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Antiplasmodial Efficacy of Fruit Extracts and Cladodes of Opuntia ficus-indica

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Abstract

Development of antimalarial drugs from medicinal plants continues to be a very appealing process. Therapeutic effects of the ethyl acetate cladodes an extract of *Opuntia ficus-indica* has been evaluated in *Plasmodium berghei* infection in mice. The extract of the *Opuntia ficus-indica* has an in vitro activity against the parasite, the average parasitemia of 4.7% from two wells at 24 hour concerning a blood control containing only 7.5%. The reduced parasitemia extract batch has proved to process more efficiently than the control group with olive oil, with 3.2% parasitemia on day 5 and 30% on day 8. However, the average parasitemia of the treated animals with the extract has been lower than the control detected one which showes very low levels of parasitemia (5%, 3.7% and 6.7%). This study presents an in vitro and in vivo evaluation of the antiplasmodial effects of two-plant extracts of *Opuntia ficus-indica* commonly used in Tunisia as a folk medicine. It is concluded that the ethyl acetate cladodes extract of *Opuntia ficus-indica* prove to be potentially useful for the development of antimalarial drug.

Keywords: Antimalaria, Percentage suppression, *Opuntia ficus-indica*, *Plasmodium Berghei*, Parasitaemia

1. Introduction

Malaria is a serious and lethal disease and mortals affecting the tropical areas, in particular Africa. In sub-Saharan Africa, this infectious disease, causing numerous deaths, is endemic due to the warm climate (Dadji et al., 2011). It is recognized today that malaria is resource of poverty and a major hurdle with economic development in many countries where this disease predominantly prevails. Treatments are available and still effective for the time being. However, a disease-borne agent, Plasmodium, develops quickly as a resistance to the molecules in use (Peters, 1998; Wellems and Plowe, 2001; Djaman et al., 2004; Pradines et al., 2010).

The development and spread of drug resistant strains of the causative *Plasmodium Berghei* agent has limited the effectiveness of the currently used antimalarial drugs. Hence, the need for new antimalarial drugs. Previous findings of antimalarial agents such as quinine and artemisinin extracted from medicinal plants have also enhanced the possibility of discovering new antimalarial drugs from plant source (Schwikkard et al., 2002).

In this context, the present study exposes an attempt made to explore the potential antiplasmodial cladodes of *Opuntia ficus-indica* selected terrestrial medicinal plants to fight *Plasmosdium Berghei.*

2. Applied Materials and Methods

2.1. Materials

2.1.1. Plant materials

Cladodes of *Opuntia ficus-indica* L. (Cactaceae) spineless (2-3 week-old) and fruits of the precocious cultivar (Ain Amara) from the FAO collection located in the region of Kairouan,Tunisia, were harvested in August 2010. The second spineless, belated fruits were harvested from the region of Nabeul, Tunisia in October 2010.

2.1.2. Animals

Determining the antipaludial activity of extracts has been implemented on an animal model of *Plasmodium Berghei* taken from the Institute Pasteur in Paris. The mice were female Swiss mice weighing 18-20 g from January (France) breeding and the animals were fed ad libitum with standard feed and had free access to water. They were also maintained under standard conditions of humidity, temperature and 12 hour light/darkness cycles. The animals were acclimatized for two weeks before the beginning of the study.

The parasitemia has been determined on day 5 and animal survivals have been evaluated on 14th day. A blood smear has been undertaken to determine parasitemia after staining with May-Grunwald-Giemsay.

2.1.3. Inoculum

The strains have been cryopreserved in the presence of 10% DMSO (Dimethyl sulfoxide) at -192° C in liquid nitrogen. A vial of 350 µl of total blood containing *Plasmodium Berghei* has been thawed by incubation at 37° C. After centrifugation, the supernatant was removed and the pellet was diluted with sterile saline. Two Swiss mice (mice 1 and 2) have been inoculated with 100 ul of blood containing parasitized erythrocytes. Maintenance of *Plasmodium Berghei* in Swiss mice was made by retro taking - orbital blood of an infested mouse, then by transferring the blood by intraperitoneal inoculation in to a healthy mouse.

2.2. Methods

2.2.1. Study of the in vitro activity of extracts

Three solvents have been used for the preparation the fruit and cladodes extracts: water, methanol and ethyl acetate. The extract is diluted in DMSO to obtain a mother solution to 1 mg/ml.

