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ScienceDirect
Nutrition Research 27 (2007) 492–497
Nutrition Research
www.elsevier.com/locate/nutres

Systemic absorption of antioxidants from mulberry (*Morus alba* L) leaf extracts using an in situ rat intestinal preparation

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Received 16 June 2006; revised 2 June 2007; accepted 5 June 2007

Abstract

Consumption of antioxidant-rich foods is thought to be capable of increasing our body antioxidant levels, but little is known about their systemic bioavailability. The aim of this study was to demonstrate the systemic absorption of antioxidants from the aqueous and ethanol extracts of mulberry leaves by using an in situ rat intestinal preparation. After an overnight fast, the carotid artery of an anesthetized rat was cannulated, followed by isolation of a short length (8-13 cm) of the duodenum, jejunum, or ileum. Blood samples were collected from the artery before and at various times after administration of the extract or vehicle into the isolated intestinal segment. The collected plasma samples were assayed for their total antioxidant activity (TAA). There was a transient increase in the plasma TAA for animals given the aqueous extract at the ileum, but not when given at the duodenum or jejunum. This suggests that the water-extracted antioxidants need to be hydrolyzed, probably by bacteria, before absorption. For animals given the ethanol extract, significant increase in the plasma TAA occurred at different times for duodenal, jejunal, and ileal segments. This indicates that the ethanol-extracted antioxidants are more readily absorbed. The absorption of these antioxidants probably involves several different mechanisms, and may be influenced by the vehicle of administration. In conclusion, the antioxidants in mulberry leaves are absorbed to different extents throughout the small intestine of rats. Furthermore, this in situ preparation may be used to screen for systemic bioavailability of antioxidants in herbal samples.

Keywords: Antioxidants; Bioavailability; Intestinal absorption; Mulberry (*Morus alba* L); Rat; Total antioxidant activity

1. Introduction

Mulberry (*Morus alba* L) plant was originally cultivated in China where the leaves were used as food for silkworms [1]. There is an increasing interest on mulberry leaves because the leaves were found to have antihyperglycemic effect [2-4], skin whitening property [5], protection against cerebral ischemia [6], and antiatherogenic effect [7]. The antioxidative effects of mulberry leaves have been mainly attributed to quercetin rutinoside (rutin), quercetin 3-glucoside (isoquercitrin), and quercetin 3-(6-malonylglucoside) [8].

It is widely believed that consumption of antioxidant-rich foods will help to improve health status. Therefore, it is important to show that dietary antioxidants are bioavailable. The ability to absorb a significant amount of dietary antioxidants is a prerequisite for their bioavailability. Absorption and bioavailability studies on selected dietary antioxidants have been carried out in human and in in vitro preparations, such as cell culture and isolated organs, whereas studies involving in vivo or in situ animal models are relatively few. A simple and inexpensive in vivo or in situ

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doi:10.1016/j.nutres.2007.06.004

animal model would be useful to allow for a relatively quick assessment of a large number of consumable extracts with purported antioxidant properties.

Although mulberry leaves have been extensively studied for their beneficial effects, and some of the active compounds have been identified, absorption and bioavailability studies on these compounds in the plant extracts have not been reported. Therefore, the aim of the present study was to demonstrate the systemic absorption and bioavailability of antioxidants present in the mulberry leaf extracts, by using a relatively simple in situ animal model developed in this laboratory. This study is important because we showed that this in situ animal model can be used in functional food studies, where information on the absorption and bioavailability of dietary antioxidants is still limited. The aqueous and ethanol extracts used in this study have previously been shown to have high antioxidant capacity based on Trolox equivalent antioxidant capacity (TEAC) assays done in our laboratory.

2. Methods and materials

2.1. Chemicals and solvents

The 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, heparin, and urethane were purchased from Sigma-Aldrich (St Louis, Miss). Absolute ethanol (AR grade) was purchased from R&M Chemicals (Essex, England, UK). All other chemicals were purchased from Merck (Darmstadt, Germany). Ultrapure water produced from Elgastat Maxima (Elga Ltd, Buckinghamshire, England, UK) was used to prepare all solutions and dilutions in this study.

