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# Stage-Specific Changes on *Plasmodium yoelii yoelii* Following Treatment with *Hintonia latiflora* Stem Bark Extract and Phytochemical-Antioxidant Evaluation

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## Abstract

Malaria endemic zones are mostly located on third world countries, where antimalarials are not easily found or patients cannot afford them, and in consequence, they must turn toward natural products or phytomedicines. In the present study, the effect of *Hinotnia latiflora* (*Hl*) methanolic stem bark extract (*Hl*MeOHe) on the ultrastructure of the asexual intraerythrocytic stages of *Plasmodium yoelii yoelii* (*Pyy*) after a Peters' four-day oral treatment was assessed by transmission electron microscopy (TEM), as well as the parasite development on blood smears, analyzed by light microscopy. Likewise, extract was subjected to qualitative tests adopting standard procedures for identification of phytoconstituents; its antioxidant activity was evaluated according to the method of Brand-Williams and by the radical cation decolorization assay. Results showed higher percentage of rings and lower percentage of trophozoites and schizonts in the treated animals, in comparison with those of the control groups, which demonstrated lower percentage of rings and trophozoites, and schizonts in higher number. Images of TEM showed in some treated parasites, mild parasite membranes, organelle swelling and ribosomal depletion. The phytochemical profile demonstrated that the extract contains alkaloids, tannis, steroids, terpenoids, flavonoids, phenolics and saponins. The obtained values of the half maximal inhibitory concentration (IC50) in µg/mL,

for both antioxidant assays were of 423.83 and 202.95 respectively. It is concluded that *Hl*MeOHe altered the development of the intraerythrocytic asexual stages and the ultrastructure of *Pyy*, and due to its phytochemical constituents, showed an *in vitro* antioxidant activity.

## Keywords

*Hintonia latiflora*, *Plasmodium yoelii yoelii*, Malaria, Ultrastructural Changes, Phytochemicals, Antioxidant, Natural Products

## 1. Introduction

Malaria is still a public health problem in third world countries where people cannot afford or have access to the conventional antimalarial drugs. It is estimated that 80% of malaria patients living in poor endemic areas treat themselves with plants and never assist to any formal health facility [1] [2]. Artemisinin combination therapies in these countries are often used to treat children and pregnant women, consequently, patients at lower risk, attempt to find less expensive and more accessible alternatives such as natural products. *Artemisia annua*, Cinchona bark, *Cryptolepis sanguinolenta* and *Cochlospermum planchonii*, are some of the plants officially approved to be used as phytomedicines to treat malaria in Africa [1] [3]. In Mexico, the stem bark of *Hintonia latiflora* (Sessé & Moc. Ex. DC.) Bullok Rubiaceae, commonly known as copalchi, is frequently consumed to treat gastrointestinal disorders, diabetes and malaria [4]; in Europe, capsules of copalchi micronized cortex are consumed as an antioxidant supplement [5]. In previous studies, the stem bark of *Hl* has demonstrated *in vitro* and *in vivo* antimalarial efficacy in a good to moderate range, respectively, and an excellent antipyretic effect on murine malaria model [6] [7], however, more studies need to be done concerning its toxicity, pharmacokinetics and biological activity in order to obtain valuable clinical information to sustain their use as an adjuvant treatment to control malaria, as, even though the World and Health Organization (WHO) promote the use of natural products, a synthetic or natural medicine must achieve good scientific evidence of safety and efficacy before being commercialized [2]. There are no published data showing the effect of *Hl*MeOHe on the development of the asexual intraerythrocytic stages and morphology of the parasite, therefore, in the present study, the development of the asexual stages and the ultrastructural changes of *Pyy* induced by the treatment with *Hl*MeOHe were evaluated, as well as its phytochemical profile and *in vitro* antioxidant activity.

## 2. Methods

### 2.1. Animals

15 CD-1 male mice weighing 28 g were used for the study and were obtained from the Faculty of Medicine, UNAM vivarium. Mice were divided into 3

groups of five mice each. Animal management was performed according to the Mexican Official Norm NOM-062-ZOO-1999 for the production, care, and use of laboratory animals in accordance to international guidelines and approved by the Ethical and Research committee at School of Medicine, UNAM (project 095/2016).

