UNIVERSITI TEKNOLOGI MARA

CHARACTERIZATION OF THE ANTI-INFLAMMATORY AND ANTINOCICEPTIVE ACTIVITIES OF *Erythroxylum cuneatum* (Miq.) Kurz

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ABSTRACT

Erythroxylum cuneatum (*E. cuneatum*) which can be found in the tropical regions of South America, Africa, Southeast Asia, and Australia was used traditionally in treating diabetes mellitus, bodily discomfort and also as a tonic for miscarriages. However, not many studies have been conducted on this plant to prove the folkloric claims. This study was performed to evaluate the ability of *E. cuneatum* aqueous extract (ECAE) as an anti-inflammatory and antinociceptive agent. Phytochemical screening revealed that the hexane, dichloromethane, ethyl acetate and butanol extracts contained alkaloids, flavonoids, tannins and saponins. These compounds have been found to possess antioxidant properties with strong ferrous ion chelating activity (EC_{50}: 615.9 µg/mL) however, ECAE appeared not to have any effect on xanthine oxidase inhibitory assay. ECAE had low cytotoxicity effect on the macrophage RAW 264.7 cells with IC_{50} of 140 µg/mL. Following a 6-h of incubation with lipopolysaccharides (LPS), cell treated with ECAE (35, 70 and 140 µg/mL) significantly reduced IL-1β and IL-6 activities. ECAE also significantly reduced COX-2 activity. Reduction of COX-1 activity was confirmed by Western Blot analysis whereby COX-2 detected in cells treated with ECAE was significantly lower than untreated cells. ECAE can be considered as a dietary antioxidant by significantly increasing the superoxide dismutase (SOD) and catalase activities. ECAE (400 mg/kg, p.o.) significantly reduced paw edema volume after carrageenan injection in acute inflammation study. Significant increase in TNF-α was observed after 4 h of treatment with ECAE (400 mg/kg, p.o.). IL-1β was also increased after treatment with ECAE (50 and 200 mg/kg, p.o.). However, ECAE (400 mg/kg, p.o.) significantly decreased IL-1β concentration. No significant changes were observed in IL-6, COX-1 and -2 levels. Analyses on blood biochemistry and haematology were within normal range. Histological examination of major organs and macrophage presence on liver were unremarkable. In chronic inflammation study, ECAE did not elicit significant reduction in granuloma formation. No significant changes were observed in cytokine levels, COX activity, blood biochemistry and haematology analyses. No abnormal morphological changes were observed in the liver, kidney and stomach. Lower number of macrophages were detected in the liver after treatment with ECAE (200 and 400 mg/kg, p.o.). Furthermore, significant increase was detected in catalase activity without any difference in SOD and GPx activities. Lastly, ECAE (100, 200 and 400 mg/kg, p.o.) also revealed its potency as an antinociceptive agent by reducing the number of abdominal constriction following injection of acetic acid and by increasing the time latency in tail withdrawal test after immersion of the tail in warm water bath. As a conclusion, ECAE showed iron chelating activity, but was not able to inhibit xanthine oxidase with formidable antinociceptive and anti-inflammatory properties.

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CHAPTER ONE
BACKGROUND

1.1 Background of Study

Inflammation is a process of the body protection against infection, burn, toxic chemicals, allergens and other noxious stimuli (Das et al., 2009a; Ibrahim et al., 2017). Redness, swelling, heat and pain are the characteristic of inflammation reaction (Ibrahim et al., 2017). Persistent inflammation can cause chronic diseases such as cancer, diabetes, neurodegenerative, cardiovascular, pulmonary, metabolic, auto-immune and neoplastic diseases (Buodonpri et al., 2009; Lucas et al., 2006).

Non-Communicable Diseases (NCDs) or chronic diseases which are cardiovascular diseases (like heart attacks and stroke), cancer, chronic respiratory diseases (such as chronic obstructed pulmonary disease and asthma) and diabetes, contribute to an estimated 73% of total deaths in Malaysia (IPH, 2015; Kew et al., 2015). The biggest contributor is cardiovascular diseases with an estimated 35% of deaths occur in individuals aged less than 60 years, which are mainly our working population (IPH, 2015). In 2012, Institute of Public Health, ranked hypertension, smoking, diabetes, high cholesterol and high BMI as the biggest contributors to both disabilities adjusted life-years (DALY) and deaths (IPH, 2015). According to World Health Statistics (2017a), probability of dying in Malaysia from diseases such as cardiovascular disease, cancer, diabetes and chronic respiratory disease in 2015 are 17.1% and the statistic is higher compared to neighboring countries including Thailand (16.2%) and Singapore (10.1%). Furthermore, the top 10 drugs prescribed in Malaysia were for diabetes and cardiovascular disorders (Roughead et al., 2013; Siti Fauziah et al., 2014). In 2005, it was reported that 3.6% of Malaysian population was on anti-diabetic medication and increased in the following years which is 3.97% in 2007 (Ministry of Health Malaysia, 2007; Ministry of Health Malaysia, 2010). According to Malaysian Statistics on Medicine 2009 & 2010, the use of drugs in diabetes treatment is still on the rise in 2009 and 2010 (4.89% and 5.97%, respectively) (Ministry of Health Malaysia, 2014). Data provided also showed that treatment for cardiovascular disorder from 2005 until 2010 was increased accordingly (Ministry of Health Malaysia, 2007, 2010, 2014). These data strongly suggest that