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Nano-Phytosome of Quercetin Could Protect Liver from Plasmodium Berghei in Mouse Model

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Abstract

Background and purpose: Malaria is one of most important diseases especially in tropical regions. Flavonoids are known to have beneficial properties that could be profitable. This study aimed to evaluate the effects of nano-phytosomes of Quercetin (NQ) on liver damages of mice infected with *Plasmodium berghei*.

Methods: A total of sixty male BALB/c mice were intra-peritoneally infected by administration of 10^6 *P.berghei*-infected RBCs. Animals were acclimatized for 7 days and divided into 5 groups including 1) Mice received 0.9% isotonic saline and considered as negative control (NC) and infected mice treated with saline or positive control (PC), 3) treated with 2 mg/kg body weight of Hydroxychloroquine sulfate (HF) for 4 days (HF), 4) treated with 10 mg/kg body weight of NQ for 4 days (NQ) and 5) treated with 10 mg/kg body weight of NQ and 2 mg/kg body weight of HF for 4 days (NQ+HF). Histo-pathological parameters and pro-inflammatory cytokines were also evaluated.

Results: Administration of *P. berghei* could increase scores for histo-pathological parameters and levels of pro-inflammatory cytokines ($P<0.0001$). Administration of HF and NQ could alleviate adverse effects of *P. berghei* on histo-pathological parameters ($P<0.05$).

Conclusion: A combination of NQ and HF could show the best response. It means that NQ can be used as adjuvant therapy for treatment of infection induced by *Plasmodium berghei*.

Keywords: Malaria, Pro-inflammatory cytokines, Quercetin, Liver damages

Introduction

Malaria is one of most important diseases especially in tropical regions and it is a big challenge for most people in all over world. *Plasmodium* spp. is main factor for malaria. Organization like WHO has estimated to be 200 million people involved with malaria [1]. The different factors such as parasite growth, proliferation, and ability of host immune responses could influence pathogenesis of malaria [2]. The different symptoms has been reported for patients involved with malaria including ague, anemia and cerebral and kidney disorders and splenomegaly [3]. It has been accepted to use animal model for study of animals due to limitations for nature and interpretation under in vitro conditions. *Plasmodium berghei* is known to have specific characteristics and red blood cell (RBC) tropisms have been used as experimental models in human model [4]. Liver is site for infection of malaria and is involved in the diseases. Inflammatory parameters can be used as markers assessment of liver damages. Some synthetic agents have commonly been used for treatment of malaria such as chloroquine, pyrimethamine, and sulfadoxine-pyrimethamine. There is increasing interest for application of natural agents for treatment of diseases and/or as adjuvant therapy. Natural anti-inflammatory agents may protect patients with malaria from inflammation. In this connection, the Flavonoids are known to have beneficial properties including antioxidant, antimicrobial, anticancer and anti-inflammatory [5]. Quercetin is a one of polyphenolic flavonoids which is found in fruits, vegetables and herbs and is used for health benefits such as antioxidant, anti-inflammatory and antimicrobial activities [6, 7]. It is known to have major limitations in bioavailability and absorption [8-10]. Inefficiency of absorption could be attributed to inappropriate lipid solubility, big size of molecules that cannot be absorbed through passive diffusion in intestine-bloodstream pathway, and destroy of phenol moiety of Quercetin by bacteria in gastric space [11-14]. It is essential to use a noval carrier for Quercetin [15]. Phytosome is one novel delivery system that could improve absorption and bioavailability of phyto constituent [16]. It seems that Quercetin could protect liver from damages and inflammation by its antioxidant and anti-inflammatory properties. This novel study aimed to evaluate the application of Nano-phytosomes of Quercetin (NQ) as main agent and/or adjuvant therapy for treatment of infection induced by Plasmodium berghei in mice model.

Materials and methods

Chemical agents

Hydroxychloroquine sulfate (HF) was prepared from Biogen Company (India). We prepared soybean phosphatidylcholine, cholesterol, methanol and dichloromethane from Merck Company (Darmstadt, Germany) and Quercetin from Sigma Aldrich Company (Steinheim, Germany). All other substances were prepared from analytical grade.