## 2.2. Parasites

*P. yoelii yoelii* lethal strain was obtained from the London School of Hygiene and Tropical Medicine and maintained by serial passages in CD1 mice.

## 2.3. Plant Material

Stem bark of *H. latiflora* was collected at the UNAM Chamela's Biological Station, Jalisco México, latitude 19°29'52.90375 N, longitude 105°02'41.33116W, H: (Ell. Height) 77.679 m, identified and prepared as described by Rivera *et al.* (2014) [7]. A voucher sample (collect number 7772) of the plant was deposited at Dr. Salvador Nava y Esparza Herbarium (UAMIZ) collect number 83,519 by Jhony Anacleto. The stem bark of *Hl* was prepared as described by Rivera *et al.*, (2014) [7]. Permission to collect stem bark samples was obtained from Chamela UNAM Research Institute of Biology.

## 2.4. Extraction

Sixty grams of dried powdered stem bark of *Hl* was extracted with methanol in a solid-liquid system for 72 h. The solvent was evaporated in vacuo to afford 10 g of extract [7]. Methanol extract was used because it was reported previously that this extractant showed the higher biological activity and lower toxicity to the mice [7].

## 2.5. Biological Experiment

Mice were infected according Rivera *et al.* (2013) [8]. A four-day suppression test [9] was used to evaluate the effect of the extract on the parasites. By oral gavage, five mice received 1000 mg/kg of *Hl*MeOHe and five others received the vehicle (tween 80%) at a concentration of 0.04%; five mice remained as *Pyy*-untreated control group. Selection of the extract dose, was made regarding the LD10 work dose reported previously [7]. On the fifth day post-treatment, a blood smear was made to all mice and the percent individual parasitemia was estimated [6]. The development of the intraerythrocytic asexual stages was evaluated by light microscopy, classifying the parasites into three groups: rings, trophozoites, and schizonts [10]. At $5^{th}$ day post infection (PI), 1000 cells on blood smears were counted per mouse to obtain the percentage of rings, trophozoites and schizonts. The results obtained from both *Hl*MeOHe and tween 80 group, were compared with those obtained on the untreated *Pyy* mice.

At fifth day post-treatment, 25 μL of peripheral blood from a cut of mice tail vein from the treated and untreated animals was collected in 200 μL Eppendorf®

tubes and immediately mixed with citrate 3.8% in a ratio of 9 parts blood to 1-part anticoagulant. The samples were mixed by gentle inversion. Whole blood samples were centrifuged at $1500 \times g$ for 10 minutes at room temperature to obtain infected erythrocytes for TEM [11].

## 2.6. Transmission Electron Microscopy

Blood samples were fixed for 1 h in 2.5% glutaraldehyde in sucrose-cacodylate buffer, then, centrifuged at 1500 rpm, washed three times in cacodylate buffer and postfixed for 1 h with 2% osmium tetroxide in sucrose-cacodylate buffer. The samples were dehydrated in a graded 30% to 100% ethanol series at 4°C for 10 min each, and embedded in Araldita 6005 epoxy resin (Electron Microscopy Sciences). Thin sections were stained with lead citrate and uranyl acetate and observed using a JEM 1010 electron microscope [11]. All studies were made by duplicate.

## 2.7. Phytochemical Evaluation

The analysis was conducted as described by Yadav and Agarwala (2011) [12]. 10 mg of extract was mixed with a few drops of HCl for the detection of alkaloids, turbidity in the suspension indicated the presence of alkaloids. Tannins were detected by dissolving 0.01 g of extract in 0.2 mL of water and a few drops of $FeCl_3$ 0.1%, the change to dark blue indicated the presence of tannins. Steroids were detected by mixing 10 mg of extract with 0.4 mL of acetic anhydride and 0.4 ml of sulfuric acid; the change to red violet indicated the presence of steroids. For the detection of terpenoids, 10 mg of extract was dissolved in 0.4 mL of chloroform and 0.6 mL of concentrated sulfuric acid without shaking, formation of brown ring indicates the presence of terpenoids. Flavonoids were detected by mixing 5 mL of plant extract with a few drops of ethanolic $FeCl_3$, the formation of red color indicated the presence of flavonoids. For total phenols, 5 mL of alcoholic solution of the plant extract was mixed with $FeCl_3$ solution. The change in color to dark blue indicated the presence of phenols. Saponins were detected by dissolving 10 mg of the extract in 1 mL of distilled water and few drops of olive oil; the formation of emulsion indicated the presence of saponins.

