Thesis Title: Stevioside and Steviol Nephrotoxicity and their Relationship to Urinary Enzymes Excretion

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Date of Graduation: 27 October B.E. 2535 (1992)

ABSTRACT

Stevioside, a diterpene glycoside, is the principle sweet constituent in Stevia rebaudiana Bertoni, leaves. Steviol, an acidic aglycone, is a product of enzymatic hydrolysis of stevioside. They were subjected to investigate for their effects on kidney. This study was performed to evaluate the relative indices of the urinary enzymes levels by comparing with the changes in blood urea nitrogen (BUN) and plasma creatinine levels simultaneous with the light and electron micrographs of the kidney in vivo. BUN levels were increased at various time intervals (0 to 96 hours) after subcutaneous injection (S.C.) with stevioside (1.5 g/kg BW) in rats. The maximum change in BUN was observed approximately 180.28% ($p<0.001$) at 9 hours after stevioside injection. BUN levels were then decreased after this time. Effect of stevioside

(1.5 g/kg BW,S.C.)on the other plasma biochemical parameters and urinary enzymes levels simultaneous with the light and electron micrographs of the kidney were then determined. At 9 hours after S.C. injection of stevioside ; plasma creatinine, plasma glutamic pyruvic transaminase (PGPT) and plasma glutamic oxaloacetic transaminase (PGOT) were significantly increased by 131.71 % ($p<0.001$), 58.69 % ($p<0.01$) and 161.69 % ($p<0.01$), respectively. It is likely that stevioside is capable of inducing nephrotoxicity with evidences of the excretion of enzymes in urine. Stevioside caused a significantly increased in glucosuria, alkaline phosphatase (AP) and ɣ-glutamyl transpeptidase (ɣ-GTP) but no significant change in proteinuria, N-acetyl-β-D-glucosaminidase (NAG) and glutathione-S-transferase (GSH-S-TF). The histopathological alterations of the kidney caused by stevioside (1.5 g/kg BW, S.C.) both in light microscope and electron microscope were the renal proximal tubular damage at 9 hours after administration. Most of the "degenerative" stage of renal proximal convoluted tubular cells were found whereas the distal convoluted tubular cells showed normal appearance.

The effects of stevioside and steviol on p-aminohippurate (PAH) accumulation were studied by using slices prepared from rat kidney cortex. Administration of stevioside (1.5 g/kg BW, S.C.) to rats significantly decreased the ability of the slices to accumulate PAH at 9 hours after administration. The degree of inhibition was 63.42% ($p<0.001$). The addition of either stevioside

(6.25-100.0 μM) or steviol (1.5625-100.0 μM) in medium could inhibited PAH accumulation in the slices after 30 minutes incubation. This inhibitory effect was a dose-dependent manner. The inhibition on PAH accumulation of stevioside and steviol was plateau from 25.0 μM and 50.0 μM, respectively. Steviol is more potent than stevioside to inhibit PAH uptake in the slice.

There was no effect of stevioside on lipid peroxidation in the plasma and renal cortical slices at various time intervals after stevioside (1.5 g/kg BW, S.C.) administration in rats. The addition of either stevioside or steviol (both in 100.0 μM) to medium had also no effect on lipid peroxidation in rats renal cortical slices incubated at various time intervals.

Therefore, the results suggested that stevioside induced nephrotoxicity at the proximal convoluted tubules rather than glomerulus and other tubules as indicated by the changes in plasma biochemical parameters, urinary enzymes, PAH accumulation and histopathologic changes of the kidney. The mechanism by which stevioside induced nephrotoxicity did not via the lipid peroxidation.