Original Article

Analysis of Anti-toxoplasma Immunoglobulin G and Immunoglobulin M Antibody Levels after Intervention with *Curcuma Longa* Extract on Early Pregnant Mice with Acute Toxoplasmosis

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Abstract

Background: *Curcuma longa* has strong anti-inflammatory effect. This study aims to evaluated the level of anti-Toxoplasma immunoglobulin G and immunoglobulin M (IgG-IgM) antibody after intervention with *C. longa* extract in early pregnant mice with acute toxoplasmosis. **Materials and Methods:** We evaluated 20 early pregnant mice that were divided into five groups, four mice in each. Group 1-4 received injections of *Toxoplasma gondii* tachyzoites. Three days later, G1 and G2 were given orally 125 mg/kg/day and 500 mg/kg/day of *C. longa* extract, respectively. G3 was given 60 mg/kg/day of spiramycin (positive control), and G4 was given 0.2 ml of distilled water (negative control). G5 underwent no intervention at all. Blood samples were obtained serially (before and 3 days after injection of tachyzoites, 3 days and 7 days after intervention) to assess anti-Toxoplasma IgG-IgM antibody levels by enzyme-linked immunosorbent assay methods. **Results:** Anti-Toxoplasma IgG-IgM antibody levels increased significantly 3 days after injection of tachyzoites ($P < 0.05$), but decreased significantly ($P < 0.05$) 3 days, and 7 days after administration of *C. longa* extract dose 125 mg, 500 mg, and spiramycin 60 mg, and there was no significant difference between these three groups. Anti-Toxoplasma IgG-IgM antibody levels increased significantly ($P < 0.05$) 3 days, 6 days, and 10 days after injections of tachyzoites on G4. The IgG-IgM antibody levels fluctuated on G5 and considered as insignificant ($P > 0.05$). **Conclusion:** The administration of *C. longa* extract at a dose of 125 mg/kg/day for 7 days effectively decreased anti-Toxoplasma IgG-IgM antibody level in early pregnant mice with acute toxoplasmosis.

Keywords: *Curcuma longa*, early pregnancy, immunoglobulin G, immunoglobulin M, toxoplasmosis

Introduction

Toxoplasmosis is a zoonotic disease caused by the *Toxoplasma gondii*.[1] Humans can be infected through several ways,[2] if infected during early pregnancy can lead to congenital abnormalities, fetal death, and abortion.[3] *T. gondii* infection will trigger the production of antibody level. The antibodies level is associated with virulence, and the number of parasites.[4,5] Examination of immuno-globulin G and -immuno-globulin M (IgG-IgM) antibody to taking diagnosis and also to determine the efficacy of a therapy.[6,7] The diagnosis of acute infection depends on detection of Toxoplasma-specific IgG and IgM antibodies.[8] IgG avidity test and IgM analysis are considered to be suitable methods for determining acute infections. Recently, recombinant fusion proteins have been

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used in an attempt to improve serologic tests for diagnosis of *T. gondii*.[9,10]

Appropriate therapy in early pregnancy in the acute phase of toxoplasmosis will help to decrease the probability of transmission to the fetus, and prevent potential damage in case of transplacental infection. However, these therapies may be teratogenic and/or may sometimes generate intolerance to women. Therefore, it is very important to treat only patient whose acute infection has been proven. Consequently, an accurate diagnosis of this infection phase is necessary.[11]

As revealed by Serranti *et al*., until now there has been no ideal drug as toxoplasmosis therapy in pregnant women.[12] Spiramycin is still the drug of choice, but it proves unable to eradicate infection in the fetus. Spiramycin works as bacteriostatic and bactericidal proven to decrease the rate of growth of bacteria/parasite load.[13,14] Damage to embryonic tissue cells/placental tissue cells may occur directly from the parasite, but embryonic cell tissue damage or placental tissue may also occur indirectly from excessive pro-inflammatory levels[15-17] that cannot be controlled/influenced by spiramycin.

Yellow turmeric (Curcuma longa) extract, which has been shown to be an anti-inflammatory agent[18] and also has antimicrobial, although it is more likely to be simply anti-inflammatory.[19,20] This study aims to demonstrate the effectiveness of the *C. longa* as a treatment in early pregnant mice with acute toxoplasmosis by analyzing anti-Toxoplasma IgG-IgM antibody level, hoping that our result will provide additional information for further research.

