Effect of Spiramycin and Moringa leaf feeding in improving placenta, heart, histopathological neurons and glia in fetal models with toxoplasmosis

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ABSTRACT

Background: Toxoplasmosis is a protozoan disease caused by Toxoplasma gondii that can cause fetal abortion, stillbirth, death, or fetal and neonatal abnormalities. Spiramycin prophylactic purpose is to prevent fetal infection in toxoplasmosis. While Moringa oleifera contains bioactive compounds that benefit from treating oxidative stress and infections. This study aims to evaluate the effect of Spiramycin and Moringa leaf extract in improving the placenta, heart, histopathological neurons and glia in feral rat models with toxoplasmosis.

Methods: An experimental study with a post-test-only control group design was done using 24 pregnant female rats divided into the control group receiving spiramycin therapy (P1) and two intervention groups that were given spiramycin and ethanol extract from Moringa leaves at a dose of 240 mg/kg body weight (P2) and combination of spiramycin and ethanol extract from moringa leaves at a dose of 420 mg/kgBW (P3). A histopathological examination was then performed.

Results: Administration of spiramycin and ethanol extract of Moringa leaves at 420 mg/kg body weight had the same histopathological examination results as spiramycin administration. In a histopathological examination of toxoplasmosis pregnant rat placenta and toxoplasmosis fetal rat heart was effective in repairing gliosis in neurons and neuroglia of toxoplasmosis rat fetuses.

Conclusion: Administration of spiramycin and ethanol extract from Moringa leaves was effective in repairing gliosis in neurons and neuroglia of toxoplasmosis rat fetuses.

Keywords: Moringa oleifera, Placenta, Spiramycin, Toxoplasmosis.


INTRODUCTION

Toxoplasmosis is a systemic protozoal disease caused by Toxoplasma gondii that affects warm-blooded animals, including humans.1 Parasites are known infectious diseases that affect humans and animals. Toxoplasmosis occurs in all countries with a 10-90% seroprevalence.3 The prevalence of toxoplasmosis in Indonesia ranges from 3.1% to 70%, not only for pregnant women but also for groups at risk of infection, such as people who like to eat raw meat and vegetables and keep cats at home also occurs.4 Transmission of toxoplasmosis can occur through two mechanisms: vertically from mother to fetus through the placenta and horizontally through ingestion of undercooked meat, contaminated milk and raw vegetables.5 Toxoplasmosis infection during pregnancy causes neonatal morbidity and mortality.6 T. gondii can cross the placenta, infect the fetus, and cause fetal abortion, stillbirth, neonatal death, and fetal and neonatal abnormalities.4,7,8 T. gondii infection causes fetal death, prematurity, fetal growth retardation, fever, pneumonia, hepatosplenomegaly, and thrombocytopenia, affecting the eyes and brain.9,10 Infection with T. gondii causes oxidative stress, causing an imbalance between the free radical production and cellular defense systems, i.e., increased free radical production or decreased antioxidant defense activity, or both, leading to reduced angiogenesis. Increased trophoblast invasion inhibits and induces endothelial dysfunction and causes acute atheroma.11 Cellular responses by T. gondii because T. gondii infects all cell types, including various cells within the uteroplacental. T. gondii infections also occur in the uteroplacental. This is also because the uteroplacental leukocyte population consists of macrophages, NK cells, CTL T cells (CD8+ T
cells), and CD4+ (Th) T cells.12

