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Screening of Cytotoxicity and Antiplasmodial Activity of *Xanthium strumarium* L

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ABSTRACT

Screening of medicinal plants along with safety parameters are the primary target in the development of new medicines. Thus the present work has been undertaken to evaluate *in vitro* antiplasmodial efficacy, cytotoxicity and selectivity index of ethanolic leaves extract of *Xanthium strumarium* (ELEXS). *In vitro* antiplasmodial activity of ELEXS has been evaluated through Schizont inhibition assay. The cytotoxicity of plant extracts has been determined through standard colorimetric methylthiazoletetrazolium (MTT) assay by using Hela cancer cell line. ELEXS has been found to exert significant *in vitro* antiplasmodial activity with an IC_{50} 4 µg/ml and cytotoxicity with an IC_{50} 40 µg/ml. The selectivity index was found to be 10, which offers potential safer therapy. The significant *in vitro* antiplasmodial activity and high selectivity index stresses the need to isolated active antiplasmodial components and characterize their exact mechanism of action.
Key words: Antiplasmodial, Cytotoxicity, *In vitro*, *Plasmodium berghei*, Selectivity index, *Xanthium strumarium*.

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INTRODUCTION

Malaria is the most widespread and devastating parasitic disease in the world with an estimated 300-500 million clinical cases and 1.2–2.8 million deaths annually [1]. Prompt treatment with effective antimalarials is seriously compromised with the rising problem of resistance to classical antimalarials drugs such as chloroquine and sulfadoxine/ pyrimethamine and the problem of recrudescence of artemisinin and its derivatives [2]. Thus, it becomes imperative to search new antimalarial compounds having effective mode of action [3, 4].

Plants have a long history of their use in the treatment of malaria. Most of the present-day medicines have been developed from traditional medicinal plants. *Xanthium strumarium* L. (cocklebur), belongs to compositae family, is an annually growing herb in wild. The genus Xanthium includes 25 species, among which two species *X. indicum* and *X. strumarium* have been reported in India [5]. The whole plant especially leaves, root and fruit is used in traditional medicine. Leaves of plants have been found to exert *in vitro* and *In vivo* antitrypanosomal effect [6]. *In vivo* antiplasmodial activity of ethanolic leaf extract of *Xanthium strumarium* has shown promising suppressive, preventive and curative antiplasmodial activity [7], however, it's *in vitro* antiplasmodial efficacy and cytotoxicity has not been evaluated. Thus in the present work it's *in vitro* antiplasmodial efficacy has been determined along with its therapeutic safety.

MATERIALS AND METHODS

Plant material

Leaves of *X. strumarium* were collected in the month of September, from the Mandi district of Himachal Pradesh. Voucher specimen (17920) was deposited in the herbarium of Department of Botany, Panjab University, Chandigarh, India, where identification of plants was confirmed by expert taxonomist.

Preparation of extract

The leaves of *X. strumarium* were collected air dried and pulverized into powder. Dried and powdered leaves (300 g) were stirred in 500 ml ethanol and kept overnight at room temperature. The solvent extract was filtered, and solvent was evaporated to dryness in a rotary evaporator. The resulting residue was stored in screw-capped vials at –4°C temperature until tested.

Animals

BALB/c mice weighing 25-30 g of either sex were obtained from the Central Animal House, Panjab University, Chandigarh, India. The animals were housed in standard plastic cages acclimatized for a period of 30 days. The mice were maintained on standard feed and water ad

libitum. Approval for the study was obtained from the Animal Ethics Committee, Panjab University, Chandigarh CPCSEA 45/1999.

Maintenance of Parasitic Strain

The chloroquine sensitive *Plasmodium berghei* (NK65 strain) was maintained *In vivo* in BALB/c mice in our laboratory by weekly inoculation of 1×10^7 *P. berghei* infected red blood cells in naive mice.

Schizont maturation inhibition assay

Short term *in vitro* culture of blood stages of *Plasmodium berghei* was maintained by using candle jar method [8]. RPMI–1640 (Gibco) supplemented with 0.06% (w/v) HEPES, 5% (w/v) sodium bicarbonate; antibiotics – gentamycin (50 µg/ml), penicillin (100 Ul/ml) and streptomycin (100 µg/ml) along with 10% (v/v) inactivated fetal calf serum (FCS) was used as culture medium. Normal and *P. berghei* infected red cells were mixed in a proportion to have 2-4% parasitemia at 0 h.