## 2.8. *In Vitro* Antioxidant Capacity Determinations

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity of the extract was determined according to the method of Brand-Williams [13]. DPPH radicals have an absorption maximum at 518 nm, which disappears with reduction by an antioxidant compound. The DPPH$^•$ solution in methanol (0.2 mM) was prepared daily. 150 μL of this solution was mixed with 50 μL of methanolic plant extract solution at concentrations of 25, 50, 125, 250 and 500 μg/mL. Absorbance reading was taken at 518 nm 5 min after initial mixing. Butylated hydroxytoluene (BHT) and ascorbic acid were used as positive controls in the same concentrations (25, 50, 125, 250 and 500 μg/mL). All determinations were car-

ried out in triplicate. The percentage of inhibition of ABTS$^{+\bullet}$ was calculated using the following formula:

% inhibition = [(AB − AE)/AB] × 100, where AB = absorbance of the blank sample, and AE = absorbance of the plant extract.

The free radical scavenging capacity of the plant extract was performed using the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation decolorization assay [14], which is based on the reduction of ABTS$^{+\bullet}$ radicals. ABTS was dissolved in deionized water to a 7 mM concentration. ABTS radical cation (ABTS$^{+\bullet}$) was produced by reacting ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12 - 16 h before use. The ABTS$^{+\bullet}$ solution was then diluted in ethanol to an absorbance of 0.7 (±0.02) at 734 nm. Plant extract (50 μL) at concentrations of 25, 50, 125, 250 and 500 μg/mL were mixed with 150 μL of the ABTS$^{+\bullet}$ solution. Absorbance reading was taken at 734 nm 10 min after initial mixing. BHT and ascorbic acid were used as positive controls in the same concentrations (25, 50, 125, 250 and 500 μg/mL). All determinations were carried out in triplicate. The percentage of inhibition of ABTS$^{+\bullet}$ was calculated by using the same formula as given above.

### 2.9. Statistical Analysis

Obtained data were analyzed with one-way analysis of variance (ANOVA) and Tukey's test using GraphPad Prism® software, version 7. All analyzed data had a normal distribution, and statistical significance was set at $P < 0.05$.

### 3. Results

### 3.1. Antimalarial Efficacy

All mice showed parasites in their blood on the fifth day of sampling. At 5$^{th}$ day, the mean parasitemia for the *Pyy* and Tween 80 control group was of 41% and 38.30% respectively, while the *Hl*MeOHe treated mice showed a parasitemia of 5.60%. Mice from *Pyy* and Tween group died at 6 days postinfection (PI) with parsitemias over 69%, while *Hl*MeOHe treated mice died at 14 days PI with a parasitemia of 22.6% (Table 1). At 5$^{th}$ day PI, on the infected untreated group, the percentages of *Pyy* intraerythrocytic stages were observed as follows: rings 7.4, trophozoites 26.4 and schizonts 7.2 and in the Tween control group, the observed percentages were: 7.2 rings, 24.2 trophozoites and 6.9 schizonts. In the *Hl*MeOHe treated group, rings, trophozoites and schizonts were seen in percentages of 15.2, 7.06 and 1.4, respectively (Table 2).

### 3.2. Light Microscopy Observations

Under light microscopy, *Pyy* and *Pyy*-Tween 80 mice, showed a high number of infected red blood cells in peripheral blood at 5 days PI; rings, trophozoites, schizonts, and merozoites with visible pigment, were observed on the blood smears. Smears of *Hl*MeOHe treated mice showed a small number of infected

**Table 1.** Effect of 1000 mg/kg$^{-1}$ of methanolic stem bark extract of *Hintonia latiflora* on CD-1 *Pyy* infected mice.