The test has been carried out realized in 96-well microplates with flat bottom. Each well contains 100 µl of blood and 1 µl of the extract to be tested. The 100 µl of blood solution has been formed by 0.3 ml from the mouse 1 and 0.4 ml of the mouse 2 in which we added a cell culture medium (RPMI) to complete in reach the level of 2 ml.

Of heparin has been added to the bottom of the tube along with 20 µl antibiotic (penicillin and streptomycin to 10mg/ml). Mouse 1 parasitemia has attained, while 25%, that of the wells has been comprised between 2 and 3%.

The test product is compared to three controls:

-Control1: wells containing only the blood

- Control 2: wells containing blood and 1µl of DMSO

- Control 3: wells containing blood and 1 µl of Chloroquin

Two wells are constructed for extract and control purposes.

The micropatch is then placed at 37 °C in an atmosphere of 5% CO2.

Then, a follow-up study analysis of the parasitemia relevant to each of the wells after 24, 48 and 72 hours has been executed by mean of smears colored by May-Grunwald-Giemsay.

2.2.2. Study of the in vivo activity of extract

The extract was administered to mice at a dose of 10 mg/kg by force-feeding. For this purpose, we prepare 10 mg of the ethyl acetate extract powder from the cladodes to be tested, which has been diluted in 5 ml of water for Chloroquin and 5 ml of olive oil for the extract to be tested.

2.2.2.1. Mice Infestation by Plasmodium

The blood of many parasitized mice donors has been taken via retro orbital puncture with a heparinized Pasteur pipette in advance. The mice blood was then Poole (2 ml of blood have been collected).

The blood sample obtained has then been diluted to 10 000th the number of red blood cell, calculated by counting on a Malassez cell.

After that, a smear has been then made from the blood Poole to calculate parasitemia and the number of parasitized erythrocytes per milliliter determined. So, the blood was starting to get diluted to a parasitemia of 10^8 parasitized erythrocytes/ml or 10^7 parasitized erythrocytes /100 µl, corresponding to the injection volume.

2.2.2.2. In vivo test according to Peters

On day 0, all 24 mice were infected by the inoculum containing 100 µl 10^7 parasitized erythrocytes in an intraperitoneal

way. For 4 days from day 0, mice have received 0.1 ml by force-feeding of different substance depending on their lot at a dose of 10 mg /kg.

The mice were divided into three batches of 8 mice:

- Lot 1: control receiving 0.1 ml olive oil only
- Lot 2: control receiving 0.1 ml of Chloroquin
- Lot 3: mice receiving 0.1 ml of extract ethyl acetate from the cladodes of *Opuntia ficus-indica*.

On D5, D8 and D10, the parasitemia of all mice was calculated from a caudal blood smears. Then the mean parasitemia by a batch was performed.

The average of the parasitemia by lot has been then realized. The mortality of mice was followed until day 18.

3. Results

3.1. In vitro anti-malaria test

Determining of parasitemia smears on a 48 and 72 hour basis turns out to be more difficult than determining them on a 24 hour interval. Indeed, the red cell count was complicated because the erythrocytes were very pale. This is probably due to their ageing.

So, to color smear in a 72 hour interval we used the usual protocol applied to color skin protozoa (see Table 1).

The entirety of water extracts, methanol fruits and cladodes were inactive and the two extracts derived from ethyl juice of two different cultivars acetate were not significantly at lower antiplasmodial activity. Actually, the ethyl acetate derived cladodes proved to have an *in vitro* activity against *Plasmodium Burghei*.

At a 24 hour basis the group treated with chloroquin demonstrated a significant ($p<0.05$) parasite suppression (1.50 ± 0.15 %), compared to all other wells. However, no significant difference ($p>0.05$) was noticed among the wells treated with 1mg/ml crude ethyl acetate cladodes extract of *Opuntia ficus-indica*. The well which was not treated at all, gave the highest average proportion of parasite (7.5 ± 0.75 %). The power of the antimalarial chloroquin (1,7 ± 0.11 % ; 1,8 ± 0.17 %) and extract (10± 1.01 % ; 11± 1.68 %) turns out to weaken over time after 48 hours and proves to weaken even more after 72 hour period.

Table 1 below highlights the determined parasitemia average of the various wells.