The antiplasmodial activity of the extract was checked according to WHO method, which is based on the assessment of the inhibition of schizont maturation [9]. Cells containing more than three nuclei were considered as schizonts. The stock solution of extract was prepared by dissolving known quantity of extract in DMSO (20 mg/ml), which was further diluted with RPMI–1640, to achieve the required concentration (1 µg – 100 µg/ml) before being tested in 24-well microtiter plate. Each concentration was run in triplicate wells along with 10 µM chloroquine (positive control) and solvent ≤0.02% (negative control). One milliliter of complete medium was added to each well in microtiter plate having 100 µl of test/standard drug solution. The culture plates were incubated at 37°C in a candle jar (5% CO_2, 17% O_2, 78% N_2). After 21 hrs of incubation, thin blood smears from each well were prepared, fixed in methanol and stained with Giemsa's stain. Erythrocytic blood stages (rings, trophozoites, and schizonts) of parasite were counted under light microscope and their differential percentage was calculated among 500 cells. Fifty percent inhibitory concentration (IC_{50}) is defined as the drug concentration corresponding to 50% inhibition of schizont development as compared to the control. Percentage of schizont maturation inhibition was calculated by 100[(A-B)/A], where A is the average schizont maturation in the untreated control well and B is the average schizont maturation in the extract or drug treated wells. IC_{50} value was determined by plotting a graph between various concentrations of extract and percentage of schizont maturation inhibition corresponding to that concentration.

Cytotoxicity on mammalian cells and Selectivity index

The human cervix carcinoma cells HeLa were seeded into 24-well microtiter plate at 5000 cells per well. After 24 hrs, cells were washed and maintained with different concentrations of extract for 5 days, at 37°C, under 5% CO_2 atmosphere. The cytotoxicity of plant extracts was determined using the colorimetric methylthiazoletetrazolium (MTT) assay

[10] scored as a percentage of absorbance reduction at 405 nm of treated cultures versus untreated control cultures. The stock solution of extract was prepared by dissolving known quantity of extract in DMSO (10 mg/ml), which was further diluted with RPMI–1640, to achieve the required concentration (10 µg – 100 µg/ml) before being tested in 24-well microtiter plate. IC_{50} values on cell growth were obtained from drug concentration-response curves. Results were expressed as the means ± standard deviation determined from three independent experiments. The selective index was determined by the ratio of the IC_{50} value on cancer (HeLa) cells to the IC_{50} value on *Plasmodium berghei*. Extract with high selectivity offer the potential of safer therapy.

Statistical analysis

Appropriate statistical analysis was carried out by Student's t test using Graphpad Prism software (3.0), where a p value <0.05 was considered as significant.

RESULTS

Schizont maturation inhibition assay

There was almost a three-fold increase in the percentage of parasitemia in control well after 21 hrs of incubation as compared to the 0 h smear. ELEXS was found to inhibit *P. berghei* schizonts maturation in dose-dependent manner, with an IC_{50} 4 µg/ml after 21 hrs (Fig. 1). Maximum schizont maturation inhibition (88.1%) was observed with 100 µg/ml concentration of extract, while concentrations of 80, 40, 20, 10, 5, 4, 3, 2 and 1 µg/ml extract showed 79.6, 72.2, 65.5, 56.6, 54.4, 50.0, 48.1, 46.9 and 56.2% inhibition, respectively. Chloroquine at 10 µM concentration exhibited 90.3% inhibition.

Cytotoxicity on mammalian cells and selectivity index

The Extract has been found to exert dose dependent cytotoxicity with an IC_{50} 40 µg/ml (Fig. 2). The maximum cytotoxicity (73%) was observed with 100 µg/ml concentration of extract, in the present study. The selectivity index was found to be 10, which offers potential safer therapy.

