UNIVERSITI PUTRA MALAYSIA

HEPATOPROTECTIVE EFFECT OF ANDROGRAPHOLIDE ON PARACATEMOL INDUCED LIVER DAMAGE IN BALB/C MICE

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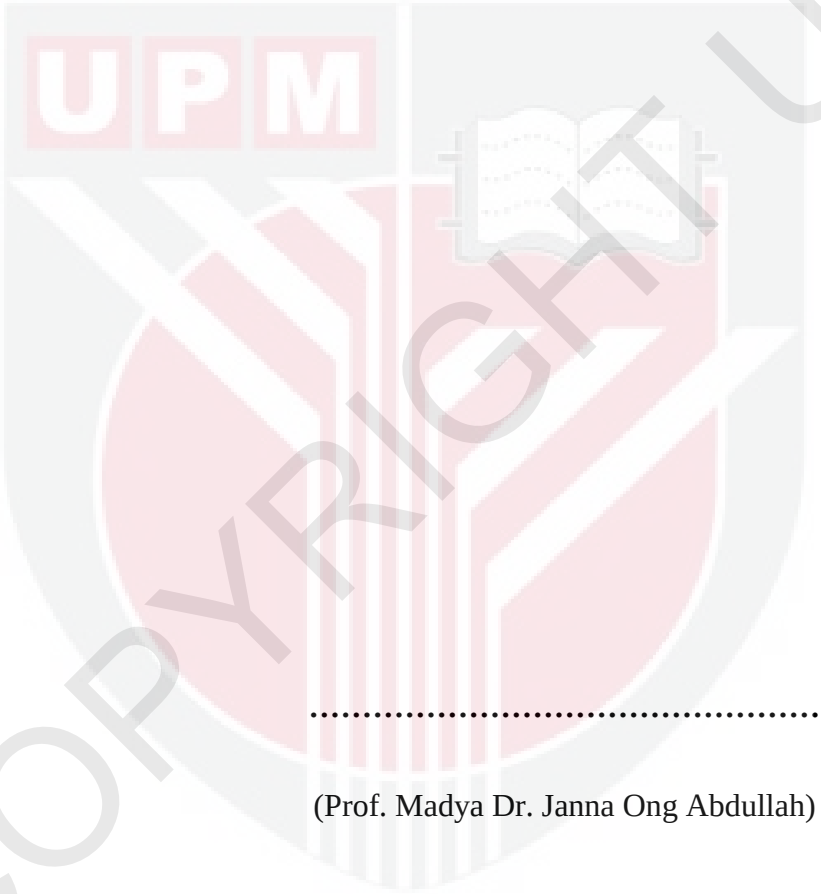

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Hepatoprotective Effect of Andrographolide on Paracatemol Induced Liver Damage in BALB/C Mice

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Abstract

Andrographis Paniculata has been widely used as hepatoprotectant for liver damage problem. The active compound called andrographolide is reported to be hepatoprotective and its protection against paracetamol-induced was better than that produced by silybin. In this study, the *in vivo* hepatoprotective effect and antioxidant effect of andrographolide were determined by comparing with the untreated and silybin (positive control) groups. Liver superoxide dismutase (SOD), malondialdehyde (MDA), ferric reducing antioxidant power (FRAP), nitric oxide (NO) levels, and serum biochemical profile including aspartate transaminase (AST),alanine transaminase (ALT), alkaline phosphatase (ALP), Total Bilirubin (TBil), triglycerides (TG), and cholesterol, also serum IL-2 and IFN-γ levels, immunophenotyping evaluation and histopathological changes were studied. Results showed that the serum enzyme biomarker levels were reduced in the paracetamol-induced mice after administration of 200 µL andrographolide extract (30mg/kg).These serum levels are including ALT (P=0.0012), AST (P=0.0011), TG (P=0.0079),Chol (P=0.0053), and TBil (P= 0.0012). Besides, comparing to silybin, the liver antioxidant levels such as SOD (P<0.0001) and FRAP were restored and the reduction of NO (P=0.0105) and MDA (P=0.0005) in the liver andrographolide treated mice were also seen. In conclusion, andrographolide was capable of recovering the damaged hepatocytes to their normal structures hence could enhance hepatoprotective and antioxidant effects *in vivo* of paracetamol-induced hepatotoxicity.

Keyword: Andrographolide, silybin, hepatoprotective effects, antioxidant effect

Kesan Ketahanan Hepato daripada Andrografolid Terhadap Kerosakan Hati BALB/C Tikus oleh Induksi *Paracetamol*

