การศึกษาเบื้องต้นถึงความเป็นพิษเฉียบพลันทางปากของผลิตภัณฑ์เสริมอาหารที่มีส่วนผสมของสารสกัดจากผลสำรอง

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บทคัดย่อ

"สำรอง" หรือ "พุงทะลาย" มีชื่อทางวิทยาศาสตร์ว่า *Scaphium scaphigerum* (G.Don) จัดอยู่ในวงศ์ *Sterculiaceae* ผลของต้นสำรองใช้เป็นสมุนไพรที่เข้าตำรับยาไทยโบราณ มีสรรพคุณในการรักษาโรคต่างๆ เช่น แก้ร้อนใน กระหายน้ำ แก้โรคท้องเสีย และช่วยในเรื่องระบบการทำงานของไตและลำไส้ใหญ่ ในงานวิจัยได้นำสารสกัดจากผลสำรองมาใช้เป็นส่วนผสมสำคัญในผลิตภัณฑ์เสริมอาหาร (SCD) ซึ่งมีส่วนช่วยในการปรับสมดุลและกระตุ้นการทำงานของระบบภูมิคุ้มกันในร่างกาย ด้วยเหตุนี้จึงเป็นที่น่าสนใจที่จะทำการประเมินความปลอดภัยในการรับประทานผลิตภัณฑ์เสริมอาหารนี้ วัตถุประสงค์ในการศึกษาครั้งนี้ คือ เพื่อศึกษาข้อมูลความเป็นพิษในการรับประทานของ SCD โดยทดสอบในสัตว์ทดลอง ซึ่งจะทำการศึกษาความเป็นพิษเฉียบพลันทางการกิน และศึกษาผลของ SCD ต่อความเสียหายของโครโมโซมในเซลล์ไขกระดูกของหนูขาว จากผลการทดลองสรุปได้ว่า SCD ในขนาดที่ใช้ทดสอบ 5,000 มิลลิกรัม/กิโลกรัม น้ำหนักตัว มีความปลอดภัยในการรับประทาน และไม่ก่อให้เกิดความเสียหายต่อโครโมโซม

คำสำคัญ: *Scaphium scaphigerum*, ผลสำรอง, ความเป็นพิษเฉียบพลันทางปาก, การวิเคราะห์ โครโมโซม

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Preliminary Studies on Acute Oral Toxicity Effects of Dietary Supplement Derived from *Scaphium scaphiugerum* (G.DON) Fruit.

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ABSTRACT

Scaphium scaphigerum fruit (Malva nut; in Thai "Phungthalai" or "Samrong") has been used as food and a traditional medicine in many Asian countries. It has been used in folklore medicine to treat many diseases such as fever, diarrhea, kidney and large intestine disorders. In this research, the water extract of Malva nut is an active ingredient of dietary supplement which is proposed to be used as a health promoting agent and immunostimulant in human body. Therefore, it is interesting to evaluate the oral toxic effect of dietary supplement derived from *Scaphium scaphigerum* fruit (SCD). The objective of this study was to establish and investigate oral toxic effects of the SCD in animal testing. The acute oral toxicity study and chromosome analysis of the SCD were conducted by following the Test Guidelines No. 420 and No. 475 of the OECD guidelines for testing of chemical, respectively. The results of this study showed that an oral administration of the SCD at a dose of 5,000 mg/kg body weight to Wistar rats did not cause toxicity or death and did not cause chromosomal aberrations in the rat bone marrow cells.

