Abstract

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Stevioside is a major sweet constituent from *Stevia rebaudiana*. It is one of the most important natural sweeteners which has been used for many years to sweeten a variety of foods including sea foods, pickled vegetables, dessert items, soft drinks and confectionary. However, safety evaluation for human consumption is questionable when steviol, aglycone of stevioside is reported to be mutagenic towards *S. typhimurium* (TM 677) in the presence of S-9 fraction from liver of the rat. Although, no mutagenicity of stevioside and crude extracts was detected against *S. typhimurium*, *E. coli* and *B. subtilis* and no significant acute, subacute and chronic toxicity in mice and rats was observed. Stevioside was found to be degraded to steviol by rat intestinal microflora *in vitro* and steviol was almost totally absorbed from large intestine. Therefore, the most effective approach to investigate the toxicological evaluation of stevioside and steviol is the use of most susceptible animal species such as hamster.

In vitro digestibility of stevioside by various digestive enzymes was investigated. Stevioside was incubated with salivary α-amylase and pancreatic α-amylase, saliva, pepsin, gastric secretion, pancreatin, intestinal brush border membrane enzymes of mice, rats and hamsters as well as intestinal microflora of mice, rats, hamsters and human. None of these enzymes digests stevioside except the microflora of the rat and hamster cecal contents which hydrolyzes it to steviol, and the microflora of mouse cecal content and human fecal content which hydrolyzes it to both steviol and steviol-16,17-epoxide. Steviol-16,17-epoxide was then completely converted back into steviol. These results suggest that steviol might be the only metabolite produced by the intestinal microflora from various animal species and human.

The pharmacokinetic study of stevioside was studied in hamsters. The disposition of stevioside and its metabolites in blood at various time intervals (0-120 hours) after fed with 1000 mg/kg BW was determined. The plasma level of stevioside was high after fed with stevioside in between 1-3 hours. During this period, the level of plasma steviol, hydroxysteviol and isosteviol was also detected. At 90 hours after stevioside treatment, the level of stevioside metabolites in plasma was detected in a very small amount. These results suggest that stevioside can be absorbed to the circulation and distributes throughout the body and then it might be metabolized by various organs in the body. The tissue distribution of stevioside and its metabolites was also determined at 3 hours

after stevioside treatment (peak blood level). The total amount of stevioside and its metabolites in liver was highest. Stevioside and its metabolites that found in various tissues at peak blood level was 9.71% of dose. In addition, the excretory rate in urine and feces of stevioside and its metabolites will be determined along with the metabolic patterns. At 24 hours after stevioside treatment, stevioside and its metabolites were excreted in a large amount both in urine and feces. Stevioside can be excreted in the urine. The total amount of excreted stevioside and its metabolite was excreted in feces higher than in urine. Stevioside and its metabolites could not detected in feces and urine after fed with stevioside 5 days and 7 days respectively. The highest in vitro metabolizer of stevioside was liver when compared with kidney and small intestine. The principal in vitro metabolite produced by each these tissues was 15α-hydroxysteviol. Therefore, the results of these studies suggested that the ingested stevioside was mostly metabolized and was eliminated from the body at a high rate.