Preparation of NQ

Thin layer hydration procedure was used to prepare the NQ. Quercetin and phosphatidylcholine were firstly dissolved in methanol, but cholesterol was dissolved in dichloromethane. The resulted mixture was kept in a flask. To evaporate the mixture, a rotary evaporator was used and all the solvents were kept to produce a thin dry film. The prepared lipid thin layer had been exposed to nitrogen gas flow and maintained in an overnight and in the room temperature before hydration to ensure the complete removal of the organic solvents. The film was hydrated with distilled water in a rotary at 45°C. Several procedures were applied to reduce the phytosomes size such as bath sonication (Model 8852, cole-Palmer Instrument, Chicago, IL) in 45°C, homogenization (Heidolph, Germany) with 20,000

rpm and probe-sonication method (Sonix, Vibracell).

Animals and bacteria

A total of sixty male BALB/c mice, 10-12 weeks of age and initial weight 27±3g, were used. The mice were intra-peritoneally infected by administration of 106P.berghei-infected RBCs. We used HF as recommended by WHO in a dose of 2mg/kg body weight daily for 4 days. Mice were acclimatized for 7 days and divided into 5 groups including 1) Mice received 0.9% isotonic saline and considered as negative control (NC), 2) Non-treated infected mice that were considered as positive control (PC), 3) Infected mice treated with 2 mg/kg body weight of HF for 4 days(HF), 4) Infected mice treated with 10 mg/kg body weight of NQ for 4 days (NQ) and 5) Infected mice treated with 10 mg/kg body weight of NQ and 2 mg/kg body weight of HF for 4 days (NQ+HF). A schematic design of study is presented in Figure 1.

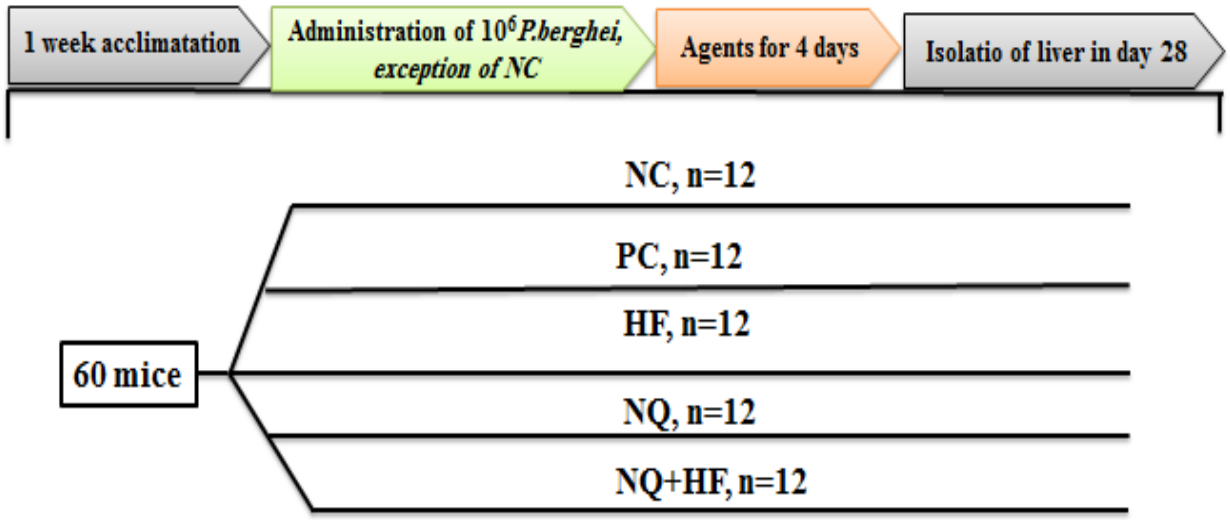

Figure 1 Experimental design and groups

Histological study and pro-inflammatory cytokines

In day 28, following scarification, liver was separated and fixed in 10% neutral buffered formalin. Samples were inserted into paraffin and histological changes were evaluated. Scores were considered in scale of 0 up to 4, including not observed (0), very mild (1), mild 2), moderate (3), and severe (4). Blood samples were collected and tumor necrosis factor TNF-α and interleukin (IL)-1β levels in the serum were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the Company guidelines.

