine (sigma), 20 g Glucose(Merck) and 5.94 g HEPES buffer (GibcoBRL) in distilled water. The solution was sterilized by pumping (Sartorius) through a 0.22 µm filter. The filtered medium was completed by adding 10% AB type human serum and 10 µg mL⁻¹ gentamicine (as a precaution against bacterial contamination). The completed medium was stored at 4°C.

In vitro test for antimalarial activity: Twenty three samples of *P. falciparum* were isolated from malaria patients, with criteria as mono-infection, fleshy rings and 1000-80000 asexual parasite per microlitre of blood. The assay procedure was based on the method of WHO (WHO, CTD/MAL/97.20) with some modifications. Briefly, 10 µL aliquots of diluted extract at four different concentrations (as mentioned in part 2.3) were dispensed into 96-well microtiter plates. Ninety microliter of a 10% haematocrite of parasitized erythrocytes (isolated samples) suspended in the medium was added to each well. Into wells that had been allocated to Chloroquine, 10 µL of the drug plus 90 µL of the parasitized erythrocytes were added. Two series of control were performed, one with parasitized blood without addition of plant extract and another with infected erythrocytes plus DMSO 5% to detect, if any, adverse effects of the solvent on growth of the parasites. The culture and incubation processes were carried on according to Trager and Jensen (1976) method. After incubation period, the red blood cells sedimented on

the bottom of the wells were transferred onto clean microscope slides to form a series of thick smears. The thick smears were stained with 5% Giemsa solution for 30 min and then examined under immersion oil lens. The number of formed schizonts with three or more nuclei were counted out of a total of 200 asexual parasites. The mean of each set of the wells was calculated and then analyzed with McNemar test. Inhibition% for each concentration was calculated (Deharo *et al.*, 2001) as:

$$\text{Inhibition\%} = \frac{\text{No. of Schizont in control wells} - \text{No. of Schizont in treated wells}}{\text{No. of Schizont in Control wells}} \times 100$$

RESULTS

Range of primary parasitaemia in considered samples was 2640-60000 parasite per microlitre of blood. The results showed that there was not significant relevance to primary parasitaemia and effectiveness of different concentrations of extract of the plant on the parasites (p<0.05). Such result appeared for Chloroquine as well. Mean of Schizont formation was 134±30.3 and 136±31.2 at the control 1 (Containing infected blood and culture medium) and control 2 (containing infected blood, culture medium and DMSO 5%), respectively. There was not significant difference between control 1 and control 2 in schizont formation (p<0.05). Mean of Schizont formation for concentrations of 12.5, 25, 50 and 100 µg mL⁻¹ of the plant was found as 12±5.7, 3.5±4.1, 2±3.8 and 0.3±1.4, respectively (Table 1). Meanwhile, Chloroquine exerted its efficacy on the parasites which resulted in a mean of 0.5±1.4 for schizont formation (Table 1). Inhibition percent of the parasites for Chloroquine and the plant concentrations are tabulated in table one. Three of twenty three samples were found as Chloroquine resistant cases using *in vitro* test that all of them showed a significant decrease in schizont formation in 100 µg mL⁻¹ concentration of *P. harmala* (Table 2).

Table 1: Schizont formation of *P. falciparum* in the control wells and wells those treated with extract of *P. harmala*

Wells	Mean of Schizont formation±SD	Inhibition (%)
Control	134±30.3	0
12.5 (µg mL ⁻¹)*	12±5.7	91
25 (µg mL ⁻¹)	3.5±4.1	97.4
50 (µg mL ⁻¹)	2±3.8	98.5
100 (µg mL ⁻¹)	0.3±1.4	99.8
Chloroquine	0.5±1.4	99.6

* Different Concentrations of extract of *P. harmala*

Table 2: Comparative schizont formation of Chloroquine- sensitive and Chloroquine - resistant *P. falciparum* strains in response to Chloroquine and four concentrations of *P. harmala*

Chloroquine (CQ) and <i>P. harmala</i> (P.h)	Schizont formation±SD				
Strains	P.h 12.5 ($\mu\text{g mL}^{-1}$)	P.h 25 ($\mu\text{g mL}^{-1}$)	P.h 50 ($\mu\text{g mL}^{-1}$)	P.h 100 ($\mu\text{g mL}^{-1}$)	CQ
CQ-sensitive	11.5±5.9	2.78±3.9	1.47±3.5	0	0
CQ-resistant	15.5±1	8.4±0.5	5.5±4.7	2.3±4	4.1±1