| Groups (n = 5) | Animals infected at 5$^{th}$ day | Parasitemia (%) at 5$^{th}$ day | Parasitemia (%) at death time | Survival time after 5$^{th}$ day (days) |
|---|---|---|---|---|
| Pyy | 5 | 41 ± 1.80 | 69.40 ± 2.50 | 1 |
| *Hl*MeOHe (1000 mg/kg PO SID/4 days) | 5 | 5.60 ± 1.30* | 22.60 ± 1.63* | 14.20 ± 0.86* |
| Tween 8 (0.04%, PO SID/4 days) | 5 | 38.30 ± 1.63 | 70.20 ± 2.35 | 1 |

Results are reported in mean ± standard error of two independent studies. *$P < 0.05$ versus *Pyy* control group. PO = per os, SID = every 24 h.

**Table 2.** Activity of 1000 mg/kg$^{-1}$ of methanolic stem bark extract of *Hintonia latiflora* on *Plasmodium yoelii yoelii* intraerythrocytic stages.

| Groups (n = 5) | Rings (%) at 5$^{th}$ day | Trophozoites (%) at 5$^{th}$ day | Schizonts (%) at 5$^{th}$ day |
|---|---|---|---|
| *Hl* | 15.20 ± 0.97* | 7.06 ± 1.2* | 1.40 ± 0.85* |
| Control *Pyy* | 7.40 ± 0.28 | 26.40 ± 0.73 | 7.20 ± 0.37 |
| Tween 80 | 7.20 ± 0.30 | 24.20 ± 0.66 | 6.90 ± 0.44 |

Results are reported in mean ± standard error of two independent studies. *$P < 0.05$ versus *Pyy* control group.

red blood cells at 5 days PI; only a few rings and trophozoites were observed (Figure 1), and in some samples between 1 - 2 schizonts per smear were detected. It was difficult to assess parasite structural changes at a light microscope level.

### 3.3. Transmission Electron Microscopy

On the TEM images obtained from the parasites from the untreated *Pyy* and Tween 80 groups, well preserved intraerythrocytic parasites were observed. Almost all the evaluated samples showed stages of rings, trophozoites and schizonts; merozoites stages were present only in a few samples and in a reduced number; rhoptries, micronemes and dense granules were the organelles most frequently observed in merozoites. Rings were located peripherally in the erythrocytes. Almost all the evaluated ring stages presented a thin and flat discoidal appearance with cytoplasm housing their nucleus and free ribosomes. A digestive vacuole as large as 40% of the parasite volume was observed in almost all the rings. In some samples, crystallized malarial pigment was found. Trophozoites showed a thick border of cytoplasm housing organelles like nucleus, mitochondrion, and ribosomes; in some samples clusters of vesicles on the cytoplasm were seen. Schizogony budding was observed in some specimens, free merozoites were difficult to find. Different parasite stages can be observed on Figure 2.

On the treated parasites, the most common observed stages were rings, followed by few trophozoites. Schizonts were difficult to find and in some samples a very few number of free damage merozoites were observed. Parasites treated
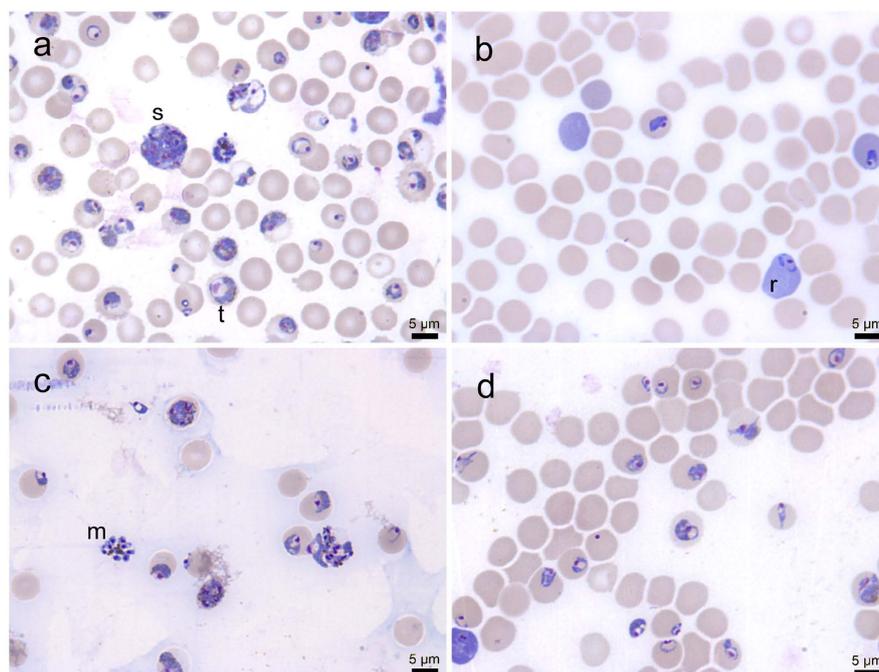
**Figure 1.** Blood smears of *Pyy* and *Hl*MOHe-*Pyy* mice. (a) 5th day sampling *Pyy* mice; rings, trophozoites, schizonts and merozoites are observed; (b) *Hl*MOHe treated *Pyy* mice at 5th day PI, only ring stages were observed; (c) *Pyy* mice at the day of the death (6 days postinfection), very low number of infected red blood cells with all the asexual parasite stages are observed; (d) *Hl*MOHe treated *Pyy* mice at 14 days PI, infected red blood cells are observed containing mostly rings and trophozoites stages; m (merozoite), r (ring), s (schizont), t (trophozoite).

