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Komunikasi Pendek/*Short Communications*

Inhibitory Effects of the Extracts of *Plantago major* Linn. on the Number and Size of Calcium Oxalate Crystals *In Vitro.*

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ABSTRAK

Kajian ini dijalankan untuk menentukan kesan aktiviti perencatan pelbagai ekstrak Plantago major ke atas kristal kalsium oksalat secara in vitro. Ekstrak, petroleum eter, n-butanol, etanol, metanol dan akues P. major digunakan pada kepekatan masing-masing10 mg/ml dan dimetilsulfoksida digunakan sebagai kawalan negatif dan zilorik sebagai kawalan positif. Kaedah slaid gel Schneider digunakan dan pengukuran bilangan dan saiz kristal kalsium oksalat (dihidrat dan monohidrat) dilakukan setiap 24 jam dengan menggunakan sistem Analisa Imej. Saiz kristal kalsium oksalat dihidrat menurun secara signifikan ($p < 0.05$) pada semua ekstrak kecuali petroleum eter berbanding dengan kawalan. Saiz kristal monohidrat menurun secara signifikan ($p < 0.05$) pada ekstrak n-butanol dan akues sahaja. Saiz kristal monohidrat menurun secara signifikan ($p < 0.05$) pada ekstrak n-butanol dan akues berbanding dengan zilorik. Bilangan kristal monohidrat menurun secara signifikan ($p < 0.05$) pada ekstrak etanol, n-butanol dan akues berbanding dengan kawalan dan etanol (juga dihidrat) dan akues berbanding dengan zilorik. Hasil ini menunjukkan kebanyakan ekstrak P. major berupaya merencat saiz dan bilangan kristal kalsium oksalat secara in vitro pada kepekatan 10 mg/ml selepas 24 jam.

ABSTRACT

This study was carried out to determine the inhibitory effects of the various extracts of Plantago major on calcium oxalate crystals in vitro. The petroleum ether, n-butanol, ethanol, methanol and aqueous extracts of P. major were each used at a concentration of 10 mg/ml and dimethylsulfoxide (DMSO) was used as a control. Modified Schneider slide gel method was used and the number and size of calcium oxalate crystals (both dihydrate and monohydrate types) were counted every 24 hrs by using the Image

Analyser system. There was a significant reduction in the size of the calcium oxalate dihydrate crystals by all of the extracts except for petroleum ether extract compared to the control ($p < 0.05$). The significant reduction in the size of the monohydrate crystals was only observed in the n-butanol and water extracts ($p < 0.05$). The size of monohydrate crystal reduced significantly in n-butanol and water compared to zylorik ($p < 0.05$). The number of monohydrate crystals reduced significantly in the ethanol, n-butanol and water extracts compared to the control and in ethanol(also dyhydrate) and water extractcompared to zylorik ($p < 0.05$). These results indicated that most of the extracts of P. major could reduce the number and size of calcium oxalate crystals in vitro at 10 mg/ml after 24 hrs.

Plantago major Linn. belonging to the family *Plantaginaceae* is a perennial herb found wild throughout the whole of Europe and temperate Asia (Burkill 1966). Every part of the plant has been used in many traditional medicines to treat various ailments. Among the many medicinal uses of the plants are for cough, diarrhoea, dysentery, urinary tract calculus, diabetes, worm infestations, haemorrhoids, inflammation, haematuria, dysuria, oliguria, pains, gastritis, gonorrhea infections and as an anti-venom (Burkill 1966; Muhamad & Mustafa 1994). In the present study we investigated the inhibitory effects of various extracts of *Plantago major* on calcium oxalate crystals *in vitro*. Calcium oxalate is the most common stone (80%) in urolithiasis which is a condition where there is a formation of calculus in the urinary system. Zyloric (*allopurinol*) is a uricosuric agent that has been clinically used for the follow up patients with stone as it reduced the production of uric acid and in fact uric acid may be a nidus for calcium oxalate stone (Ismail et al. 1994)

Crystal analysis indicated that the calcium oxalate crystals used in this study were a mixture of calcium monohydrate and dihydrate varieties (Table 1). These were the crystals usually found in the calculus of human being (Grases et al. 1990; Kataoka et al. 1990; Zhari et al. 1995). The results indicated that all of the extracts of *P. major* except for petroleum ether extract significantly reduced the size of the dihydrate crystals ($p < 0.05$). The n-butanol and aqueous extracts were the most potent inhibitors on the size of calcium oxalate dihydrate and monohydrate crystals as compared to the control ($P < 0.05$). The ethanol, petroleum ether and aqueous extracts decreased the number of monohydrate crystals significantly as compared to the control and ethanol extract on dihydrate crystals compared to zyloric ($P < 0.05$). This indicates that the extracts might have inhibited the growth or aggregation of the crystals or have dissolved the preformed crystals.