Materials and Methods

This study was conducted using 20 early pregnant mice and was divided into five groups (G1-G5) randomly, and 4 mice each group. At the end of the study, the mice were sacrificed then buried in specific places. The procedures for animal preservation and intervention in this study were according to the procedures of the U. K. Animal (Scientific Procedures) Act, 1986 and associated guidelines. This research had been approved by the animal ethics research committee of the Faculty of Medicine, University of Hasanuddin, Makassar, Indonesia.

Modeling and intervention sampling

The adult female mice Balb/c fulfilling the inclusion criteria (age 11–13 weeks, weight 18–20 g, active movement) were placed in a stable with a healthy and proven mature male mice. Mice were pregnant if a plug is found in the vagina and is said to be a 0 day pregnancy. gestational age in this study 0–2 days. The 20 early pregnant mice and were divided into five groups (G1-G5) randomly, and 4 mice each group. The G1-G4 was injected with 10 tachyzoites of *T. gondii* RH strain intraperitoneal, and G5 without infection. Three days after injection of tachyzoites, G1 and G2 were given *C. longa* extract 125 and 500 mg/kg/day respectively, G3 (positive control) was given spiramycin of 60 mg/day, and G4 (negative control) was given 0.2 ml of distilled water. Each intervention was administered orally using cannulas for 7 days. G5 underwent no intervention at all.

Examination of blood samples

Blood samples were taken from the tail vein serially (1 day before tachyzoites injection, 3 days after tachyzoites injection, and 3 and 7 days after the intervention). Anti-Toxoplasma IgG/IgM antibody levels were determined by enzyme-linked immunosorbent assay (ELISA) method, using a qualitative mouse antibody IgG (toxoplasma[TP]-IgG) ELISA kit and a qualitative mouse antibody IgM (TP-IgM) ELISA kit.

Statistical analysis

Research data were obtained, tabulated, and processed using SPSS version 20 software. The data were analyzed statistically using paired *t*-test and one-way ANOVA test. The $P < 0.05$ is used.

Results

Anti-Toxoplasma IgM antibody results were shown in Figure 1. Anti-Toxoplasma IgM antibody level increased significantly ($P < 0.05$) 3 days after tachyzoites injection. Anti-Toxoplasma IgM antibody level in G1-G3 decrease significantly ($P < 0.05$) 3 days, and 7 days after treatment with *C. longa* extract doses of 125 mg/kg/day, 500 mg/kg/day, and spiramycin 60 mg/kg/day, but no significant differences among these groups ($P > 0.05$). Anti-Toxoplasma IgM antibody level in G4 increased significantly ($P < 0.05$) at 3, 6, 10 days after injection of tachyzoites. Anti-Toxoplasma IgM antibody level in G5 fluctuated at the same period, but no significant changes ($P > 0.05$).

Anti-Toxoplasma IgG antibody results were shown Figure 2. Anti-Toxoplasma IgG antibody level increased significantly ($P < 0.05$) 3 days after tachyzoites injection. Anti-Toxoplasma IgG antibody level in G1-G3 decrease significantly ($P < 0.05$) 3 days, and 7 days after treatment with *C. longa* extract doses of 125 mg/kg/day, 500 mg/kg/day, and spiramycin 60 mg/kg/day, but no significant differences among these groups ($P > 0.05$). Anti-Toxoplasma IgG antibody level in G4 increased significantly ($P < 0.05$) at 3, 6, 10 days after injection of tachyzoites. Anti-Toxoplasma IgG antibody level in G5 fluctuated at the same period, but no significant changes were noted ($P > 0.05$).

Discussion

The infection of *T. gondii* will trigger the humoral and cellular immune response. Humoral immunity such as anti-Toxoplasma IgG-IgM antibody level increase in the event of infection of *T. gondii*.[21] In our study, after injection of T. *gondii* tachyzoites, the anti-toxoplasma IgG-IgM antibody level increased significantly ($P < 0.05$) after 3 days, 6 days, and 10 days Figures 1 and 2.

