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Effect of extracted malva nut gum on reducing high glucose levels by Caco-2 cells

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ABSTRACT

Mucilage of malva nut seeds has been commercially produced as a functional beverage in many South East Asian countries. The purposes of this research were, first, to analyze chemical compositions of different layers of malva nut seeds. Second objective was to investigate the efficacy and dose response of malva nut gum (MNG) for the reduction of glucose uptake in Caco-2 cells. Third objective was to determine the effect of 30 min pre-incubation of Caco-2 cells with various MNG preparations on the uptake of glucose. Caco-2 cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM) containing (i) different concentrations of glucose (*i.e.* 5.5, 25 mM), (ii) different sources of glucose (*i.e.* sucrose, starch), (iii) different types of dietary fiber contained in different preparations of gum extracted from malva nut seeds and guar nuts. The cells were incubated in glucose containing DMEM under two different conditions: simultaneous and 30 min pre-incubation conditions. Scanning electron microscopy showed porous surface of extracted MNG compared to malva nut seed. In 25 mM glucose concentrate containing DMEM, the optimal level of dietary fiber to significantly reduce glucose uptake was 0.25% for malva nut seed, extracted MNG and mixed gum, but 0.5% for guar gum. FT-IR showed additional bands in the mixture of each gum and glucose. The contents of glucose uptake for the cells pre-incubated for 30 min, were 50–85% lower than that of the control. These results demonstrate that MNG is effective in lowering glucose uptake by Caco-2 cells in both low and high carbohydrate concentrations.

1. Introduction

Malva nut is the common name of seeds from plants in the family Malvaceae, genus *Scaphium* (Wilkie et al., 2006), and is native to South East Asian countries such as Thailand, Myanmar, Laos, Cambodia, and Malaysia. Phonsena and Wilkie (2008) reported that *Scaphium scaphigerum* has two distinct species, *Scaphium scaphigerum* (Wall. Ex G. Don) G. Planch. and *Scaphium affine* (Mast.) Pierre. *Scaphium affine* is found in the east of Thailand and is popularly used in Thailand overall. The outer layer of the malva nut seeds, when dispersed in water, forms a weak gel and provides viscosity in the solution. Malva nut mucilage has been used to control body weight in animals (Zhao et al., 2008) and also in human studies (Namwong, Panomai, & Thaingamsilp, 2013), and has been shown to reduce plasma glucose levels in type 2 diabetic patients (Pongthananikorn & Veranitinun, 2007). Because of the health benefits and traditionally popular uses of the malva nut seeds in Thailand, they are now exported to many countries, especially China. The Chinese use malva nut seeds for stomach problems, soothing the throat and curing dry coughs (Baird & Bounphasy, 2002). The price of malva nut seeds in Thailand has increased from USD$6/Kg in 2007 to USD$25/Kg in 2014. Moreover, the International Union for Conservation of Nature (IUCN) has launched a project to promote the sustainable harvesting of malva nuts in Laos which has increased local income (IUCN, 2011).

In our study, malva nut gum was extracted from the outer layer of malva nut seeds. The extracted malva nut gum was determined for its rheological properties and retardation of glucose diffusion in a dialysis bag. Fourier Transform Infrared Spectrometer showed that alkaline-extracted malva nut gum had carboxylic bonds with the reduction of galacturonic acid contents which contributed to higher storage moduli compared to the mucilage of malva nut seeds (Srichamroen & Chavasit, 2011a). In a dialysis system, alkaline-extracted malva nut gum were more effective in retardation of glucose diffusion than water-extracted malva nut gum (Srichamroen & Chavasit, 2011b). Malva nut gum replaced wheat flour in bread formulation improved shelf life and the quality of the physical properties (Srichamroen, 2014).

Caco-2 cells are heterogeneous human epithelial colorectal adenocarcinoma cells. Over a period of postconfluent culture conditions, they are differentiated to exhibit a brush border on the apical surface and tight junction, and also express adequate amounts of brush-border enzymes which would be a model to simulate human intestinal enterocytes (Balimane & Chong, 2005). Caco-2 cell has been developed for *in vitro* digestion and absorption models to estimate the

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bioavailability of the active components when included in meals (Garrett, Failla, & Sarama, 1999). Leforestier et al. (2009) identified the increased sucrose activity and proliferation of Caco-2 cells in the presence of galacto-oligosaccharide. Watanabe, Kamata, Sato, and Takahashi (2010) examined the effect of *Acanthopanax senticosus* Harms extract on glucose uptake in Caco-2 cells and reported that the extracts inhibited glucose uptake in these cells. Zhang et al. (2015) reported the benefit of ethanol extract of *Eucommia ulmoides* leaves in inhibiting sucrose digestion which reduced glucose transport into Caco-2 cells.

No published literature has been found on the proximate composition of malva nut seeds and their efficacy to reduce glucose uptake in Caco-2 cells. Hence, the objectives of this study were to analyze chemical compositions of different layers of malva nut seeds; to investigate the efficacy and dose response of the malva nut gum and to determine suitable incubation times of the malva nut gum for glucose uptake reduction. Guar gum as a positive control was chosen for comparison with the malva nut gum since it has been known to reduce blood glucose in humans (Anderson, Akanji, & Randles, 2001).