Keywords: *Scaphium scaphigerum*; Malva nut; Acute oral toxicity; Chromosomal analysis

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Introduction

Recently, dietary supplement consumption is a new trend for the people who concern about their health. Dietary supplement has been widely believed to enhance the immune system in human body. Malva nut (in Thai "Phungthalai" or "Samrong") is the fruit of *Scaphium scaphigerum* (G.Don) which belongs to the plant family *Sterculiaceae*. This plant is distributed throughout tropical and sub-tropical regions of the world. It is mostly found in South East Asian countries, especially in Thailand, Cambodia, Malaysia and Indonesia. It is an indigenous plant normally found in the eastern part of Thailand and its fruit is locally used as a laxative. It grows to height of 4-5 meters and the characteristic of its fruit is a small brownish oval shape [3]. The fruits are known to contain a large amount of mucilaginous substance and have been used as a traditional medicine in South East Asia. The sweetened jelly from Malva nut is consumed as dessert but its principle use is for relief of sores and cough. In Thailand, the jelly made from Malva nut is consumed as food fortified which may be accepted by the consumer since there is well-established knowledge of its medicinal uses. However, there had been sparsely reports of its biological properties in terms of health promotion [4].

Although, Malva nut has been used for a long time but some toxicity reports or published data on its toxicity and safety has been limited. In this research, the acute oral toxicity study and the effect of SCD on chromosomal aberrations in rats were investigated.

Material and Methods

Animals

Healthy adult male and female Wistar rats, *Rattus norvegicus*, 5-7 weeks old were used in acute oral toxicity and chromosomal aberration tests. They were supplied by the National Laboratory Animal Center, Mahidol University, Salaya, Nakhon Pathom. They were kept in controlled conditions at the room temperature of 22-25°C and relative humidity of 60 ± 10 percent. They were housed in stainless steel cages, 5 animals with the same sex per cage and were fed with food (Pokphan Animal Feed Co., Ltd., Thailand) and water *ad libitum*.

Test material

The dietary supplement derived from *Scaphium scaphigerum* fruit (SCD) was prepared in the tablet form by Thailand Institute of Scientific and Technological Research (TISTR) which used the water extract of Malva nut and banana powder as major ingredients.

Methods

1. Acute Oral Toxicity Test

The acute oral toxicity test was conducted according to the Test Guideline (TG) No. 420: Acute Oral Toxicity – Fixed Dose Method (Limit test) of the OECD guidelines for testing of chemicals (2001) [1]. Twenty rats were assigned as control and treatment groups. Each group consisted of five male and five female rats. They were acclimatized to the laboratory environment for one week prior to experimentation. The rats were randomly chosen using the simple random sampling method and identified numbers by tail labeling. All rats were fasted for 16 hrs prior to the dosing with the test material while drinking water was available *ad libitum*.

On experimentation day, the SCD was freshly prepared in the form of suspension in distilled water and mixed well before oral

administration to the rats in the treatment group at a dose of 5,000 mg/kg body weight based on their body weights after fasting. The rats in the control group were dosed with distilled water at the equivolume as the treated rats. After dosing, all rats were further

Chromosomal Aberration Test

Based on the Test Guideline No. 475 "Genetic Toxicology: *In vivo* Mammalian Bone Marrow Cytogenetic Test – Chromosomal Analysis" of OECD Guidelines for Testing of Chemicals 1997 [2]. The SCD was freshly prepared in the form of suspension in distilled water and mixed well before oral administration for 14 consecutive days to the rats in the treatment group at a dose of 5,000 mg/kg body weight calculated based on their body weights after fasting. The rats of the negative control group were dosed with distilled water at the equivolume as the treated group. Cyclophosphamide (CP) was freshly prepared by dissolving in sterile distilled water at a dose of 50 mg/kg body weight. CP was injected intraperitoneally as a single agent to the rats as the positive control. The animals were sacrificed under CO_2 anesthesia 24 h after mutagen administration

Ninety minutes before sacrifice, all animals were injected intraperitoneally with colchicine at the dose of 3.5 mg/kg body weight. Their femurs were removed and the femoral marrow cells were flushed out with HBSS medium for chromosomal analysis [5]. Bone marrow cells preparations were made by hypotonic solution and fixative. All slides were coded and stained with 10% Giemsa [6].

In this study, metaphase cells with one or more chromosome aberrations were scored from 50 well-spread metaphases per animal at random. The types of aberration were scored and recorded strictly in accordance with the method of Ito & Ito. The severity of chromosome aberrations included the aberrations with break, exchange and multiple aberrations, but not the aberrations with gap [7].