Table 1: Determination of the parasitemia average of the various wells

	Average parasitemia of two wells to 24H	Average parasitemia of two wells to 48H	Average parasitemia of two wells to 72H
Control blood only	7.5 ± 0.75 %	12± 0.90 %	12.3± 1.03 %
Control DMSO	6± 0.55 %	12± 0.82 %	12± 1.15 %
Control Chloroquin	1,5 % ± 0.15	1,7 ± 0.11 %	1,8 ± 0.17 %
Extract ethyl acetate from the cladodes	4.7 ± 1.40 %	10± 1.01 %	11± 1.68 %

3. 2. In vivo anti-malaria test

The evaluation of the extract ethyl activity as extracted from the cladodes of the *Opuntia ficus-indica* is achieved in accordance with the Peters test (Peters and al., 1970).

Given that extracted with ethyl acetate was the only extract which proved to have an antiplasmodial in vitro activity as compared to *Plasmodium Berghei*, we decide to continue with evaluating the antiplasmodial activity in vivo exclusively with the extract by removing other inactive extracts.

The determination of mice parasitemia is made to D5, D8 and D10 by implementing a caudal smear for every mouse.

Besides, we noticed the presence of many extracellular parasites in the control group which received olive oil already treated with the extract. For this reason, we have also calculated the report (relationship) between the number of inside and outside parasites on the total number of erythrocytes. This allowed determining the extract's possible activity on the extracellular parasites.

The results of the parasitemia to D5, D8 and D10 allow for the construction of a histogram.

Figure 1 of suppressive test shows the mice parasitemia percentage after five, eight and ten day of treatment of

infection. The percentage of parasitemia of witness well and well containing Chloroquin have validated the compliance of the in vitro assay. Indeed, the witness showed that the mice infestation by Plasmodium turns out to be positive. In fact, Chloroquin reduced parasitemia by 96% compared to the control group (olive oil).

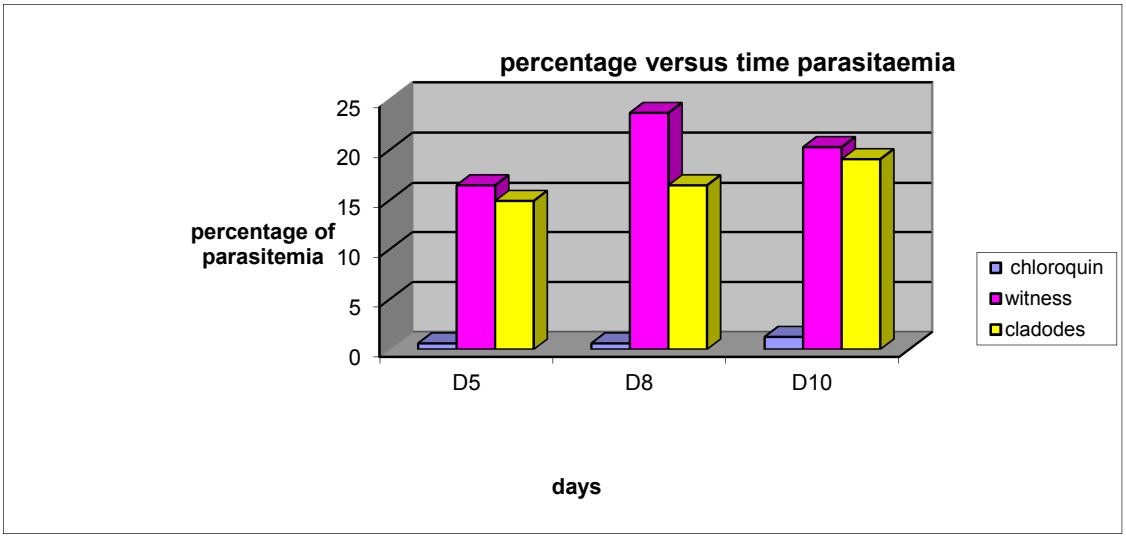

Figure 1: Evolution of parasitemia versus time

Figure 2 shows the evolution in the number of mice died within ten days of treatment as counted for each Chloroquin treated control group and those treated with this extract. The survival period has been followed up on a daily basis. No deaths have been recorded in the mice group set treated with Chloroquin. All over the period of treatment against Plasmodium Berghei. As for the mice set treated with the extract of cladodes ethyl acetate, it has been noticed that two mice died on the seventh day, three ones on the eighth day and half of them on the ninth day. It is worth noting that the number of infected mice on the start of the experiment has been equal to eight. The survival of the last remaining mice group set is discovered to be much better than the control group among which mice death has been noted to occur since the third day.