with *Hl*MeOHe, showed in general, an amorphous shape and swollen plasma and cellular membranes. In some cases, cell membranes seemed to be disintegrated in specific zones and occasionally membranous debris were observed; however, a complete disintegration of the cell membranes was never seen. Most of the parasites depicted vacuoles in their cytoplasm and ribosomal depletion. In almost all the samples, endoplasmic reticulum was difficult to observe. All parasites presented a normal food vacuole, except in some cases (around 10% of the observed parasites), where a minimally swollen membrane was detected. The nucleus membrane appeared to be a little bit swollen in some parasites. In the more severe cases, very few parasites showed a complete destruction of their organelles (approximately less than the 3% of the evaluated parasites). Images of treated parasites are depicted in **Figure 3**.

### 3.4. Phytochemical Profile and Antioxidant Activity

*Hl*MeOHe stem bark extract showed an effective free radical scavenging activity against radicals DPPH and ABTS (**Table 3** and **Table 4**). Extract radical scavenging capacity was compared with that of the ascorbic acid and BHT. In the DPPH assay, the extract showed an $IC_{50}$ value of 423.83 µg/mL, while BHT and ascorbic acid depicted $IC_{50}$ values of 805.50 and 139.84 µg/mL respectively, and
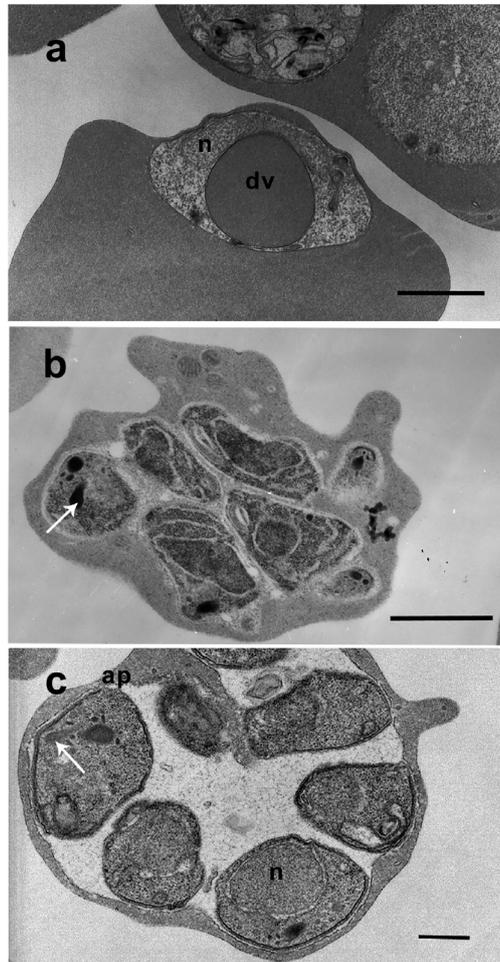
**Figure 2.** Transmission electron micrographs of the asexual intra-erythrocytic stages of *Pyy,* recovered at $5^{th}$ day sampling. In all the images, the density of the parasites and the red blood cells are the same. Intraerythrocytic parasites are surrounded by the parasitophorous vacuole membrane. (a) Ring stage surrounded by ribosomes with a sausage like nucleus and a large digestive vacuole; (b) Immature schizont with developing merozoites; (c) Schizont with mature merozoites showing the apical prominence and different organelles as nucleus, ropthries and dense granules; ap (apical prominence), arrows (rhoptries), dv (digestive vacuole), n (nucleus). Bars 1 µm.