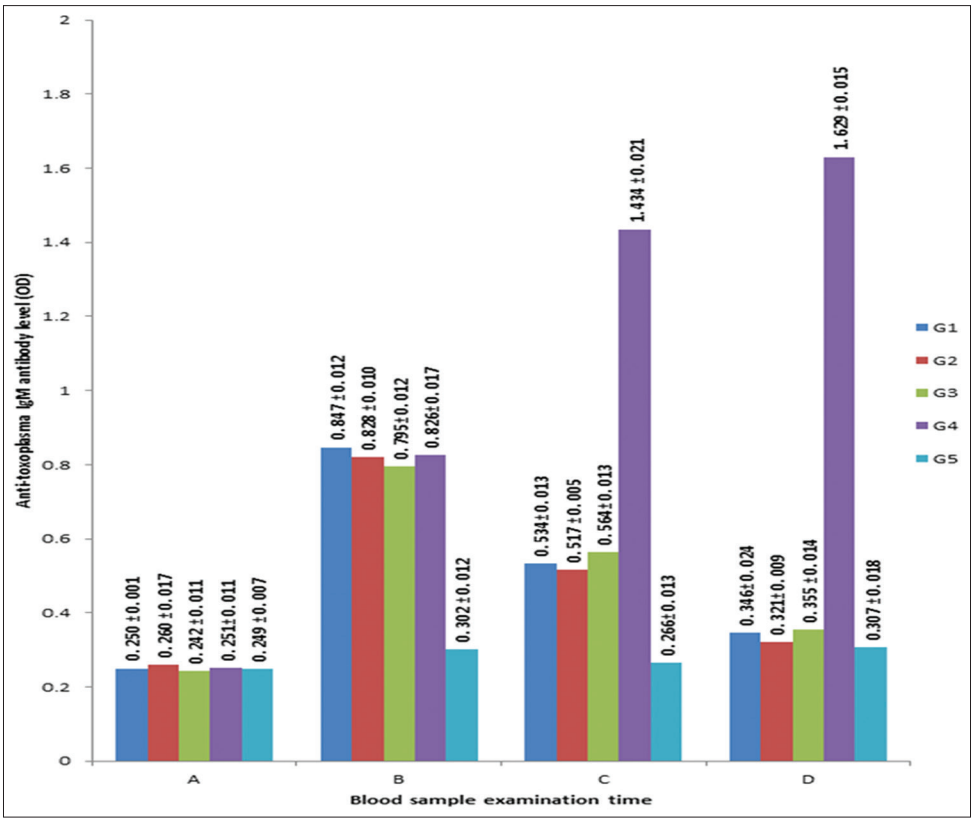

Figure 1: Anti-Toxoplasma immuno-globulin M antibody levels before and after intervention. Early pregnant mice group (g). G1 were injected of tachyzoites and intervened with *Curcuma longa* extract 125 mg/kg/day, G2 were injected of tachyzoites and intervened with *Curcuma longa* extract 500 mg/kg/day, G3 were injected of tachyzoites and intervened with Spiramycin 60 mg/kg/day, G4 were injected of tachyzoites and intervened with distilled water 0.2 ml/day, G5 were not injected nor intervented. Time of sample collection: A (1 day before tachyzoites injection), B (3 days after tachyzoites injection), C (3 days after the intervention), D (7 days after the intervention). Optical density

Spiramycin is one of the macrolide antibiotics; it inhibits the synthesis of both *in vivo* and *in vitro* bacterial proteins with various potentials. Macrolides are generally bacteriostatic, although some of these drugs may be bactericidal at very high concentrations.[13] Spiramycin is the drug of choice of toxoplasmosis, but it has been proven failed to eradicate fetal toxoplasmosis. Pyrimethamine and sulfadiazine are given when the polymerase chain reaction test is positive, as it may penetrate the placenta, but pyrimethamine and sulfadiazine are not given in suspected infections especially in the first trimester of pregnancy because of the potential teratogenic and toxic effects on fetal bone marrow.[14]

Curcumin is a major component of *C. longa*,[22] it has been shown to be a potent anti-inflammatory agent,[18,23,24] with additional antimicrobial,[25,26] and antioxidant properties.[27] Al-Zanbagi *et al*., reported that administration of C. *longa* extract dose of 100 mg/kg/day and doses of 200 mg/kg/day for 7 days, intraperitoneal injected female mice 2×10^2 toxoplasma/ml, is highly effective in inhibiting the rate of growth of tachyzoites 98.6% and 99.2%, compared to control group, as well as spiramycin dose of 100 mg/kg/day and 200 mg/kg/day inhibited tachyzoites growth rate of 71% and 94%.[17]

In our study, we found that anti-Toxoplasma IgG-IgM antibody level decreased significantly 3 days and 7 days after intervention with *C. longa* extract doses of 125 mg/kg/days, 500 mg/kg/days, and spiramycin 60 mg/kg/day. The decrease in levels of anti-Toxoplasma IgG-IgM antibody in the group that were given the extract of *C. longa* was greater than the spiramycin group (positive control), but the difference was not significant. Decreased levels of anti-Toxoplasma IgG-IgM antibodies in this study may be related to effectiveness of C. longa as anti-inflammatory,[18,23,24] antimicrobial,[25,26] and antioxidant,[27] thus inhibiting the rate of growth of tachyzoites. Based on the results of this study and the results of previous researchers who have been reported, the *C. longa* extract may be considered as an alternative treatment in early pregnancy with acute toxoplasmosis, but further research is required with larger sample and a more comprehensive research.