The main treatment for acute toxoplasmosis infection during pregnancy is to prevent mother-to-child transmission and minimize the symptoms experienced by the baby. Toxoplasmosis infections can be treated with two protocols. Spiramycin has a prophylactic purpose in preventing fetal infection. Another option is to combine pyrimethamine and sulfadiazine. Both treatments are active only in rapidly growing parasite forms and inactive in cysts.10 Spiramycin is minimally toxic to the fetus, is effectively absorbed after oral administration, diffuses rapidly through tissues to kill tachyzoites, and reduces or prevents the spread of the parasite across the placenta to the fetus.11 Pyrimethamine and sulfadiazine are indicated for severe and long-standing toxoplasmosis.14 This drug inhibits Toxoplasma gondii folic acid metabolism and acts directly on tachyzoites (the acute phase of infection) but has side effects such as hematologic abnormalities, increased serum creatinine, liver and hypersensitivity reactions.15 Moringa oleifera grows in tropical and subtropical arid to humid environments.16,17 Moringa contains bioactive compounds shown in several studies to affect humans positively. The most commonly used part of the plant is the leaves, which include vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, and saponins.18 Moringa leaves can be used for patients with inflammatory diseases due to their high concentration of antioxidants. Moringa contains beta-carotene, which has been proven to have antioxidant properties. The antioxidants contained in the moringa plant have potent antioxidant effects that repair free radicals, prevent oxidative damage to key biomolecules, and provide important protection against oxidative damage.18 Moringa leaf extract's anti-inflammatory and antioxidant properties are thought to prevent cell damage due to oxidative stress caused by T. gondii infection. Moringa oleifera is traditionally used to treat various diseases.19

Based on those mentioned above, this study aims to evaluate the effect of Spiramycin and Moringa leaf extract in improving the placenta, heart, histopathological neurons and glia in fetal rat models with toxoplasmosis.

METHODS

This research is experimental laboratory research using randomized control trials (RCT) with the research design in the form of a post-test only with a control group design. The T. gondii isolate used by the RH strain was taken from the Bogor Veterinary Research Center. Moringa leaf simplicia was obtained from Hergobinagun, Pakem Yogyakarta, and maceration was carried out at the Center for Research and Development of Traditional Medicinal Plants Tawangmangu, Central Java. Pregnant female rats were treated at the Laboratory of the Center for Food and Nutrition Studies at Gadjah Mada University, Yogyakarta. Histopathological examination of the placenta, heart, and fetal brain was conducted at the Laboratory of Anatomical Pathology, Sebelas Maret University, Surakarta.

The research subjects were 24 female rats aged 6-8 weeks with a body weight of 200-250 grams, a healthy condition, shining eyes, not dull fur, active, and good appetite. Rats were given BR II standard food while drinking was given freely (ad libitum), and the cage environment was homogeneous. Female rats were mated mono-mating: one female mated with one male. The pregnancy diagnosis was obtained after 17 hours of mating. A copulatory plug was evaluated, namely a plug covering the rat's vagina from the cervix to the vulva, as an indicator of pregnancy. A copulatory plug characterizes pregnancy. Each rat was first adapted to laboratory conditions for 1 week. At this stage, the mice were given standard BR-2 pellets and water from PAM ad libitum.

In this study, eight pregnant rats as a control group received spiramycin therapy as P1, P2 were eight pregnant toxoplasma rats who received spiramycin, and Moringa leaf ethanol extract at a dose of 240 mg/kg body weight found approximately 20% necrosis. Based on histopathological findings of Toxoplasma fetal hearts receiving spiramycin treatment, the ventricles and atria appeared relatively normal. Fetal toxoplasma hearts treated with spiramycin and an ethanol extract from Moringa leaves at 240 mg/kg showed relatively normal ventricles with atrial hemorrhages. Ventricles and atria appeared relatively normal in toxoplasma fetal hearts treated with spiramycin, and an ethanol extract from Moringa leaves at 420 mg/kg, as seen in Figure 2.
Toxoplasmosis model histopathological examination of fetal rat brains showed that neurons and glial cells in fetal toxoplasmosis treated with spiramycin exhibited moderate gliosis in 31–60% of the area. Toxoplasma fetal neurons and neuroglia treated with ethanol extracts from moringa leaves at a dose of 280 mg/kgBW showed moderate gliosis with an area of 31–60%. Glial cells treated with Toxoplasma fetal neurons and spiramycin and ethanolic extracts from Moringa leaves at 420 mg/kgBW showed mild gliosis with an area of 1-30% in Figure 3.