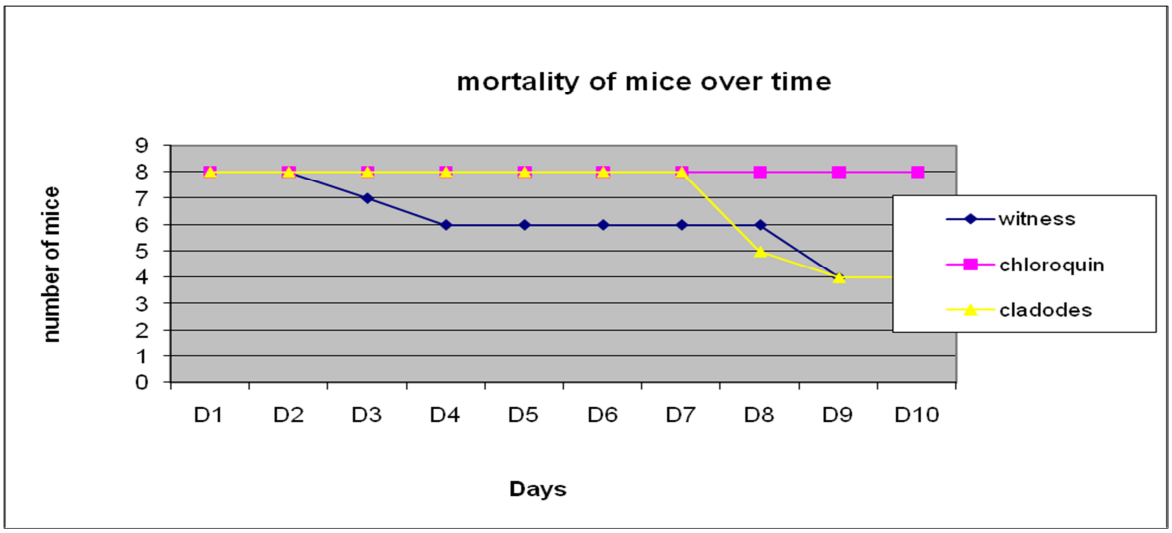

Figure 2: Mortality curve versus time

In fifth day, all the groups infected with *Plasmodium Berghei* and treated with the crude ethyl acetate cladodes extract of *Opuntia ficus-indica* and Chloroquin, showed an exponential decrease in the proportion of parasite inside the

erythrocytes, during the study period, with Chloroquin showing the highest parasite inhibition (0.62 %). However, the negative control group showed an exponential increase in parasite ratio (15.42 %). In another batch of mice treated with the extract showed a slight decrease of parasitemia (7.53 %) compared to control (olive oil), throughout the study period (Tab.2). The activity of extract weakened further in the eighth (16.50 %) and more the tenth day (19.51 %).

Table 2: Determination of parasitemia in each mice lot and average parasitemia lots

		D5		D8		D10
		Parasitemia[1] (%	R[2] (%)	Parasitemia (%	R (%)	Parasitemia (%)
Olive oil	Parasitemia of each mouse	21,0 17,0 9,0 21,0 11,0 13.5 15 14	35,0 39,0 1.2 36,0 25,0 24,0 30 22	32,0 17,0 10,0 52,0 12,0 19,0 23 28	TPP[3] TPP TPP TPP TPP TPP TPP TPP	11 20 30 20 20 20 20 20
	Average of parasitemia of the lot	15.42	30.14	24.12	TPP	20.12
Chloroquin	Parasitemia of each mouse	0.39 0.60 0.75 0.60 0.88 0.50 0.70 0.60	AP[4] AP AP AP AP AP AP AP	0.31 0.50 0.66 0.44 0.96 0.51 0.81 0.51	AP AP AP AP AP AP AP AP	1.40 0.80 2.30 1.00 0.60 1.80 1.50 0.60
	Average of paras of the lot	0.62	AP	0.63	AP	1.25
Extract ethyl acetate from the cladodes	Parasitemia of each mouse	21.0 22.0 5.0 12.7 3.2 6.7 27.0 22.0	37.7 49.0 14.0 24.0 6.4 8.0 51.0 39.0	5.30 28.6 8.60 28.0 12.0 D[5] D D	9.5 30.0 9.6 29.0 12.0 D D D	18.5 30.0 19.6 24.0 6.0 17 18 20
	Average of paras of the lot	7.53	34.0	16.50	18.0	19.51

[1]Parasitemia (%): Percentage of Parasitemia inside the erythrocytes; [2]R (%): Percentage of Parasitemia outside the erythrocytes; [3]TPP: very few parasites outside the erythrocytes; [4]AP: Absence of Parasites outside the erythrocytes; [5]D: Death of the mouse.