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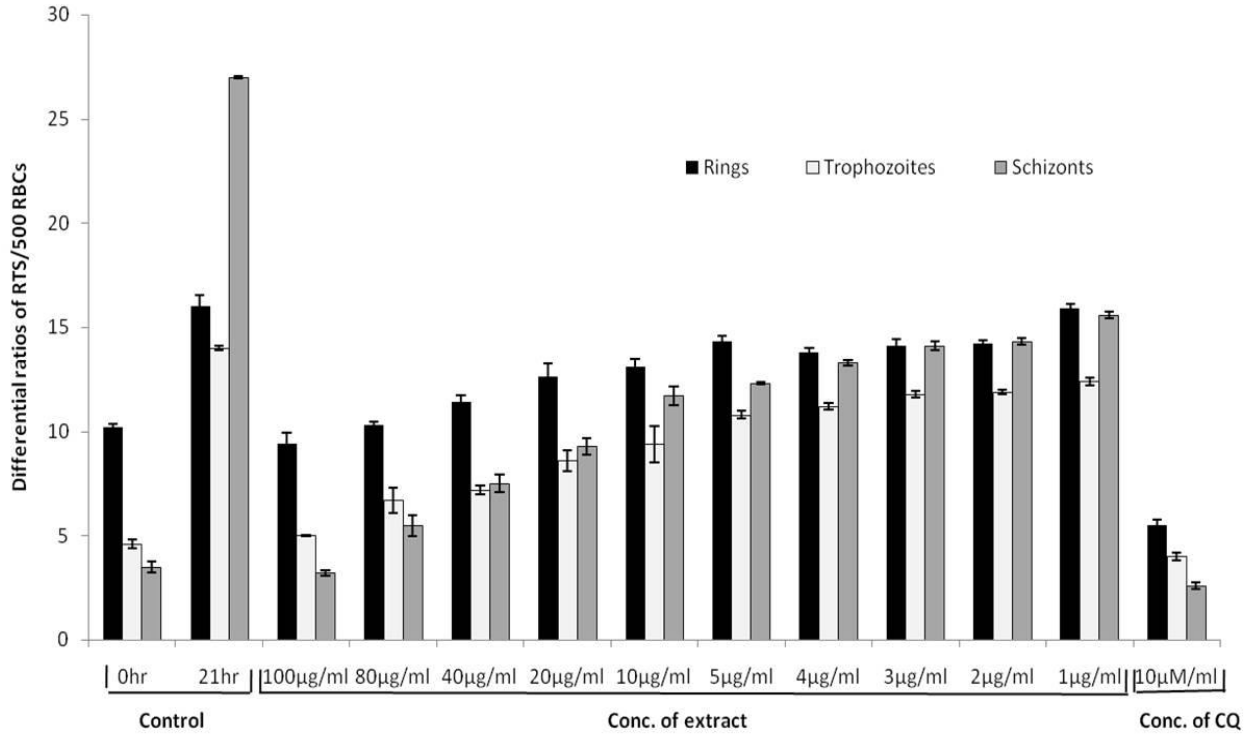

Figure 1: Effect of ELEXS on Intraerythrocytic Growth of Plasmodium berghei *in vitro*. Observations obtained are mean of three independent experiments and each concentration is added in triplicate wells every time along with positive and negative controls.

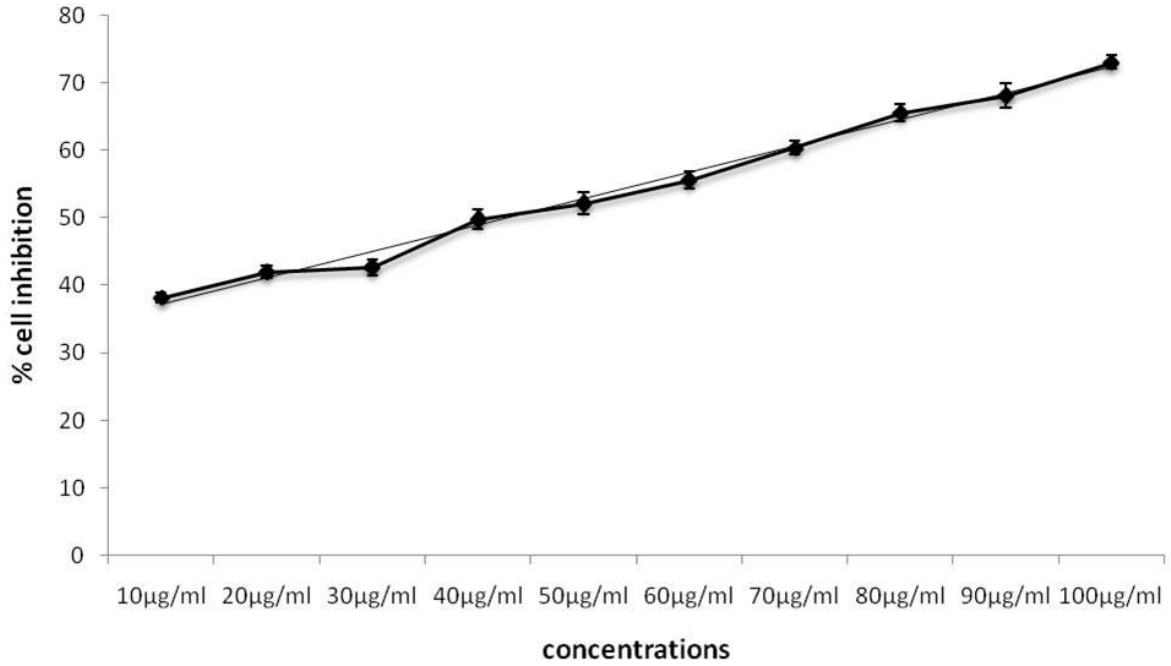

Figure 2: *In vitro* cytotoxicity of ELEXS against HeLa cell line. Observations showing percentage inhibitions of cells are obtained as the mean of three independent experiments.