Statistical analysis

The resulted data were reported as mean ± standard deviation of the mean. One-way ANOVA with Tukey's post hoc tests were applied to compare among groups. A $P<0.05$ was used as statistically significant.

Results

Histo-pathological parameters

Results for histo-pathological parameters are presented in Figure 2. As results show administration of P. berghei could increase scores for histo-pathological parameters in terms of kupffer cell hyperplasia, hemosiderosis, hepatic necrosis and periportal inflammation (comparison of NC and PC group) (P<0.0001).Administration of HF and NQ could alleviate adverse effects of P. berghei on histo-pathological parameters (P<0.05). The responses were better in HF in comparison to NQ (P<0.05). A combination of NQ and HF could have better effects on histo-pathological parameters in comparison to single form (P<0.05).

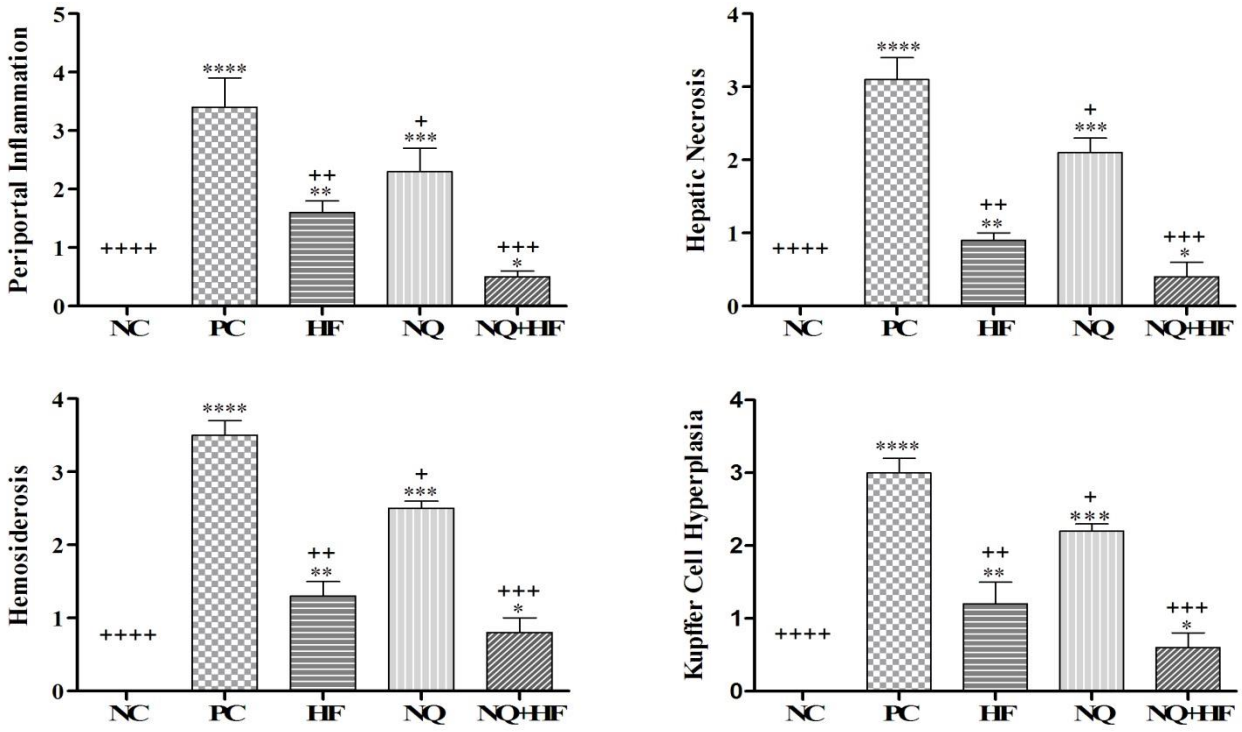

Figure 2 Effects of experimental treatments on histo-pathological parameters of mice involved with malaria. Superscript (*) shows significant difference between NC group and other groups in 0.05 (*), 0.01 (**), 0.001 (***) and 0.0001 (****).Superscript (+) shows significant difference between PC group and other groups in 0.05 (+), 0.01 (++), 0.001 (+++) and 0.0001 (++++).