4. Discussion

Noteworthy, the originality of this work lies in its novelty in evaluating it is the first to evaluate the antiplasmodial activity of plant related research *Opuntia ficus-indica*. This research as aimed on plant should be explored. This research aimed to study the antimalarial *in vitro* activity and *in vivo* activity of extracts fruit and cladodes of *Opuntia ficus-indica* from various solvent.

Smears of the witness DMSO reveal parasitemia similar to those of the witness sample containing only blood: it allows us to refute a possible activity of the DMSO on the parasites.

This is the first evidence demonstrating that extract ethyl acetate from the cladodes of the *Opuntia ficus-indica* proves to have *in vitro* activity on the parasitemia. Indeed, 4.7 ± 1.40 % average parasitemia of two wells on a 24 hour bases with regard to the control containing the only blood 7.5± 0.75 % (see Table 1).

The extract parasitemia treated batch was lower than the control group (olive oil): as the extract was reduced by

3.2 % parasitemia on day 5 and 30 % on day 8.

However, the mean parasitemia of the extract treated animals was lower than the control because 3 mice from the lot of the extract showed very low parasitemia values (5%, 3.7% and 6.7%).

It should also determine of the extract kinetics in the body as its bioavailability or its metabolism. Mice having a lower percentage of parasitemia in the batch of the extract (5%, 3.7% and 6.7%) might have a different metabolism than other mice belonging to the lot.

The mice behavior and their appearance differ between batches. The mice in the control group (olive oil) and those of the group treated with the extract did retain some. They were motionless and all clustered in a corner of their cage. Unlike the mice that had a lot Chloroquin and which kept silky hair tonic. The mortality of mice was followed until day 10 (Fig.1 and Fig 2).

The extract's low antimalarial activity was accompanied by high toxicity in mice causing their death. The dose of the extract administered orally (0.1 ml at a concentration of 10mg/ml) was too strong but the dose reduction would reduce already very too small activity (Fig. 2, Tab.2).

The presence of flavonoids and other bioactive constituents is believed to have contributed to the observed antiplasmodial activity of *Opuntia ficus-indica* racquets extract.

The ethanol extract showed significant ($p < 0.01$) dose dependent protection of mouse splenocytes against glucose oxidase-mediated cytotoxicity. It was characterized by containing a high amount of phenolics (180.3 mg/g), which might be the active compound responsible for the antioxidant properties, and anti-inflammatory activity of the *Opuntia ficus-indica* var. saboten extract (Kaur et al., 2012). Arizona prickly-pear cactus effectively inhibited cell growth in several different immortalized and cancer cell cultures *in vitro* and suppressed tumor growth in a nude mouse of ovarian cancer model (Zou et al., 2005).

An interesting study by Ahmad et al. (1996) demonstrated that the administration of a cactus stem extract (Opuntia streptacantha) to mice, horses, and humans inhibits intracellular replication of a number of DNA- and RNA-viruses such as Herpes simplex virus Type 2, Equine herpes virus, pseudorabies virus, influenza virus, respiratory syncitial disease virus and HIV-1. In addition, an inactivation of extra-cellular viruses was reported by the same authors. However, the active inhibitory components of the cactus extract used in this study was not yet investigated, and still, no further study has dealt with this specific topic (Ahmad et al., 1996)

In a previous work, Alimi et al. (2013) showed the wealth in phenolic, flavonoids and polysaccharide contents in the methanolic extract of *Opuntia ficus indica f. inermis* flowers and roots (Alimi et al., 2010), the radical-scavenging activity, the reducing power and the antiulcerogenic activity.

Thereby, it has been reported that *Opuntia ficus indica* fruit extract could prevent erythrocytes against lipid oxidation induced in vitro by organic hydroperoxide (Butera et al., 2002). But there is no information hitherto to about the in vivo effect of Opuntia fruit on rat erythrocytes.

As a conclusion, this report seems the first study to examine the effect of *Opuntia ficus-indica* on treating malaria via a reduction of parasitemia in the erythrocytes. Our results have indicated that Opuntia ficus-ndica could exert an *in vitro* and *in vivo* antiplasmodial activities in respect of *Plasmodium Berghei*.

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