2.2. Plant materials

Mulberry (*M alba* L) leaves were harvested from Field II, Universiti Putra Malaysia, Serdang, Malaysia, in August 2005. The leaves were first separated from their stems, air-dried, followed by drying in the oven at 40°C, and finally ground into fine powder using a domestic mixer (Mathur Micro Motors & Appliances Pte Ltd, India).

2.3. Extraction of the mulberry leaves

Aqueous and ethanol extracts of the mulberry leaves were prepared at 15 g% by soaking the leaf powder (1.5 g) in water (10 mL) or absolute ethanol (10 mL) for 12 hours at 4°C and 25°C, respectively. The mixtures were centrifuged at 5000 rpm for 10 minutes to obtain the supernatants, which were then maintained at −20°C until use. These procedures were modified from that used by Huang et al [9]. Because ethanol is noxious to living cells, the ethanol extract was diluted 10 times with water to obtain a 10% ethanol (1:9 ethanol/water, vol/vol) solution, which is often used as a vehicle to dissolve lipophilic compounds for such in situ absorption studies in animals [10].

2.4. Animals

Adult male and female Sprague-Dawley rats (200-240 g) were obtained from the Laboratory Animal Centre, University of Malaya, Kuala Lumpur, and were housed in polypropylene cages with free access to water and standard rat chow, composed mainly of: protein, 21%; fiber, 5%; fat, 3%; moisture, 13%; ash, 8%; nitrogen-free extract, 49% (Gold Coin Feedmills, Kuala Lumpur, Malaysia). Before the experiment, the rats were fasted 12 to18 hours by placing them individually in metabolism cages. All surgical procedures done on the animals had been approved by the Animal Care and Use Committee, Faculty of Medicine, University of Malaya.

2.5. Experimental design

Rats (n = 60) were randomly divided into 2 control groups (water and 10% ethanol) and 2 test groups (aqueous and ethanol extracts), each of which was further subdivided into 3 groups to study absorption from 3 different parts of the small intestine (duodenum, jejunum, and ileum; n = 5 per intestinal segment). The intestinal segments were isolated by using a method modified from the process described by Sim and Back [10]. A standard amount of the extract or vehicle was administered into each isolated intestinal segment and the total antioxidant activity (TAA) in the plasma was monitored for 3 hours after administration of the extract or vehicle. An increase in the plasma antioxidant level in a test group, when compared with the corresponding control group, is indicative of systemic absorption of antioxidants.

2.6. Surgical procedures and blood sampling

After overnight fasting, the rat was anesthetized with urethane (intraperitoneal, 1.75 g/kg body weight for male rats or 1.3 g/kg body weight for female rats), and the carotid artery cannulated with polyethylene tubing (PE 50). Next, a midline incision was made to expose the abdominal content, and an 8- to 13-cm segment of the small intestine was isolated at the duodenum (approximately 8 cm, measured from pyloro-duodenal junction to the ligament of Trietz), the jejunum (approximately 13 cm, taken distally from the ligament of Trietz), or the ileum (approximately 13 cm, measured at 3.0 cm from the cecum). The isolated intestinal segment was ligated at the proximal end and cannulated at the distal end using an L-shaped glass cannula before flushing gently with 4 to 8 mL of 0.9% sodium chloride (prewarmed to 37°C). The distal end was then ligated. The rat was left to stabilize for 30 minutes before the instillation of the mulberry leaf extract (0.5 mL, aqueous or ethanol) at the proximal end of the isolated intestinal segment (n = 5 for each isolated segment in each treatment or control group of animals). The control animals were given the same volume of the vehicle (water or 10% ethanol) (n = 5).

The arterial cannula was filled with approximately 0.2 mL heparinized saline (50 U/mL of heparin) to prevent clot formation at the cannula, and was drained off before each