

Abstract

THE ROLE OF CHLOROGENIC ACID IN HEPATIC FIBROSIS INDUCED BY CARBON TETRACHLORIDE IN MICE MODEL: EXPLORATION OF MACROPHAGE NUMBER, MONOCYTES CHEMOATTRACTANT PROTEIN-1 EXPRESSION (MCP-1), AND ALBUMIN LEVEL

¹ Mohammad Ghannam, ¹ Nur Arfian, MD, Ph.D., ² Dr. Dwi Cahyani Ratna Sari, MD; MS; PhD.
Department of Anatomy, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Abstract

Background: Liver fibrosis is a scarring process related to chronic liver injury of all causes and as yet no truly effective treatment is available. Chlorogenic acid (CGA) is a phenolic compound and exerts anti-inflammatory and anti-oxidant activities. This study will examine the effects of chlorogenic acid (CGA) on liver fibrosis induced by carbon tetrachloride (CCl₄) and explored the possible mechanisms of action. Experimental animals were used in this study male mice, Swiss animal strain weighing 30 ± 20 g, the age of mice is 3 months, total number of samples is 30, the main material used in this study is CCl₄ (Merck MAB13426) 0.5 mg/kg BW dissolved in olive oil (1: 1) and CGA (Sigma-Aldrich, C3878-1G), 42 mg/kg BW, 63 mg/kg BW, and 84 mg/kg BW. Mice were divided into 6 groups. CCl₄ dose 0.5 mg/kg given dissolved along with the olive oil in the ratio 1:1, twice per week, for 4 weeks of treatment injection intraperitoneally, while the CGA doses are 42 mg/kg, 63 mg/kg and 84 mg/kg given orally daily for 4 weeks. This study examined the effects of chlorogenic acid (CGA) on liver fibrosis induced by carbon tetrachloride (CCl₄) and explored the possible mechanisms of action through the exploration the effect of CGA on macrophage number, monocyte chemoattractant protein 1 expression (MCP-1), and albumin level. The results showed the CGA could reduce the effect of CCl₄ in liver fibrosis in mice model.

Objective: to investigate the effect of chlorogenic acid on the macrophage number, chemoattractant monocyte protein-1 expression, and on the albumin level in liver fibrosis caused by carbon tetrachloride.

Method: Mice were treated in Laboratory of Anatomy Faculty of Medicine, Universitas Gadjah Mada, for 4 weeks, terminated after 24 hours of treatment ends, terminations carried out by the method of cervical dislocation, were anesthetized previously with ketamine (a dose of 75 mg/kg i.m.), then performed dissection in the abdominal region at midline and perfused the liver organ was taken with formalin 10%. Then performed immunohistochemistry staining,



reverse transcription PCR (RT-PCR) to examine MCP-1 expression pathway, and measured the albumin level.

Result: the results of this study showed that the administration of CGA could reduce significantly the macrophage number in liver fibrosis induced by CCl₄ and increased significantly the serum of albumin levels through improving the antioxidant and anti-inflammatory activities in liver fibrosis induced by CCl₄, however the result of MCP-1 expression showed there was no significant effect by CGA on MCP-1 expression in liver fibrosis induced by CCl₄.

Conclusion: Group with CGA treatment had lower macrophage number compared to CCl₄ in liver fibrosis induced by CCl₄. The MCP-1 mRNA expression was not significant after the CGA administration in liver fibrosis induced by CCl₄. The administration of CGA increase significantly the serum of albumin level in liver fibrosis induced by CCl₄.

Keywords: liver fibrosis, Chlorogenic Acid, Carbon tetrachloride, Oxidative Stress, Chemokines.

INTISARI
PENGARUH PEMBERIAN ASAM KLOOROGENAT PADA MODEL MENCIT FIBROSIS HEPAR YANG DIINDUKSI DENGAN CARBON TETRACHLORIDE (CCl₄)

Kajian terhadap jumlah makrofag, Monocyte Chemoattractant Protein-1 (MCP-1), dan kadar serum albumin

Mohammad Ghannam¹, Nur Arfian, MD, Ph.D.¹, Dr. Dwi Cahyani Ratna Sari, MD., MS., PhD.¹

¹Department of Anatomy, Faculty of Medicine, Universitas Gadjah Mada. Yogyakarta, Indonesia.

Latar belakang: Fibrosis hepar merupakan jaringan parut yang disebabkan akibat cedera kronis. Hingga saat ini masih belum ditemukan terapi yang efektif untuk fibrosis hepar. Asam klorogenat (*chlorogenic acid*, CGA) merupakan senyawa fenolik yang memiliki aktivitas antiinflamasi dan antioksidan.

Tujuan: Mengkaji perubahan ekspresi Monocyte Chemoattractant Protein-1 (MCP-1), jumlah makrofag, dan kadar serum albumin pada mencit dengan fibrosis hepar setelah pemberian asam klorogenat.

Metode: Penelitian ini menggunakan mencit jantan galur Swiss (30 gram, 3 bulan) sebanyak 30 ekor. Mencit dibagi menjadi 6 kelompok yaitu, kontrol, CCl₄ dilakukan dengan cara melarutkan CCl₄(Merck MAB13426) 0.5 mg/kg BB dalam minyak zaitun (1:1), CGA (Sigma-Aldrich, C3878-1G) dengan dosis 42 mg/kgBB, CGA 63 mg/kgBB, and CGA 84 mg/kgBB. Mencit diterminasi pada minggu ke-4. Selanjutnya dilakukan pemeriksaan jumlah makrofag dilakukan dengan metode IHC, pemeriksaan MCP-1 dilakukan dengan *reverse transcription* PCR (RT-PCR).

Hasil: Pemberian CGA dapat menurunkan jumlah makrofag ($p < 0.05$) dan mRNA MCP-1 pada mencit yang diinduksi fibrosis hepar menggunakan CCl₄, dan meningkatkan kadar serum albumin ($p < 0.05$) melalui efek antioksidan dan antiinflamasi.

Kesimpulan: Pemberian asam klorogenat dapat menurunkan jumlah makrofag dan MCP-1 dan meningkatkan kadar serum albumin pada mencit dengan fibrosis hepar.

Kata kunci: Fibrosis hepar, asam klorogenat (CGA), *carbon tetrachloride* (CCl₄), stres oksidatif, kemokin