ABSORPTION AND IN VIVO EFFECTS OF ANTIOXIDANTS FROM MULBERRY (MORUS ALBA L.) LEAF EXTRACTS IN RATS

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This study was carried out to show the systemic absorption, possible mechanism of absorption, and in vivo effects of the antioxidants in the mulberry leaf extracts. We have used five animal models to achieve these objectives.

In this study, a convenient and simple in situ rat model has been optimised, that is useful for quick assessment of antioxidant absorption (Nutr. Res. 2007; see List of Publications). By using several plant extracts (mushroom, mulberry, rambutan, dragon fruit, mangosteen and cempedak), differential total antioxidant activity (TAA) absorption profiles were demonstrated with the different plants.

The in situ rat intestinal model showed that aqueous extract of mulberry leaves (15 g%) was absorbed from the ileum, but not from the duodenum and jejunum. Absorption of the ethanolic antioxidants in the mulberry leaves (1.5 g%) was detected at the duodenum for most of the 3 h monitoring period, and to a lesser extent at the jejunum and ileum. The absorption seemed to occur by passive diffusion.

Using an in vitro everted ileum preparation, absorption of mulberry leaf aqueous extract was carried out in different buffers. There was no absorption with extract in the glucose-free incubation medium. Absorption of antioxidants in the aqueous extract was seen when glucose and sodium salt were present, and in both the sodium-free and fructose-containing incubation buffers. These findings suggest that absorption at the ileum is mediated by monosaccharide-associated transporters. However, measurement of the absorption of antioxidants in the ethanol extract was affected by a spontaneously release of intestinal endogenous antioxidants into the incubation medium. The rates of absorption from both the in vitro everted duodenum and jejunum were comparable. The absorption rate was highest at the ileum. The flux of natural antioxidants has also affected absorption measurement in the in vitro non-everted gut sac model.
In the *in vitro* non-everted gut sac model, mulberry leaf aqueous extract was not absorbed from any part of the intestine. Absorption of the added antioxidants has to exceed that of the spontaneous antioxidant level for detection to be possible. With ethanolic mulberry extract, only absorption from the ileum was detected.

In the study on absorption of single compounds using the *in situ* model, rutin (glycosylated quercetin, 13.5 µg/ml), prepared at a similar concentration to that found in the mulberry leaf ethanol extract (1.5 g%), was only absorbed from the duodenum. Isoquercitrin (10.5 µg/ml), another major compound in the ethanol extract (1.5 g%), was not absorbed from any part of the intestine. Because absorption of the ethanolic mulberry extract occurred throughout the small intestine, these data suggest that absorption of single compounds is not any greater than absorption of a complex mixture of compounds. Consumption of a whole extract or whole food rich in antioxidants may give more benefits than a purified compound.

When a higher dose of rutin was instilled into the duodenal segment (2 mg) in the *in situ* intestinal model, a microbial metabolite of rutin, 3-hydroxyphenylacetic acid, was detected in the plasma. Quercetin was not found. The presence of this microbial metabolite in the plasma has not been reported previously. This suggests that microbial metabolite is a main contributor to the increase of plasma TAA after consumption of mulberry leaf extract.

In the oral feeding study, the mulberry leaf ethanol extract and pure rutin were separately shown to be absorbed from the intestine. Although there was no increase of plasma TAA after 14 days of treatment as compared to the control animals, the urine ascorbic acid equivalent of the treated animal was higher than the control. Another microbial metabolite, phenylacetic acid, was detected in the urine of days 7, 13 and 14.

In order to demonstrate tissue bioavailability, rats were first stressed by immobilisation to induce inflammation and to generate reactive oxygen species in the
target organs. Tissue bioavailability of ethanolic antioxidants in the mulberry leaves (containing about 135 µg rutin) and rutin (2 mg) were reflected in their ability to protect the stressed tissues from potential damage. The untreated stressed rats showed hypertrofies in the adrenal glands and kidneys, increased levels of nitrite and thiobarbituric acid reactive substances (TBARS) in the plasma and target tissue homogenates. Mulberry leaf ethanol extract and rutin attenuated these increases. The stress defence mechanism by mulberry extract was most dramatically seen in the adrenal glands. An increase of TAA level, which indicates absorption, was found in the adrenal homogenate. Adrenal gland is the target tissue of antioxidants in the mulberry leaf ethanol extract.

This study shows that antioxidants in the mulberry leaf ethanol extract were adequately absorbed from the intestine. Some of the dietary antioxidants can be metabolised into microbial metabolites and absorbed into the blood circulation. These metabolites retained high antioxidant activity and can reduce lesions in the target organs caused by stress.

In the course of experimentation with the *in vitro* intestinal preparations, a unique and serendipitous observation was made. There was a consistent and spontaneous release of antioxidants from the intestinal preparations into the incubation buffer. The spontaneous release of endogenous antioxidants may be associated with P-glycoprotein and multidrug resistance protein 2 (MRP2). The natural intestinal release of endogenous antioxidants could represent a normal physiological response to external stresses and pro-oxidants in consumed foods. In light of this, dietary antioxidants, even if they are not efficiently absorbed, can also contribute to the intra-luminal antioxidant protection.
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DECLARATION

I hereby declare that the work presented in this thesis is original, except for citations which have been duly acknowledged. This thesis has not been, and will not be submitted for a degree at other university.

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<td>Ascorbic acid equivalent antioxidant capacity</td>
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<td>GI</td>
<td>Gastrointestinal</td>
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<td>GLUT</td>
<td>Glucose-dependent transporter</td>
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