under same conditions; therefore, the extract demonstrated an effective antioxidant capacity. In the ABTS assay, the concentration of the extract required to quench 50% of the ABTS radical cation was higher compared to that of both controls, with values of 202.95, 2.45 and 32.59 µg/mL for the extract, BHT and ascorbic acid, respectively.

## 4. Discussion

Research on anti-malarial extracts, must include toxicity and pharmacokinetics studies as well as the evaluation of their efficacy on killing the parasites by modifying its ultrastructure, which can be very useful to identify a possible mechanism
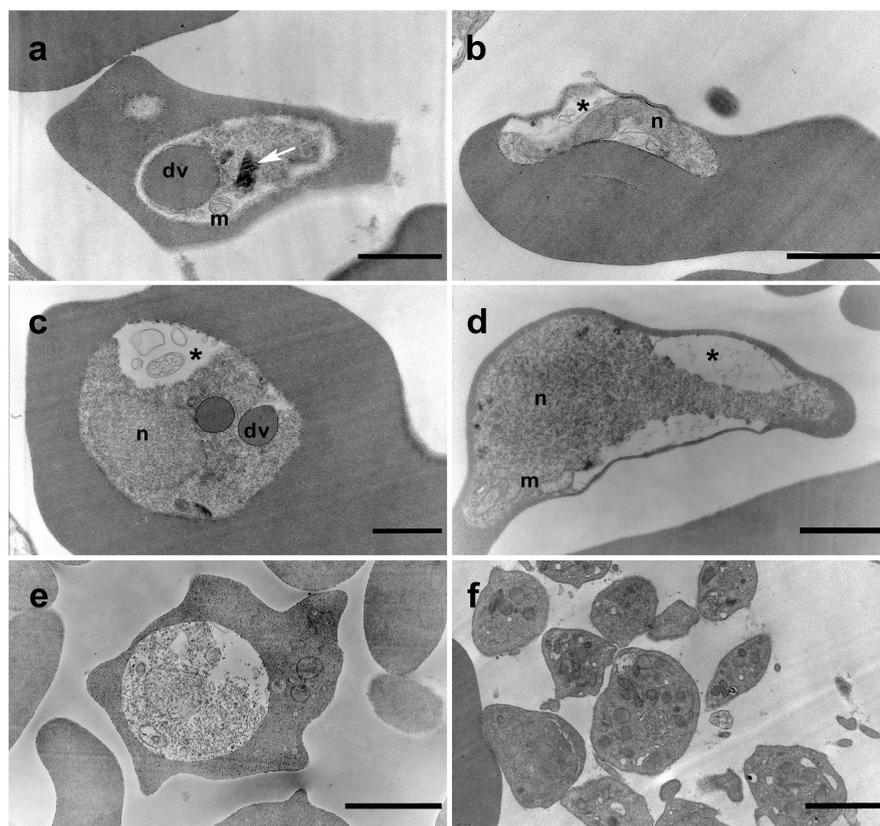
**Figure 3.** Transmission electron micrographs of the asexual intraerythrocytic stages of *Pyy-Hl*MeOHe, recovered at 5th day sampling. (a) Ring stage with a slightly swollen digestive vacuole membrane and swollen mitochondria; (b) Ring stage with a flattened like appearance, showing depletion of ribosomes (*) and undistinguishable organelles; (c)-(d) Trophozoite with extensive loss ribosomal areas, swollen mitochondria and small digestive vacuoles; (e) Trophozoite exhibiting severe damage and total disorganization; (f) Free merozoites with vacuoles in their cytoplasm, swollen organelles and small digestive vacuoles; m (mitochondria), n (nucleus), dv (digestive vacuoles), arrow malaria pigment. Bars (a)-(b) 2 μm, (c)-(d) 1μm, (e)-(f) 2 μm.

