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Effectiveness *Curcuma Longa* to Prevent Cells Damage in Early Pregnant Mice with Acute Toxoplasmosis.

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Abstract

Objective: To prove the effectiveness of Curcuma longa to prevent damage cells by analyzing FOXP3 gene expression, TNF-α level, and histopathology of placental tissue.

Methods: This study was conducted in 20 early pregnant mice, divided in 5 groups (K1-K5). K1-K4 were injected with tachyzoites. K1 and K2 were intervened with Curcuma longa 125 and 500 mg/kg/day. K3 were intervened with spiramycin 60 mg/kg/day, K4 were intervened with 0,2 distilled water, and K5 was not injected and intervened. IgG-IgM levels, FOXP3 mRNA expression, TNF-α level was examined serially, and placental mice were taken for histopathology examination 7 days after intervention.

Results: FOXP3 mRNA expression in K1 and K2 increased significantly 7 days after intervention compared to K4 ($p < 0.05$), however the increased of expression in two groups has no significant difference. TNF-α level in K1 and K2 decreased significantly 7 days after intervention compared to K4 ($p < 0.05$), however the decrease of level in two groups has no significant difference. Hemorrhagic and necrotic cells were not found in K1 and K2, but were found in K3 (75%) and K4 (100%).

Conclusion: Curcuma longa 125 mg is effective to prevent cells damage in early pregnant mice with acute toxoplasmosis.

Key words: Curcuma longa, Cells damage, Early pregnancy, FOXP3 gene, TNF-α, Toxoplasmosis

Efektivitas Ekstrak *Curcuma Longa* Mencegah Kerusakan Sel Pada Mencit Hamil Muda dengan Toksoplasmosis Akut.

Abstrak

Tujuan: Membuktikan efektivitas *Curcuma longa* mencegah kerusakan sel dengan menganalisis ekspresi gen FOXP3, kadar TNF-α, dan histopatologi jaringan plasenta.

Metode: Penelitian dilakukan pada 20 tikus hamil muda, dibagi dalam 5 kelompok (K1-K5). K1-K4 diinjeksi 10 takizoid toksoplasma intra abdominal. Tiga hari pasca injeksi takizoid, K1 dan K2 diintervensi dengan Curcuma longa 125 dan 500 mg / kg / hari. K3 diintervensi dengan spiramisin 60 mg / kg / hari, K4 diintervensi dengan 0,2 ml air suling dan K5 tidak diinjeksi dan tidak diintervensi. Kadar IgG-IgM, ekspresi mRNA FOXP3, kadar TNF-α diperiksa secara serial dan plasenta tikus diambil untuk pemeriksaan histopatologi 7 hari pasca intervensi.

Hasil: Ekspresi mRNA FOXP3 pada K1 dan K2 meningkat bermakna ($p< 0.05$) 7 hari pasca intervensi dibandingkan dengan K4 ($p <0,05$), tetapi peningkatan ekspresi pada kedua kelompok tidak terdapat perbedaan bermakna. Kadar TNF-α pada K1 dan K2 menurun bermakna 7 hari pasca intervensi dibandingkan dengan K4 ($p <0,05$), tetapi penurunan kadar pada dua kelompok tidak terdapat perbedaan bermakna. Hemoragik dan nekrotik sel tidak ditemukan pada K1 dan K2, tetapi ditemukan pada K3 (75%) dan K4 (100%).

Kesimpulan: Curcuma longa 125 mg efektif untuk mencegah kerusakan sel pada tikus hamil muda dengan toksoplasmosis akut.

Keywords: *Curcuma longa,* kerusakan sel, hamil muda, gene FOXP3, TNF-α, toxoplasmosis

Introduction

Toxoplasmosis is an infection caused by the parasite toxoplasma gondii[1-3]. Toxoplasma infection will stimulate the cellular and humoral immune response of the body[4-6]. The Fork head box P3 (FOXP3) gene as a regulator of the balance of cellular immunity through T- helper cell[7]. The role of FOXP3 gen is to suppress T helper cells especially Th1 (TNF-α) [8]. When the infection occurs, FOXP3 gene expression decreases and the TNF-alpha levels increase [9,10]. An excessive increase of TNF-alpha levels can cause thrombosis, ischemia, and necrotic cells. If this condition occurs in early pregnancy, it can cause damaged cells and abortion[11-13]