Pro-inflammatory cytokines

The data for pro-inflammatory cytokines are shown in Figure 3. The levels of inflammatory cytokines were significantly higher in PC group in comparison to NC group (P<0.0001). Administration of NQ, HF and specially their combination could significantly decrease levels of pro-inflammatory cytokines (P<0.05).

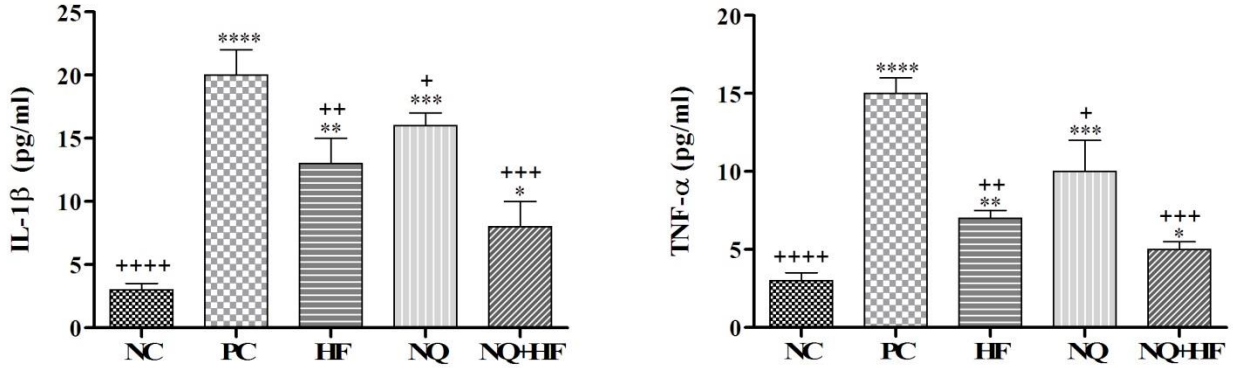

Figure 3 Effects of experimental treatments on serum pro-inflammatory cytokines of mice involved with malaria. Superscript (*) shows significant difference between NC group and other groups in 0.05 (*), 0.01 (**), 0.001 (***) and 0.0001 (****).Superscript (+) shows significant difference between PC group and other groups in 0.05 (+), 0.01 (++), 0.001 (+++) and 0.0001 (++++).

Discussion

Malaria is a disease that needs more attentions because it is not still exterminated and the best control procedure is application of a true treatment procedure. Anti-malarial drugs have been used for treatment of malaria but it is essential to investigate their toxicity and damage [17]. The resulted findings for histo-pathological parameters in positive group show that proliferation of the parasites in the blood. As malaria progresses, hepatocytes are destroyed and merozoites are transferred into the blood circulation. Our findings for cytokines confirm such claim. Our findings for kupffer cell hyperplasia showed to be lower in the treated groups and had difference with PC group; showing that the agents could prevent proliferation of liver macrophages.Following the rupture the RBCs, hemoglobin splitting is done and hemosiderosis occurs [18]. In the present study HF and NQ, especially in combination form could alleviate the adverse effects of malaria. The Flavonoids are known to have most common group of polyphenolic compounds and have antioxidant, antimicrobial and anti-inflammatory properties [5].In summary, NQ showed better activity in combination with HF. NQ has low water solubility and its application in nano-phytosom form could improve solubility and help more absorption. Activated host immune system increases fight against infection, whereas overproduced inflammation causes to produce the tissue damage or even multiple organ failure. It has been shown that the flavonoids and triterpenoids may encourage to treat that could be attributed to antimicrobial properties [19]. Nuclear factor-kappa B (NF-κB) is one mediator for inflammatory response which increases inflammatory cytokines including TNF-α and IL-1β [20]. These findings suggest that NQ does not have effects such as HF, but in combination with HF could increase its efficiency.

Conclusion

In conclusion, this study for the first time showed that NQ can alleviate adverse effects of malaria in terms of liver damages and inflammation. Future studies are needed to evaluate the effects of NQ on improvement of conditions in mice with malaria, but our findings suggest that NQ can be used as adjuvant therapy in treatment of malaria.

Ethical Considerations

Compliance with ethical guidelines

Approval for this study was obtained from Lorestan University of Medical Sciences Research Committee.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of interest

The authors declared no conflict of interest.

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