**DISCUSSION**

Oxidative stress caused by *Toxoplasma gondii* infection can reduce angiogenesis, inhibit trophoblast invasion, induce endothelial dysfunction, and cause acute atheroma.\(^\text{11}\) *T. gondii* infection infects the placental syncytiotrophoblast. These multinucleated cells form the outer layer of the placenta and make direct contact with maternal blood through the two stages of attachment and intracellular replication.\(^\text{21}\) In this study, the administration of spiramycin can repair the placenta infected with *Toxoplasma gondii*. This is consistent with research by Dunay IR et al.. Spiramycin was found to be administered after a toxoplasmosis diagnosis, a potent macrolide antibiotic that concentrates in the placenta. Spiramycin has a good safety profile and achieves good placental concentrations.\(^\text{22}\) Administration of spiramycin and an ethanol extract of Moringa leaves at a dose of 420 mg/kgBW had the same effect as administration of spiramycin on the histopathological appearance of the placenta of pregnant rats in a toxoplasmosis model.

*T. gondii* infection induces histopathological changes in rat hearts, including increased tissue lesions characterized by focal, multifocal, and diffuse inflammatory infiltrates, with no findings, mild, moderate to severe There was up to toxoplasma myocarditis often presents as progressive cardiac dysfunction. Other rare complications include conditions such as arrhythmia and heart failure. Signs of myocardial *T. gondii* infection include sinus tachycardia, atrial fibrillation, atrioventricular (AV) block, and bundle branch block. This disorder can, under certain circumstances, lead to hemodynamic depression and congestive heart failure.\(^\text{25}\) In this study, the administration of spiramycin produced the same histopathological images of normal hearts as spiramycin and an ethanolic extract from moringa leaf at a dose of 420 mg/kg body weight. Spiramycin administration in this study showed improvement with a reduction in the incidence of gliosis from 31% to 60%. Spiramycin works by inhibiting protein synthesis and cell proliferation or inhibiting the inflammatory cascade.\(^\text{24}\) This is consistent with a study by Beesa et al. 2021, administration of spiramycin induced a higher number of brain cysts compared to untreated rats.\(^\text{25}\) Similarly, the study by Farooq F et al. Spiramycin is used to reduce cyst burden in murine toxoplasmosis.\(^\text{26}\) This study found gliosis and no necrosis of neurons and glia. Unlike most other tissues, the blood-brain barrier (BBB) tends to make it difficult for cells, pathogens, and proteins to enter the brain from the blood. The blood-brain barrier (BBB) is composed of tightly bound endothelial cells that are densely packed and supported by a network of robust basement membranes. In addition, pericytes and astrocytes surround the endothelium, providing structural and biochemical support and preventing substances from crossing the endothelium and entering the brain parenchyma.\(^\text{27}\) This is inconsistent with his Hartati S et al. study, where histopathological examination found necrotic areas in the lung, liver, heart, muscle, and central nervous system with macrophage infiltration. Pathological changes caused by toxoplasmosis can occur in a variety of ways. Organs include the brain, neurons, microglia, liver parenchyma, heart, skeletal muscle, fetal membranes, and leukocytes.\(^\text{28}\) Gliosis is a nonspecific alteration of glial cells in response to central nervous system injury, most often associated with the proliferation or hypertrophy of several different glial cell types, including astrocytes, microglia, and oligodendrocytes.\(^\text{29,30}\)

Administration of spiramycin in combination with moringa leaf ethanol extract at a dose of 420 mg/kgBW was more effective in ameliorating gliosis in the brain, as indicated by a 30% reduction in the number of gliosis. Administration of moringa leaf ethanol extract can interfere with protein synthesis and cell proliferation.
with intracellular tachyzoite invasion and replication and impair cellular tachyzoite release.\textsuperscript{31} Our study limitation was we determined the gestational age of the rat based on an estimate only and could not control the level of stress in animals.

**CONCLUSION**

Administration of spiramycin and an ethanol extract of Moringa leaves at 420 mg/kg BW produced similar histopathological results to those of spiramycin administration in Toxoplasma-infected rats’ placenta and Toxoplasma-infected fetal rat hearts. It effectively repaired neuronal and glial gliosis in fetal rats with toxoplasmosis.

**CONFLICT OF INTEREST**

The authors have no conflicts of interest to disclose.

**ETHICAL CONSIDERATIONS**

This study was approved by the Health Research Ethics Committee of Dr. Moewardi Hospital Surakarta No. 1.198/IX/HREC/2022.

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**AUTHOR CONTRIBUTION**

All authors contributed to the study from the conceptual framework, data gathering, and analysis until the study’s results were interpreted upon publication.

**REFERENCES**