Curcuma longa is a plant that belongs to the rhizome group that is easy to grow and found in Asia. *Curcuma longa* from ancient times has been used as a traditional medicine and herbal therapy. Curcumin is a major component of curcuma longa has been widely studied for its potent anti-inflammatory[14], anti-oxidant[15], anti-parasite[16], and anti-micro organism[17, 18]. This study aims to prove the effectiveness of curcuma longa extract to prevent the cell damage of placental tissue in early pregnant mice with acute toxoplasmosis by analyzing the expression of foxp3 mRNA, TNF-α levels and histopathology examination of placental mice after intervention.

Material and Methods

This research is experimental pre and post-test control groups design using 20 Balb/c female mice that were conditioned into 1-3 day(s) pregnancy that fulfill inclusion criteria (mice age 9-11 weeks, weigh 17- 20 grams, willing to eat and drink). The early pregnant mice were divided into five groups (K1 – K5), 4 mice each group. K1 – K4 were injected with 10 tachyzoites of T. gondii RH strain intra-peritoneal, and K5 without infection. The *Curcuma longa* extract get use was obtained by maceration, and the curcuminoid compound was evaluated using KLT densitometry and spectrophotometry. Curcuminoid level in *C. longa* extract obtained were at 25.5%. Three days after injection of tachyzoites, K1 and K2 were each given *C. longa* extract dose of 125 and 500 mg/kg/day, K3 was given spiramycin dose of 60 mg/kg/day (positive control), K4 was given 0.2 ml distilled water (negative control), and K5 was not intervention. Each intervention was administered for 7 days.

Blood samples were taken serially (one day before tachyzoites injection, 3 days after tachyzoites injection, and 3 days and 7 days after intervention). Examination of anti-toxoplasma IgG-IgM antibody levels and TNF-α level were examined using enzyme-linked immune-sorbent assay (ELISA), (Qualitative mouse toxoplasma antibody IgG (T-IgG) ELISA Kit Cat. No: MBS9310461 and Qualitative mouse toxoplasma antibody IgM (T-IgM) ELISA Kit Cat. No: MBS9310461) and The level of TNF-α was determined with ELISA Kit (LS-Bio: Life-Span Bio-Sciences, Inc. FOXP3gene expression by Quantitative Real-time polymerase chain reaction (q-PCR). Quantitative Real-time polymerase chain reaction (q-PCR) examination was performed using a PCR system (Applied Bio-system-Rad BT004129 USA machine) used SYBR power Green PCR Mix. FOXP3 primer is : FW-TTT ACT CGC ATG TTG CCT ACTT and RV- TCA AAT TCA TCT ACG GTC CAC ACT (NM_ 001199347.1), and standard of normality is GAPDH FW-CAT GGC CTT CCG TGT TCCT AND RV-GCG GAC GTC AGA TCCA (M32599.1). The examination of FOXP3 expression according to the standard procedure of q-PCR. The expression of the FOXP3 gene is inferred in the form of numbers seen on the computer monitor screen. Seven days after the intervention, the mice were sacrificed to remove the uterus containing the placenta. Examination of placental histopathology in

all samples was done to study and assessed the degree of damage of placental tissue in the form of the congestive capillary, extracellular edema, hemorrhagic and necrotic cells.

The data were processed using SPSS software and the data were statistically analyzed using paired t-test to determine the TNF-α level and FOXP3 gene expression before and after intervention. One-way Anova test was conducted to determine the difference of TNF-α level, and FOXP3 gene expression among these 5 groups. Histopathology finding were reported in percentage, and chi-square test was performed to identify cells damage between groups. A values of $p < 0.05$ was considered significant.

Results

Table 1 Anti-Toxoplasma IgM Antibody Level before and after Intervention.