**Table 3.** Phytochemical analysis of Methanolic extract of *Hintonia latiflora* stem bark.

| Chemical tested group | *Hl*meOHe |
|---|---|
| Alkaloid | + |
| Tannin | + |
| Steroid | + |
| Terpenoid | + |
| Flavonoid | + |
| Phenolic | + |
| Saponin | + |

+ = detected.

of action [10]. In the present study, the effect of 1000 mg/kg of *Hl*MeOHe on the development of the asexual intraerythrocytic stages and on the ultrastructure of *Pyy*, was evaluated. Likewise, the phytochemical profile and the *in vitro*

**Table 4.** Antioxidant capacity of *Hintonia latiflora* methanolic stem bark extract $IC_{50}$ (μg/mL).

|  | DPPH | ABTS |
|---|---|---|
| *Hl*MeOHe | 423.83 ± 7.64 | 202.95 ± 6.48 |
| BHT | 805.50 ± 8.22 | 2.45 ± 0.07 |
| Ascorbic acid | 139.84 ± 4.43 | 32.59 ± 1.32 |

Results are the mean ± standard error of three independent experiments, *P < 0.05 versus BHT and ascorbic acid.

antioxidant activity of the extract were performed. It is important to carry out phytochemical and antioxidant profiles, even if some constituents of the plant are partially known, because the variations in the chemical composition and consequently, on the biological activity of herb compounds, are associated mainly with the geographic origin where the plant was grown and collected [15].

The antimalarial efficacy obtained with the 4-day test, regarding the parasitemia and the mice survival time after 5th day, showed that all treated mice maintained lower parasitemias and presented a survival time of 14 days with respect to the control groups; these results agree with those reported by Rivera *et al.* (2014) [7]. The percentage of rings observed on the blood smears of the treated animals at 5th day sampling, was almost double the percentage observed on the control groups, and in consequence, the percentage of trophozoites and schizonts decreased in the treated mice. These results showed that the extract may have a parasitostatic effect, retarding the development from rings to schizonts stages. Nevertheless, the development of the parasite continued and the treated mice finally died despite the low parasitemia reported at the day of the death. Cell-cycle delays and recrudescence in malaria, have been reported after treatment with atovaquone, atovaquone plus proguanil or mefloquine [16] [17], and several studies have identified ring-stage quiescence mechanism of survival during exposure to monotherapies with artemisinin drugs [18] [19] [20] [21]. In other studies, it was observed that 50% of nonimmune patients, experience treatment failure if artemisinins are given as monotherapy [22]. Natural products tested *in vivo* and *in vitro* against malaria, like hydroethanolic crude extract of *Ajuga remota* and a natural triterpene obtained from olive pomace, also reported a parsitostatic effect, delaying the development of mature rings or trophozoites [23] [24].

It is not known how *Hl* stem bark extracts are metabolized *in vivo* and *in vitro*, nonetheless, the results obtained in this work suggest that its constituents could be rapidly metabolized and eliminated within hours in the treated mice, and in consequence, constituents could not remain in plasma time enough to kill the parasites. It is important to consider that the schizogonic rhythm of rodent malarias in the blood of mice varies from one species to the other [25]. Murine antimalarial drugs studies are performed mostly with asynchronous strains that are less sensitive than synchronous strains because of the delayed penetration of merozoites into red blood cells [25].

*Hl*MeOHe damaged the ultrastructure of the asexual intraerythrocytic stages of *Pyy*, hence, morphological alterations were observed on different parasite stages. As cited by Sachanonta *et al.* (2011) [10], it is important to guarantee that the evaluated changes in the specimens treated with drugs or other substances, are the consequence of the exposure to these molecules than a result of a wrong TEM process. In the present study, proper TEM conditions were used to ensure that the ultrastructural changes on *Pyy*, were a direct effect of *Hl* extract.