2. Materials and methods

2.1. Materials

The malva nut seeds used in the present study were obtained from the same source as reported previously (Srichamroen & Chavasit, 2011a, 2011b; Srichamroen, 2014). The seeds, which had been freshly harvested, were obtained from local markets in Chonburi province in the east of Thailand, during March and April. These malva nut seeds were identified as *Scaphium affine* (Mast.) Pierre by Dr. Pranee Nangngam of the Department of Biology, Naresuan University and the plant name was checked through the Internet (www.theplantlist.org.). The dried seeds with voucher specimen No. 003567 were deposited in the Phitsanulok Naresuan University Herbarium in the Department of Biology, Faculty of Science, Naresuan University, Phitsanulok Province, Thailand.

Media cultures, guar gum, glucose, sucrose, starch, and all chemicals used in this study were of analytical grade and purchased from Sigma Chemical Co. (St. Louise, MO, USA). Caco-2 cells were purchased from CLS Cell Lines Service GmbH (Eppelheim, Germany).

2.2. Chemical composition analyses

Malva nut seeds were separated in three parts: outer layer, middle layer, and inner seed. The chemical composition of the three parts was determined according to the AOAC method (AOAC, 1990). The total nitrogen content was determined by using Kjehdahl's method, and the standard conversion factor of 6.25 was used to calculate the crude protein content. The ash content was determined by heating samples in a muffle furnace at 550 °C for 8 h. Carbohydrate was calculated as:

$$100 - \text{moisture content} - \text{crude fat} - \text{crude protein} - \text{crude fiber} - \text{ash}$$

2.3. Extraction method

The outer layer of malva nut seeds was ground to create a 0.5 mM powder and kept in a freezer at −20 °C until used in the further experiments. Malva nut gum was extracted as described previously in (Srichamroen & Chavasit, 2011a) by dispersing the ground powder in distilled water at a ratio of 1:100 (w/v) and placing the container in a boiling water bath for 1.5 h. The resulting slurry was cooled to room temperature, and NaOH added to a final concentration of 0.05 M NaOH, and then treated with absolute ethanol at a ratio of 1:1 (v/v). The precipitate that formed was removed to recover the malva nut gum by filtration. After adjusting the gum to pH 7.4 with 1 N HCl, it was kept in airtight containers in a freezer at −20 °C until used.

2.4. Scanning electron microscopy (SEM)

Powders of outer layer of malva nut seed and extracted malva nut gum were determined for its surface morphology in the scanning electron microscope (LEO model 1455 VP, LEO Electron Microscopy Ltd., Cambridge, England). Sample was mounted on an aluminum sample holder (12 mm diameter) with double sided conductive carbon tape and a line of carbon paint was painted around the base of the sample to improve conductivity from the top of the seed to the taped surface. The sample was then sputter coated with gold (Sputter Coater Model SC 7620, Quorum Technologies Ltd., UK) and placed in the SEM chamber for examination and photographed using a 10 kV of electron beam-accelerating voltage.

2.5. Preparation of gum material

There were four samples of material used in the present study: malva nut seed powder, extracted malva nut gum, guar gum, and the combination of extracted malva nut gum and guar gum (called mixed gum), at a ratio of 1:1. All four gum preparations were prepared at 0.5%, 1% and 1.5% on a weight-*per*-weight basis (w/w) with deionized water, which were dispersed in boiling water for 5 min, with gentle stirring of the solutions to ensure homogeneity. A correction was later made for any loss of water due to evaporation. The gums were then diluted with twice concentrated Dulbecco's Modified Eagle's medium to a ratio of 1:1. The final concentration of each gum preparation was 0.25%, 0.5%, and 0.75% (w/w).

2.6. Preparation of incubation media

Cell culture media at twice concentration were prepared prior to mixing with gum solutions to a ratio of 1:1. When the mixture was completely dissolved, 2 ml of solution was transferred to a monolayer in a 24-well Transwell® (Costar, MA, USA). Media used in this study were Dulbecco's Modified Eagle's Medium (DMEM) that were variously formulated with (i) 5.5 mM D-glucose (ii) 25 mM D-glucose (iii) 25 mM sucrose or (iv) 25 mM starch. D-glucose at 5.5 mM in cell lines was chosen because it approximates normal blood glucose levels *in vivo,* while 25 mM D-glucose was analogous to blood glucose levels in a diabetic condition within the cell culture system (Sigma-Aldrich, 2014). Other ingredients in the final concentration of media in the test solution was 10% Foetal Bovine Serum (FBS), 1% non-essential amino acid (NEAA), 2 mM L-glutamine, 100 U/ml penicillin G and 100 µg/ml streptomycin.

2.7. Cytotoxic study

To investigate the toxic effect of the four gums on Caco-2 cells, cytotoxicity against cell metabolism was determined by monitoring the reduction of 3-(4,5-dimethylthiazol-2-yl) − 2,5-diphenyltetrazolium bromide (MTT) to formazan (Ekmekcioglu, Feyertag, & Marktl, 1998). Cells were seeded in a 96-well plate at 10,000 cells/well and treated with 0.75% (w/w) concentration of each gum supplemented in the medium containing 5.5 mM glucose, then incubated at 37 °C for 24 h. MTT (5 mg/ml) solution at 100 µl was added to each well and incubated in the dark at 37 °C for 30, 60, 90, 120 min. After each interval time, medium was removed and 150 µl dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. Absorbance was measured at 595 nm in a microplate spectrophotometer (iMark, Bio-Rad Laboratories, Inc., California, USA). The percentage of survived Caco-2 cell was calculated by the following formula:

$$\text{Percentage of survived Caco} - 2 \text{ cell} = \frac{\text{survived Caco} - 2 \text{ cell incubated with gum} \times 100}{\text{survived Caco} - 2 \text{ cell incubated without gum}}$$