Group (K)	Anti-Toxoplasma IgM Antibody level				Cut Off Point (OD)
	A1	A2	A3	A4	
K1	0,250 ± 0,001	0,847 ± 0,012	0,534 ± 0,013	0,346 ± 0,024	0,327
K2	0,260 ± 0,017	0,828 ± 0,010	0,517 ± 0,005	0,321 ± 0,009	
K3	0,242 ± 0,011	0,795 ± 0,012	0,564 ± 0,013	0,355 ± 0,014	
K4	0,251 ± 0,011	0,826 ± 0,017	1,434 ± 0,021	1,629 ± 0,015	
K5	0,249 ± 0,007	0,302 ± 0,012	0,266 ± 0,013	0,307 ± 0,018	

Legend. K1: Group injected with tachyzoites and intervered with *C. Longa* dose of 125 mg/kgbb. K2: Group injected with tachyzoites and intervered with *C. Longa* dose of 500 mg/kgbb. K3: Group injected with tachyzoites and intervered with Spiramycin dose of 60 mg/kgbb. K4: Group injected with tachyzoites and intervered with distilled water 0,2 ml. K5: Group was not injected with tachyzoites and was not intervered. A1:one day before tachyzoites injection, A2: Three days after tachyzoites injection, A3 and A4 Three and seven days after intervention.

Table 2 Anti-Toxoplasma IgG Antibody Level before and after Intervention.

Group (K)	Anti-Toxoplasma IgG Antibody level				Cut Off point (OD)
	A1	A2	A3	A4	
K1	0,162 ± 0,008	0,348 ± 0,015	0,449 ± 0,023	0,441 ± 0,013	0, 302
K2	0,153 ± 0,006	0,362 ± 0,021	0,462 ± 0,012	0,455 ± 0,008	
K3	0,154 ± 0,010	0,340 ± 0,008	0,439 ± 0,004	0,461 ± 0,021	
K4	0,155 ± 0,009	0,354 ± 0,010	0,753 ± 0,007	1,365 ± 0,009	
K5	0,132 ± 0,012	0,154 ± 0,010	0,152 ± 0,015	0,139 ± 0,015	

Legend. K1: Group injected with tachyzoites and intervered with *C. Longa* dose of 125 mg/kgbb. K2: Group injected with tachyzoites and intervered with *C. Longa* dose of 500 mg/kgbb. K3: Group injected with tachyzoites and intervered with Spiramycin dose of 60 mg/kgbb. K4: Group injected with tachyzoites and intervered with distilled water 0,2 ml. K5: Group was not injected with tachyzoites and was not intervered. A1:one day before tachyzoites injection, A2: Three days after tachyzoites injection, A3 and A4 Three and seven days after intervention.

Table 3 FOXP3 Gene Expression before and after Intervention.

Group (K)	FOXP3 Gene Expression							
	A1		A2		A3		A4	
K1	9,98 ±	0,14	7,98 ±	0,21	12,03 ±	0,13	9,88 ±	0,23
K2	9,96 ±	0,14	8,07 ±	0,17	12,12 ±	0,32	9,87 ±	0,13
K3	10,02 ±	0,15	8,54 ±	0,09	11,93 ±	0,12	10,14 ±	0,15
K4	10,18 ±	0,12	8,85 ±	0,15	7,09 ±	0,15	6,12 ±	0,14
K5	10,00 ±	0,19	10,20 ±	0,14	10,06 ±	0,19	9,90 ±	0,16

Legend. K1: Group injected with tachyzoites and intervered with *C. Longa* dose of 125 mg/kgbb. K2: Group injected with tachyzoites and intervered with *C. Longa* dose of 500 mg/kgbb. K3: Group injected with tachyzoites and intervered with Spiramycin dose of 60 mg/kgbb. K4: Group injected with tachyzoites and intervered with distilled water 0,2 ml. K5: Group was not injected with tachyzoites and was not intervered. A1:one day before tachyzoites injection, A2: Three days after tachyzoites injection, A3 and A4 Three and seven days after intervention.

Table 4 TNF-α Level before and after Intervention.