The most frequently observed lesions on the treated parasites were ribosomal depletion and cytoplasmic vacuolization, and in some cases, disruption and swelling of cell and organelles membranes; equivalent ultrastructural changes have been reported on plasmodium parasites after exposure to quinine, piperaquine and artesunate [10]. Disruptions in cell and organelle membranes were observed in *Plasmodium falciparum* parasites exposed *in vitro* to artemisisnin. [26] Even in the more severe cases of parasite destruction, the digestive vacuoles appeared to preserve their integrity, therefore, it seems that *Hl*MeOHe does not interfere with the physiological function of this organelle, as most antimalarials do. Same results have been obtained by other authors; Ellis *et al.* (1985) [27], observed ultrastructural changes as membrane and ribosomal organization disruption on *Plasmodium berghei* after 30 minutes treatment with artemisinin but no changes were noted in the digestive vacuoles. The effect of Qinghao extract and artemisinin on the ultrastructure of *Plasmodium berghei* by a Peters' four-day oral treatment, was evaluated by You You TU research team; their results showed that treated parasites depicted cell and organelle membrane swelling, and in extreme cases, the structure of some parasites disappeared but residual food vacuoles were still contained inside the infected erythrocytes [28]. The absence of free ribosomes observed in some treated specimens, may suggest, that the mode of action of the *Hl*MeOHe could be related with the protein synthesis of the parasite. Moreover, the disruption of some membrane-bound organelles suggested that some extract constituents could be involved in both oxidative phosphorylation and anaerobic glycolysis effects which can act as a sink for excess intracellular calcium [29] [30], thus, more studies need to be done to identify the mechanism by which *Hl* affects malaria parasites.

In our CD1 mice, *Pyy* produces pathology findings and clinical features of complicated malaria; infected mice die with high parasitemia (>80%), severe anemia, hemoglobinuria and hypoglycemia six to seven days PI and always exhibit multi-organic involvement [31]. Pathogenesis in complicate malaria is originated by three main mechanisms: cytoadherence, oxidative stress and reactive oxygen species (ROS) production and exacerbated release of proinflammatory citokines, predominantly tumor necrosis factor [32]. ROS are produced during hemoglobin degradation by the intracellular parasite, during the adherence of infected red blood cells to the endothelium and during the production of proinflammatory citokines [33] [34] [35]. Additionally, malaria infection decreases the antioxidant defense system [36]. Antioxidants limit the ROS oxidative damage to biological systems, therefore, because of their antioxidant constituents, the

use of natural products is increasingly growing.

The phytochemical profile revealed that *Hl*MeOHe, contains among other metabolites, flavonoids, phenols, tannins and terpenoids. In general, polyphenols have demonstrated benefits as antioxidants. Flavonoids have a planar structure with hydroxyl groups and double bond in position C2-C3, which give them capacity as a chelators, free radical scavengers and inhibitors of enzymes that produce free radicals [37]. It is known that secondary metabolites, such as alkaloids, flavonoids, tannins and other phenolic compounds are responsible of antioxidant and antimicrobial activities in most plants [38]. Oxidative stress is one of the main pathological mechanisms by which the parasite produces severe damage to the host, therefore, the antioxidant capacity of the *Hl*MeOHe, could explain, at least in part, the beneficial effects showed in the treated mice. The survival time of these animals, could be due to a boosting of the immune system or maybe to an inhibition of the exacerbated production of proinflammatory cytokines produced by the antioxidants metabolites of the extract; phenolic constituents and flavonoids exhibit anti-inflammatory properties and reduce the levels of prostaglandin, thus reduce fever. This action was observed in a previous study done by our research group, in which a marked decrease of body temperature was observed in mice following the first few minutes after *Hl*MeOHe treatment [7].

## 5. Conclusion

The methanolic extract of the stem bark of *Hintonia latiflora*, delayed the development of the asexual intraerythrocytic stages of *Pyy*, specifically from rings to trophozoites and schizonts, moderately altered the parasite ultrastructure and depicted an *in vitro* antioxidant activity that could help the host immune system during a complicated malarial infection. Nevertheless, the use of the stem bark of *Hintonia latiflora* as an antimalarial treatment must be taken with caution as it seems to just delay the parasite development. Our results leave an open door to continue the research with *Hl*, regarding primarily on its possible action into the host immune system.

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