Statistical analysis

The data was analyzed and expressed as mean ± SEM. Each treatment group was compared to the control group. The Student's t-Test was done to observe the significant differences between individual treated group and the control groups. The level of significance was established at $p < 0.05$

Results

After dosing, all treated rats received 5,000 mg/kg body weight of the SCD showed normal appearances and survived through the termination. Necropsy findings at the termination did not show any gross pathological lesions. The mean of body weight gain of the treated rats did not show significant difference from those of control rats. The results demonstrated that the oral LD_{50} of the SCD in Wistar rats was more than 5,000 mg/kg body weight. The mortality data and the means of body weight gain of the rats in the control and treatment groups were shown in Table 1 and 2, respectively.

The chromosomal analysis of each rat, the number of cells at metaphase stage was scored and the number of cells with structural chromosome aberrations was evaluated. Different types of structural chromosome aberrations were listed for all groups. Gaps were recorded separately but not included in the total aberration frequency. The mitotic index (M.I.), which is the fraction of cells in a given population that undergo mitosis at a given time, indicates cell proliferation activity. The number of M.I. was determined by counting 500 cells for each rat. Changes in the M.I. reflect the cytotoxic effect of the test material [5]. In this study, at least 2,000 cells per animal were counted for M.I. determination. The results of chromosomal analysis demonstrated that M.I. of SCD-

treated group (Group2) was decreased but did not show significant difference from negative control group (Group1). This means that the SCD at the given dose did not show cytotoxic effect on rat bone marrow cells.

Furthermore, there was no significant difference in chromosome aberration between SCD-treated and negative control groups. This means that the SCD at the given dose had no effect on chromosome damage to the rat bone marrow cells.

The results of chromosomal analysis in rat bone marrow cells at metaphase stage after receiving the SCD at a dose of 5,000 mg/kg body weight by oral administration for 14 consecutive days were summarized in Table 3.

Discussion

Malva nut has been used as a traditional medicine and food fortified [4]. Although, it has been used for a long time but some toxicity report or published data on its toxicity and safety in use have been limited. There was no previous study on toxic effect of Malva nut in animal model. In this study, the acute oral toxicity – fixed dose method (Limit test) of the OECD guidelines for testing of chemicals [1] is initially conducted with the aim to establish a dosage regimen in subchronic and other studies and may provide initial information on the mode of toxic action of a test substance. Dosing animals with test material at 5,000 mg/kg body weight is generally considered high enough and if no mortality or significant toxic signs could be observed in animals receiving this dose level, no higher doses are required for further study [8]. Our results showed that the dietary supplement derived from the water extract of

Malva nut (SCD) at a given dose did not cause toxicity or death of the tested rats. Therefore, the oral LD_{50} of the SCD in both sexes of Wistar rats was more than 5,000 mg/kg body weight.

Mammalian bone marrow cytogenetic test is a useful-short term technique for elucidating the mechanism as well as to identify the substances for their clastogenic activity [2]. In this study, *in vivo* metaphase chromosome analysis was conducted in order to evaluate the effect of the SCD on rat bone marrow cells. Our results showed that the SCD at a dose of 5,000 mg/kg body weight did not show cytotoxic effect on rat bone marrow cells and did not cause chromosome aberrations. However, long term consumption may increase cytotoxic risk of the user. Therefore, further study on the cytotoxic effect of the SCD in the animal model is recommended.

Conclustion

In conclusion, our results demonstrated that an oral administration of the SCD at a dose of 5,000 mg/kg body weight did not cause toxicity or death and did not show any gross pathological lesions on necropsy of the tested rats. The acute oral LD_{50} of the SCD in both sexes of Wistar rats was more than 5,000 mg/kg body weight. Moreover, the SCD at a given dose did not cause chromosome aberrations in the rat bone marrow cells. These toxicity studies might provide enough evidences to consider further studies in subchronic toxicity and others. Further studies are needed for safety evaluation of the SCD in human consumption.