Group (K)	TNF-α Level (pg/ml)			
	A1	A2	A3	A4
K1	36,738 ± 0,015	301,708 ± 0,025	130,23 ± 0,013	81,976 ± 0,010
K2	31,310 ± 0,013	285,244 ± 0,018	144,102 ± 0,013	88,611 ± 0,015
K3	38,219 ± 0,013	305,425 ± 0,017	137,139 ± 0,013	85,870 ± 0,013
K4	35,814± 0,013	292,432 ± 0,020	352,750 ± 0,019	558,436 ± 0,020
K5	36,517 ± 0,008	36,517 ± 0,007	34,410 ± 0,010	36,517 ± 0,005

Legend. K1: Group injected with tachyzoites and intervered with *C. Longa* dose of 125 mg/kgbb. K2: Group injected with tachyzoites and intervered with *C. Longa* dose of 500 mg/kgbb. K3: Group injected with tachyzoites and intervered with Spiramycin dose of 60 mg/kgbb. K4: Group injected with tachyzoites and intervered with distilled water 0,2 ml. K5: Group was not injected with tachyzoites and was not intervered. A1:one day before tachyzoites injection, A2: Three days after tachyzoites injection, A3 and A4 Three and seven days after intervention.

Table5. Histological Finding after Intervention

Group (K)	Histological Finding			
	Congestive Capiler (%)	Extracellular Edema (%)	Haemorrhagic (%)	Necrotic cell (%)
K1	100	50	0	0
K2	100	75	0	0
K3	100	100	75	75
K4	100	100	100	100
K5	100	0	0	0

Legend. K1: Group injected with tachyzoites and intervered with *C. Longa* dose of 125 mg/kgbb. K2: Group injected with tachyzoites and intervered with *C. Longa* dose of 500 mg/kgbb. K3: Group injected with tachyzoites and intervered with Spiramycin dose of 60 mg/kgbb. K4: Group injected with tachyzoites and intervered with distilled water 0,2 ml. K5: Group was not injected with tachyzoites and was not intervered. A1:one day before tachyzoites injection, A2: Three days after tachyzoites injection, A3 and A4 Three and seven days after intervention.

Discussion

This study was conducted in mice that were conditioned 1-3 days of pregnancy and acute toxoplasmosis. The anti-toxoplasma IgG-IgM antibody levels increased significantly 3 days after injection of T. gondii tachyzoites (Table 1 and 2), it means that the mice have been acute toxoplasmosis before intervention. Our previous study also found that anti-toxoplasma IgM and IgG antibody levels increased significantly 24 hours and 48 hours after injection of 10 T. gondii tachyzoites[5]

The expression of FOXP3 gene in this study decreased significantly ($p < 0.05$) 3 days after injection of 10 T. gondii tachyzoites. The other researchers also reported decreased the FOXP3 gene expression during acute infection[9-10]. Three and Seven days after intervention with C. longa extract dose of 125 and 500 mg/kg/days, the FOXP3 gene expression increased significantly ($p < 0.05$), however the increased of FOXP3 gene expression in two groups has no significant difference. The curcumin can increase the expression of the FOXP3 gene through the transforming growth factor-β (TGF-β) pathway. The curcumin induces conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-β induction of transcription factor FOXP3[19] Several researchers also reported that FOXP3 gene expression increased 7-day after intervention with curcumin[20-21].

In this study the TNF-α levels increased significantly ($p < 0.01$) 3 days after tachyzoites injection and decreased significantly ($p < 0.05$) 3 and 7 days after C. longa extract intervention dose of 125 and 500 mg / kg / day, however the decrease of TNF-α level in two groups has no significant difference. Guo *et al,* also reported that TNF-α levels increased 7 days after curcumin intervention dose of 100 mg[22].

In this study, the histopathology examination of placental tissue 7 days after intervention with curcuma longa extract was not found hemorrhage and necrotic cells, but in the positive control group found hemorrhagic and necrotic cells were 75% of sample and 100% in negative control group.

Based on the findings this study it can be concluded that *Curcuma longa* extract dose of 125 mg / kg /day for 7 days effectively to prevent the damage of placental tissue cells in early pregnant mice with acute toxoplamosis. *Curcuma longa* can prevent cell damage by increasing foxp3 gene expression and decreasing TNF-α levels. The use of curcuma longa extract at dose of 125

mg / kg / day for 7 days may be considered as supportive therapy in early pregnancy with acute toxoplasmosis, however further research is needed.

Conclusion

Curcuma longa extract dose of 125 mg/kg/ day for 7 days orally effective to prevent cells damage placental tissue in early pregnant mice with acute toxoplamosis.

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