Conclusion

The administration of *C. longa* extract dose of 125 mg/kg/day for 7 days effectively decreased anti-Toxoplasma IgG-IgM antibody level in early pregnant mice with acute toxoplasmosis.

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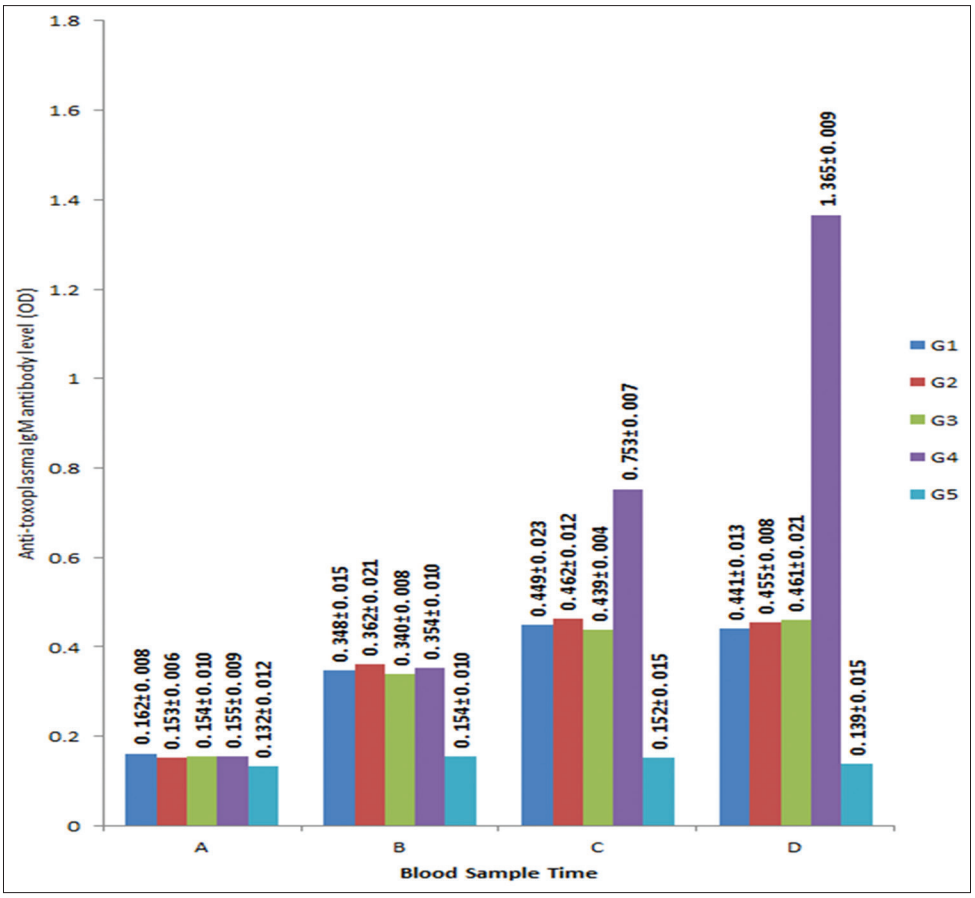

Figure 2: Anti-Toxoplasma immuno-globulin G antibody levels before and after intervention**.** Early pregnant mice group (g), G1 were injected with tachyzoites and intervened with *Curcuma longa* extract 125 mg/kg/day, G2 were injectected with tachyzoites and intervened with *Curcuma longa* extract 500 mg/kg/day, G3 were injected of tachyzoites and intervened with Spiramycin 60 mg/kg/day, G4 were injected of tachyzoites and intervened with distilled water 0.2 ml/day, G5 were neither injected nor intervented. Time of sample collection: A (1 day before tachyzoites injection), B (3 days after tachyzoites injection), C (3 days after the intervention), D (7 days after the intervention). Optical density

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Conflicts of interest

There are no conflicts of interest.

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