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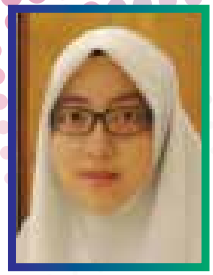

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Title : THE ENHANCEMENT OF INSULIN SENSITIVITY BY STEVIOSIDE FROM *Stevia rebaudiana* Bertoni IN *IN VITRO* AND *IN SILICO* MODELS

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The adoption of unhealthy, sedentary lifestyles has triggered the prevalence of metabolic diseases like diabetes and obesity. A condition referred to as insulin resistance has been found to be the precursor to these diseases. It commonly manifests itself in most if not all Type 2 diabetic cases. A cure is yet to be found and side effects from current drugs create complications among patients. Thus, alternative therapies from natural, plant-based products like stevioside are becoming a more preferred option. Stevioside that is extracted from *Stevia rebaudiana* Bertoni has impeccable sweetening potential, which provides an interesting aspect to its proposed antidiabetic potentials. Hence, in-depth investigations were conducted to analyse how stevioside can manifest its effects towards insulin sensitivity in *in-vitro* and *in-silico* models. Like many herbal products, scientific data on stevioside's efficacies has been scarce. Its safety of consumption was hence tested through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay on 3T3-L1 adipocytes as an *in-vitro* model. No IC_{50} was observed as cell viability was only slightly reduced, signifying its non-cytotoxicity towards adipocytes. Preceding the assay, the cells were tested using Oil Red O to confirm differentiation which was also successfully achieved. Stevioside was observed to increase glucose uptake in adipocytes better than a drug both in normal an insulin resistant states based on the glucose uptake assay that was conducted. Through Western blotting, expression of the phosphorylated tyrosine (pY20) protein on the insulin receptor (IRβ) was also observed to be enhanced by stevioside. This suggests that stevioside has a high probability of interacting with the insulin receptor in improving insulin sensitivity and increasing glucose uptake. Stevioside's actions are therefore, upstream rather than downstream of the insulin signalling pathway and were confirmed through computer simulations. Prior to that, a protein model was constructed using the MODELLER software. Multiple sequence alignment of the human and mouse insulin receptor sequences was first conducted through ClustalW. A human insulin receptor 3D structure with PDBID; 3LOH was selected as the protein template to model the *Mus musculus* insulin receptor. Subsequent docking of the stevioside ligand was conducted via AutoDock Vina and had managed to reveal possible binding sites. Interestingly, stevioside was observed to share the same binding region to that of insulin on the insulin receptor. Henceforth, insulin binding was analysed through radioimmunoassay (RIA) with radioactively tagged 125I-insulin; quantified using a γ-counter. Stevioside was seen to reduce insulin binding but not as severe as S961 treatments; that are positive controls to insulin binding inhibition. In conclusion, stevioside enhances insulin sensitivity in adipocytes by increasing glucose uptake and enhancing expressions of pY20 on IRβ of the insulin signalling pathway. Computer simulations of insulin receptor-stevioside interactions have also revealed docking of stevioside onto a site shared by insulin binding on the receptor. This was confirmed through stevioside's reduction in total insulin binding analysed through RIA. Therefore, stevioside may have a role in manifesting its effects through the insulin receptor towards improving insulin sensitivity; upstream of the insulin signalling pathway by possibly binding to the insulin receptor.