In conclusion, the various extracts of *P. major* showed inhibitory effects on the size and number of calcium oxalate crystals *in vitro*. Further study need to be carried out to establish a precise mechanism of action of the different extracts as well as identifying their bioactive compounds.

TABLE 1. Calcium oxalate crystals after 24 hr exposure to various extracts of *Plantago major* in vitro. * $p < 0.05$ vs control, ♦ $p < 0.05$ vs zyloric (unpaired t-test)

Group	n	Number of crystals (mean ± SEM).		Size of crystals (mean ± SEM) in μm^2	
Type of crystals		Dihydrate	Monohydrate	Dihydrate	Monohydrate
DMSO	10	12.83 ± 3	35.8 ± 3.2	190.2 ± 17.01	72.01 ± 9.02
Methanol	10	11.7 ± 4.4	30.5 ± 4.3	106.5 ± 23.2*	55.8 ± 9.3
Ethanol	10	7.2 ± 0.83 ♦	13 ± 1.3* ♦	102.2 ± 12.5*	61.9 ± 7.4
Petroleum ether	10	14.7 ± 3.7	16.3 ± 3.3*	140.8 ± 33.2	62.1 ± 11.8
n-Butanol	10	24.2 ± 4.1	27.7 ± 2.8	65.9 ± 18.5*	29.7 ± 6.3* ♦
Water	10	19.3 ± 2.5	15.2 ± 1.4* ♦	108.5 ± 17.6*	34.1 ± 5.4* ♦
Zyloric	10	22.17 ± 3.9	24.8 ± 3.2	128.9 ± 21.5*	57.2 ± 6.04

MATERIALS AND METHODS

The whole plant of *Plantago major* was collected from Cameron Highland and was identified by a botanist from the Malaysian Agricultural Research & Development Institute (MARDI), Serdang, Selangor. The whole plant was shade-dried at room temperature for a week and Soxhlet extracted with methanol, ethanol, petroleum ether, n-butanol and water. Each extract was diluted with DMSO to prepare a concentration of 10 mg/ml. DMSO was used as a blank control.and zyloric as a positive control. The slides were coated with 1.5 ml 1% bactoagar and each slide was equally divided into two areas. Eight equal wells with a distance of 1.25 x 0.5 cm were made when the gel was about to solidify. Calcium oxalate crystals were prepared by mixing equal amount of (20 ml) of calcium chloride and ammonium chloride solutions. The ions seeped through the gel and form a longitudinal area of calcium oxalate crystals. The formed crystals were placed in the horizontal wells and the samples and control (sample size, n = 10) were in the longitudinal wells. After 24 hours the slides were read under Image Analyser system (3.0 Karl Zeiss, Germany) to measure the size and number of the calcium oxalate crystals.

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Ulasan Buku/*Book Review*

Basic Medical Laboratory Techniques by Barbara Estridge, Anna Reynolds and Norma Walters. Stamford U.S.A: Thomson Learning International Division. 2000. 4th Edition. 120 pages, illustrated. Reviewed by Baharudin Omar.

This is a paperback book which does an excellent job of explaining the theory and techniques of medical laboratory procedures. The authors present descriptions of selected procedures and illustrate the fundamental principles underlying them. Basic information about instruments are also included and they are written in a cohesive manner and are easily comprehensible by beginners in the field of medical instrumentations. Manual procedures of medical laboratory techniques, which could be useful in smaller diagnostic and/or biomedical laboratories in Malaysia, are succinctly put forward by the authors of this book.

The laboratory techniques are covered in eight units: Introduction to the Medical Laboratory, Basic Hematology, Basic Haemostasis, Urinalysis, Basic Clinical Chemistry, Basic Clinical Microbiology and Basic Parasitology. Illustrations are numerous (325 illustrations and photographs and 120 tables) and they are highly useful as adjunct measures to the readers' understanding of the procedures. Clear colour photographs (altogether 48 of them) help students in identifying specimens pertaining to the broad fields of basic medical science, such as hematology, biochemistry, microbiology and parasitology.

Each unit is organized in a pleasing format containing a list of unit objectives, a brief overview of the unit, a list of references and resources, and lessons. Each lesson is formulated to help students to know what is expected of them and this can be self tested by answering review questions found at each unit.

An interesting feature of this book is the Glossary. It includes definitions of the glossary terms from all the lessons covered and pronunciations of some of the difficult terms. The appendices contain information such as addresses of health care regulatory agencies and professional societies but are limited to the American agencies and societies. However, the web sites given for each agency or society could provide useful information for students, whereby such sites might contain educational articles and links to various disciplines of biomedical science.

The approach followed by the book allows the reader to easily categorize, and better comprehend, the mass of information which has

rapidly accumulated in the field of medical laboratory diagnosis. The book provides a useful summary of knowledge of principles of laboratory techniques and is recommended to investigators and students alike.

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