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DISCUSSION

Plants have been a prime source of effective conventional drugs for the treatment of various parasitic diseases since ever [11]. The search for medicines, which undoubtedly began in prehistorical times, has led to compounds such as morphine, atropine, tubocurarine, digoxin, quinine and artemisinin like compounds. The success of quinine and artemisinin has opened new horizon in antimalarial drug research and increased interest in plants with medicinal properties [12, 13]. The isolation of active antiplasmodial components from reputed traditional antimalarial medicinal plant species such as *Creptolepis sanguinoleta, Dichroa febrifuga, Neurolaena lobata, Vernonia brachycalyx, Khaya grandifolia* and *Nardostachys chinensis* has shown promising *in vitro* as well as *in vivo* antiplasmodial activity [14, 15]. Evaluation of *in vitro* as well as *in vivo* antiplasmodial activity of medicinal along with its safety parameters is very necessary before going for isolation and purification of active constituents.

ELEXS has shown promising suppressive, preventative and curative antiplasmodial efficacy during our previous study, however, *in vitro* cytotoxicity, antiplasmodial efficacy and selectivity index has not been evaluated yet. The antiplasmodial activity of the extract was qualified as active when IC_{50} is < 5 µg/ml. the extract having activity beyond 10 µg/ml was consider inactive [16]. The selectivity index (SI) is defined as the ratio of the cytotoxicity on HeLa cell line to the antiplasmodial activity. Those that showed high SI (>10) should offer the potential for safer therapy [17]. *P. berghei* serves as an important source for determining *in vitro* antiplasmodial efficacy of plant extracts in developing countries, where maintenance of *Plasmodium falciparum* culture is difficult. ELEXS has been found to exert significant antimalarial activity with an IC_{50} 40 µg/ml as determined by drawing linear dose response curve between different concentrations of ELEXS and percentage of schizont maturation inhibition obtained at that concentration. The extract exerted unusually dose dependent inhibition of intraerythrocytic parasitic growth. The extract has been found to exert high *in vitro* cytotoxicity with an IC_{50} of 40 µg/ml. These results further supports our previous results of acute toxicity test carried out in BALB/c mice [7]. Present study showed that inspite of high cytoxicity the selectivity index of ELEXS was high, which indicates the promising efficacy and safety in choosing this traditional medicinal plant for further isolation and characterization of active antiplasmodial principle.

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REFERENCES

[1] Nain V, Sahi S, Verma A. Malar J 2010; 9: 258. doi: 10.1186/1475-2875-9-258.
[2] Kannan R, Kumar K, Shal D, Kukretis P, Chauhan VS. Biochem J 2005; 385(2): 409-418.
[3] Golenser J, Waknine JH, Krugliak M, Hunt NH, Gran GE. Inter J Parasitol 2006; 36(4): 1427-1441.
[4] Ibezim EC, Odo U. Afri J Biotechnol 2008; 7: 349-356.
[5] Oudhia P. Asian J Microbiol Biotechnol Environ Sci 2001; 3(1-2): 89-92.
[6] Talakal TS, Dwivedi SK, Sharma SR. J Ethnopharmacol 1995; 49(3): 141-145.
[7] Chandel S, Bagai U, Vashishat N. Parasitol Res 2011; doi: 10.1007/s00436-011-2611-1.
[8] Trager W, Jensen JB. Bull WHO 1977; 55: 363-365.
[9] World Health Organisation. *In vitro* Micro-Test (Mark III) for the assessment of the response of *Plasmodium falciparum* to chloroquine, mefloquine, quinine, amodiaquine, sulfadoxine/pyrimethamine and artemisinin. Geneva, 256 WHO, CTD/MAL/97; 2001. p 20.
[10] Mosmann T. J Immunol Methods 1983; 65: 55-63.
[11] Moon HI. Phytother Res 2010; 24: 941–944. doi: 10.1002/ptr.3104
[12] Rosenthal PJ. J Exp Biol 2003; 206: 3735-3744.
[13] Chandel S, Bagai U. IJMR 2010; 131: 440-444.
[14] Wright CW. Phytochem Rev 2005; 4: 55–61.
[15] Bhatnagar S, Das P. J Herbs Spices Med Plants 2007; 13(1): 103–132.
[16] Deharo E, Bourdy G, Quenevo C, Munoz V, Ruiz G, Sauvain M. J Ethnopharmacol 2001; 77: 91-98.
[17] Ramazani A, Zakari S, Sardari S, Khodakarim N, Djadidt ND. Malar J 2010; 9: 124. doi:10.1186/1475-2875-9-124.