DISCUSSION

The extract of *Peganum harmala* showed an inhibitory effect on 23 isolates of *P. falciparum* with 91, 97.4, 98.5 and 99.8% inhibition for concentrations of 12.5, 25, 50 and 100 $\mu\text{g mL}^{-1}$, respectively. McNemar test showed a significant difference between all four concentrations of *P. harmala* and control in schizont formation ($p < 0.05$). The results showed that there was not significant difference in inhibitory effect between 50 and 100 $\mu\text{g mL}^{-1}$ concentrations of *P. harmala* and Chloroquine on 20 Chloroquine- sensitive isolates of *P. falciparum* (Table 1) but 100 $\mu\text{g mL}^{-1}$ concentration of the plant was about two-fold more effective than Chloroquine on the three Chloroquine-resistant *P. falciparum* isolates (Table 2). Effectiveness of *P. harmala* on *Plasmodium berghei* using *in vivo* test in sorian mice was considered by Motevalli *et al.* (2003).

The investigator also showed that extract of the plant had not any toxic effect on the mice at the concentration of 100 $\mu\text{g mL}^{-1}$ or less than that (Motevalli *et al.*, 2003). In another investigation Sathiyamoorthy *et al.* (1999) demonstrated that extract of *P. harmala* seeds could fully inhibit the growth of a cultured strain of *P. falciparum* in concentration of 500 $\mu\text{g mL}^{-1}$.

These initial *in vitro* results clearly indicate that extract of *P. harmala* can prevent growth of *P. falciparum* either Chloroquine sensitive or Chloroquine-resistant strains. Present findings also indicated that DMSO 5% can be used as a safe plant solvent for *P. falciparum*.

ACKNOWLEDGMENTS

We are grateful to Mr. Sayedzadeh and our colleague in the Iranshahr Public Health training and Research station for their technical support. Financial support from the Institute of Public Health Research, Tehran University of Medical Sciences is gratefully acknowledged.

REFERENCES

- Deharo, E. *et al.*, 2001. A search for natural bioactive compounds in Boliva through a multidisciplinary approach. Part V. Evaluation of the malarial activity of plants used by the Tacana Indians. *J. Ethnopharmacol.*, 77: 91-98.
- Mahdavi, M. and J. Masood, 2002. Scolicidal effect of alcoholic, aqueous and total alkaloids of *Peganum harmala* L. (Syrian rue) against hydatid cysts protoscolices. *Fac. Med. J. Tehran Univ. Med. Sci.*, 16: 3.
- Motevalli Haghi A. *et al.*, 2003. Evaluation of the effectiveness of ethanolic of *Peganum harmala* L. against *plasmodium berghei* in comparison with Chloroquine Saurian mice *in vivo* test. *School of Public Health and Institute of Public Health Research J. Tehran Univ. Med. Sci.*, 2nd year, No., 2: 37-53.
- O'Neill, M.J., D.H. Bray, P. Boardman, J.D. Phillipson and D.C. Warhurst, 1985. Plants as sources of antimalarial drugs part. 1. *In vitro* test method for the evaluation of crude extracts from plants. *Planta Med.*, 51: 394-398.
- Sathiyamoorthy, P. *et al.*, 1999. Screening for cytotoxic and antimalarial activities in desert plants of the Negev and Bedouin market plant products. *Pharm. Biol.*, 37: 188-195.
- Simonsen, H.T., J.B. Nordskjold, U.W. Smitt, U. Nyman, P. Palpu, P. Joshi and G. Varughese, 2001. *In vitro* screening of Indian medicinal plants for antiplasmodial activity. *J. Ethnopharmacol.* 2001 Feb., 74: 195-204.
- Trager, W. and J. Jensen, 1976. Human malaria parasite in continuous culture. *Sciences*, 193: 673-675.
- WHO, 2001a. The use of antimalarial drugs, Report of a WHO Informal consultation, 13-17 November, WHO /CDS/RBM, 33: 8-16.
- WHO, 2001b. *In vitro* micro-test (mark III) for the assessment of the response of *plasmodium falciparum* to Chloroquine, Mefloquine, Amodiaquine, Sulfadoxine/Pyrimethamine and Artemisinin. CTD/MAL/97.20
- Zafar, M.M. *et al.*, 1990. Screening of *Artemisia absinthium* for antimalarial effects on *plasmodium berghei* in mice: A preliminary report *J. Ethnopharmacol.*, 30: 223-226.