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Abstrak

Andrographis paniculata (Hempedu Bumi) telah digunakan secara meluas sebagai *hepatoprotectant* untuk masalah kerosakan hati. Komponen aktif dipanggil Andrografolid dan dilaporkan sebagai *hepatoprotective* dan perlindungannya terhadap *paracetamol* adalah lebih baik daripada yang dihasilkan oleh *silybin*. Dalam kajian ini, kesan ketahanan hepato *in vivo* dan kesan antioksidan Andrografolid ditentukan dengan membandingkan dengan kumpulan yang tidak dirawat dan kumpulan (kawalan positif) *silybin*. *Superoxide dismutase* hati (SOD), *malondialdehyde* (MDA), ferik mengurangkan kuasa antioksidan (FRAP), tahap *oksida nitrik* (NO) , dan profil serum biokimia termasuk transaminase Aspartate (AST), transaminase alanina (ALT), phosphatase alkali (ALP), Jumlah Bilirubin (TBil), trigliserida (TG), dan kolesterol, juga tahap serum IL-2 dan IFN-γ , penilaian immunophenotyping dan perubahan histopatologi telah dikaji. Hasil kajian menunjukkan bahawa tahap penanda bio serum enzim telah dikurangkan dalam tikus induksi *paracetamol* selepas pemberian 200µL ekstrak Andrografolid (30mg / kg) Tahap serum-serum tersebut adalah termasuk ALT (P = 0.0012), AST (P = 0.0011), TG (P = 0.0079), Chol (P = 0.0053), dan TBil (P = 0.0012). Selain itu, berbanding dengan *silybin*, tahap antioksidan hati seperti SOD (P <0,000) dan FRAP telah dipulihkan dan berlaku pengurangan NO (P = 0.0105) dan MDA (P = 0.0005) dalam hati tikus yang dirawat menggunakan Andrografolid juga dilihat. Kesimpulannya, Andrografolid mampu memulihakan hepatosit yang rosak kepada struktur biasa maka boleh meningkatkan kesan ketahanan hepato dan kesan antioksidan *in vivo* yang disebabkan oleh induksi *paracetamol* yang telah menyebabkan ketoksikan hepato.

Kata kunci: Andrografolid, Silybin, kesan ketahanan hepato, kesan antioksidan

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NAPQI	N-acetyl-p-benzoquinoneimine
TNF-α	Tumor necrosis factor
NO	Nitric oxide
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
NAC	N-acetylcysteine
MDA	Malondialdehyde
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
TBil	Total Bilirubin
FRAP	Ferric reducing antioxidant power
NK cell	Natural Killer cell
IL-2	Interleukin 2
IFN-γ	Interferon-γ

CHAPTER 1

INTRODUCTION

Hepatotoxicity is the most widespread pathology worldwide which up to 83% of general liver diseases cases. Liver damaged can happened because of prolonged use of high doses of certain drugs, exposure to chemicals, infectious agents, and air and water pollutants. Due to the lack of liver protective drugs available, hepatoprotective herbal medicine are commonly used in the treatment of liver damage (Ai *et al.,* 2013). Furthermore, there is increasing evidence that free radicals and reactive oxygen play a role in some steps that would initiate and regulate the development of liver diseases independently (Alam *et al*., 2012). Paracetamol is a commonly used antipyretic and analgesic agent which can lead to hepatotoxicity. The over dosage of taking up paracetamol could be fatal. It is reported that its hepatotoxicity is due to toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI) that is detoxified by gluthatione (Yang *et al*., 2009). In paracetamol over dosage, excess NAPQI is formed and later would bind to proteins resulting in hepatic necrosis. N-acetylcysteine is the normal therapy for paracetamol which it restores hepatic gluthatione that detoxifies NAPQI (Yang *et al.*, 2009). Even though N-acetylcysteine is used for the treatment of paracetamol toxicity, some cases do not respond.

It is reported that *Andrographis paniculata* is widely used in the Indian traditional system of medicine as a hepatoprotective and hepatostimulative agent and has also been reported to have antioxidant effects against different hepatotoxins (Akbar *et al*., 2011). It is an herbaceous plant in the family of *Acanthaceae* which has been

widely used in traditional medical Asian countries (Dhiman *et al.*, 2012). Some of the pharmacological activities of this plant include the antiflammatory, antioxidant, hypoglycemic and hepatoproctective activities (Darbar *et al*., 2009). The stem and leaves of *A.paniculata* composes of large number of chemical constituents such as lactones, diterpenoids, diterpene glycosides, flavonoids and flavonoids glycosides. However, the bioactive component of *A.paniculata* is andrographolide which is found in the leaves (Jutti *et al*., 2010). This andrographolide has a bitter taste, is a colourless and crystalline bicyclic compound, and has low aqueous solubility in water but is soluble in acetone, methanol, chloroform, and ether (ZADE et al., 2013).

Thus, the purpose of this study is to evaluate the hepatoprotective effect of chemically synthesized andrographolide extract on paracetamol-induced liver damage in BALB/C mice. We carried out the *in vivo* studies using 20 BALB/C mice with four different treatment groups. The liver damage in the mice were induced using paracetamol. One of the treatment groups would be treated with silybin that act as positive control. Silybin is the isomer of silymarin and it is a major and most active component that is used to self- treat liver disorders (Singh *et al*., 2012).

In short, the objectives of this study are:

1. To study the *in vivo* hepatoprotective effect of andrographolide on paracetamol induced liver damage in BALB/C mice
2. To determine the effect of andrographolide on *in vivo* antioxidant level in liver samples

CHAPTER 6

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