Table 1 The mortality data of the rats in the control and treatment groups.

Treatment/Dose	*Mortality rate		
	Male	Female	Total
Control group Distilled water equivolume to the treatment group	0/5	0/5	0/10
Treatment group "SCD" 5,000 mg/kg body weight	0/5	0/5	0/10

Table 2 The means of body weight gain of the rats in the treatment and control groups

Sex	Treatment/Dose	*Mean of body weight gain (g)	
		Day 8	Day 15
Male	**Control group** Distilled water equivolume to the treatment group	18.40 ± 0.51	37.60 ± 0.87
	Treatment group "SCD" 5,000 mg/kg body weight	18.40 ± 0.24	43.20 ± 1.93
Female	**Control group** Distilled water equivolume to the treatment group	18.80 ± 0.58	45.20 ± 3.89
	Treatment group "SCD" 5,000 mg/kg body weight	19.20 ± 0.49	40.60 ± 0.92

Table 3 Chromosomal analysis of rat bone marrow cells receiving the SCD for 14 consecutive days

Group	Treatment	[a,b]%M.I.±SEM	[a,c]Type of chromosomal aberration			Total cells with aberration
			Break	Exchange	Multiple aberrations	
1	Distilled water	6.03 ± 0.31	0	0	0	0
2	SCD	5.54 ± 0.20	0	0	0	0
3	CP	*2.86 ± 0.09	*2.17 ± 0.72	*2.67 ± 1.04	*8.50 ± 1.59	*13.33 ± 1.66

[a] Data were expressed as Mean ± SEM, n= 6 and analyzed statistically by Student's t-Test.
[b] Mitotic Index (M.I.) was based on 2,000 cells per animal.
[c] Chromosome aberration was based on 50 cells per animal.
* p<0-05; Significant difference from Group 1 and 2.

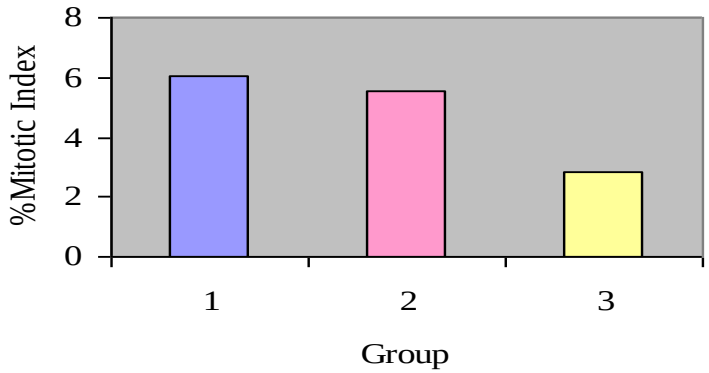


Figure 1 The %M.I. of rat bone marrow cells treated with the SCD for 14 consecutive days.

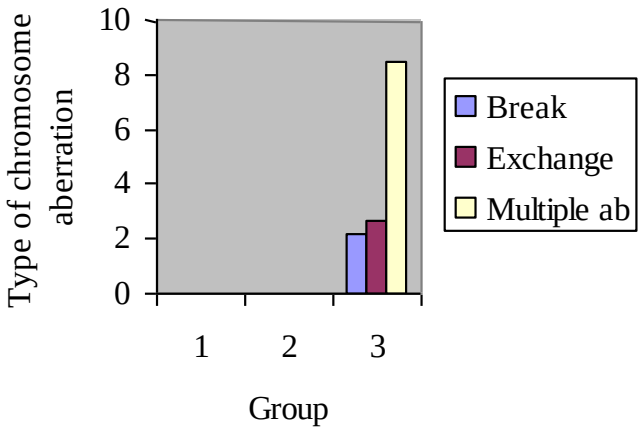


Figure 2 The type of chromosomal aberrations of rat bone marrow cells treated with the SCD for 14 consecutive days.

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