Malays. Appl. Biol. (2015) 44(2): 143–145

GLYCOGEN SYNTHASE KINASE-3β (GSK3β) INHIBITION BY KAEMPFEROL MEDIATES THE ANTI-HYPERGLYCAEMIC EFFECT OF *Gynura procumbens*

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Gynura procumbens, a tropical herbaceous shrub locally known as "Sambung Nyawa", is ubiquitously distributed in Southeast Asia and traditionally used as remedy for fever, inflammation, hypertension and diabetes (Perry 1980). The scientific basis for some of these claims including anti-inflammatory (Iskander *et al*., 2002), anti-hypertensive (Hoe *et al*., 2011) and anti-hyperglycaemic (Algariri *et al*., 2013) properties have been evaluated. Amongst these, the anti-hyperglycaemic activity of *G. procumbens* has been extensively investigated (Hassan *et al*., 2010; Chong *et al*., 2012; Algariri *et al*., 2013). One of the phytoconstituents of *G. procumbens*, kaempferol has been reported to exhibit glucose-lowering effects in soleus muscle mediated through PI3K/Akt pathway (Cazarolli *et al*., 2009). In addition, kaempferol has been shown to protect pancreatic beta-cells from hyperglycaemia-induced apoptosis and dysfunction (Zhang & Liu 2011) as well as to exhibit anti-oxidant effects (Choi 2011) through activation of Akt. A critical downstream component of PI3K/Akt pathway in insulin signaling and control of glycogen metabolism is glycogen synthase kinase-3β (GSK3β).

GSK3, a serine/threonine kinase initially identified by Embi *et al*. (1980) is constitutively active under basal conditions and is phosphorylated and inhibited by Akt during insulin stimulation (Cross *et al*., 1995). The kinase is now recognised to be involved in the regulation of a multitude of cellular functions such as protein synthesis, regulation of transcription factors, proliferation, apoptosis and inflammatory response to infections. Dysregulation of GSK3 activity is implicated in a diverse array of human pathologies including Type 2 diabetes, Alzheimer's disease and cancer (Wang *et al*., 2014). Our previous findings indicate that the anti-hyperglycaemic effects of *G. procumbens* fractions in streptozotocin-induced diabetic rats involved increased phosphorylation of liver GSK3β (Ser9) (Chong *et al*., 2012). Here, we postulate that the anti-hyperglycaemic effect of *G. procumbens* involving inhibition of GSK3β is mediated by kaempferol. Therefore the present study aims to evaluate whether the anti-hyperglycaemic effects of *G. procumbens* aqueous extract and kaempferol each involve phosphorylation of GSK3β.

Our results revealed that *G. procumbens*, kaempferol, and metformin each improved glucose consumption of hyperglycaemic HepG2 cells in a concentration dependent-manner (Figure 1). At a concentration of 0.1 μg/mL, *G. procumbens*, kaempferol and metformin each increased glucose consumption by 51%, 81.5% and 86.3% respectively. Increased phosphorylations of GSK3β (Ser9) were detected in both *G. procumbens*- and kaempferol-treated HepG2 cells by 22.56- and 2.50-fold respectively as compared to non-treated control. Phosphorylated GSK3β was not detected in cells cultured in low or high glucose media in the absence of test extract or compound (Figure 2). This strongly suggests that the anti-hyperglycaemic activity of *G. procumbens* and kaempferol each is mediated through phosphorylation and inhibition of GSK3β.

Involvement of GSK3β in insulin signaling pathway is well-documented. In normal glucose metabolism, insulin receptor activation leads to phosphorylation and activation of Akt and consequently inhibition of GSK3β (Gao *et al*., 2011). In type 2 diabetic mice, phosphorylation of GSK3β (Ser9) was significantly lower than normal mice indicating over-activity of GSK3 under diabetic conditions (Henriksen & Dokken 2006). Pharmacological inhibitors of GSK3 have been reported to improve glucose uptake in hyperglycaemic cell cultures and in insulin-resistant rat skeletal muscle (Meijer *et al*., 2004; Wagman *et al*., 2004). Our current findings showed that treatment of hyperglycaemic HepG2 cells with

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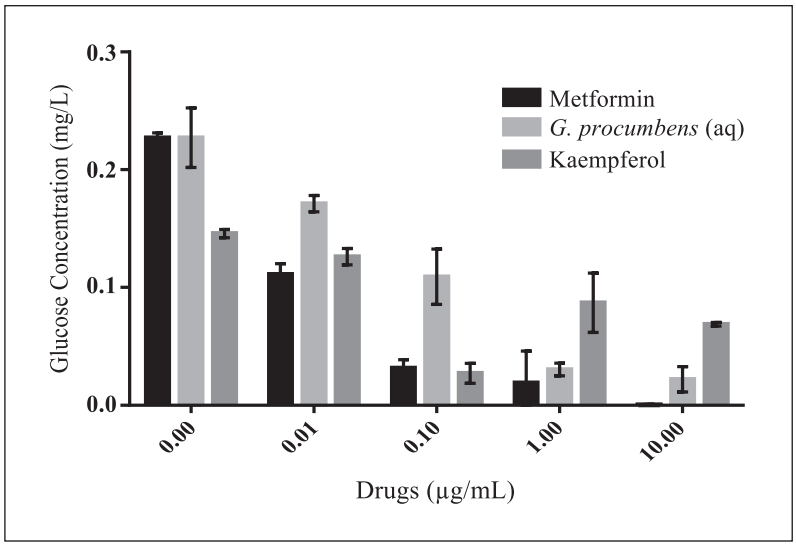

Fig. 1. Dose-effect of glucose consumption in HepG2 cells treated with or without *G. procumbens* aqueous extract, kaempferol or metformin at different concentrations for 24 hours. The glucose concentration remaining in suspension was measured and normalised to cell number. Data presented as mean ± S.D.

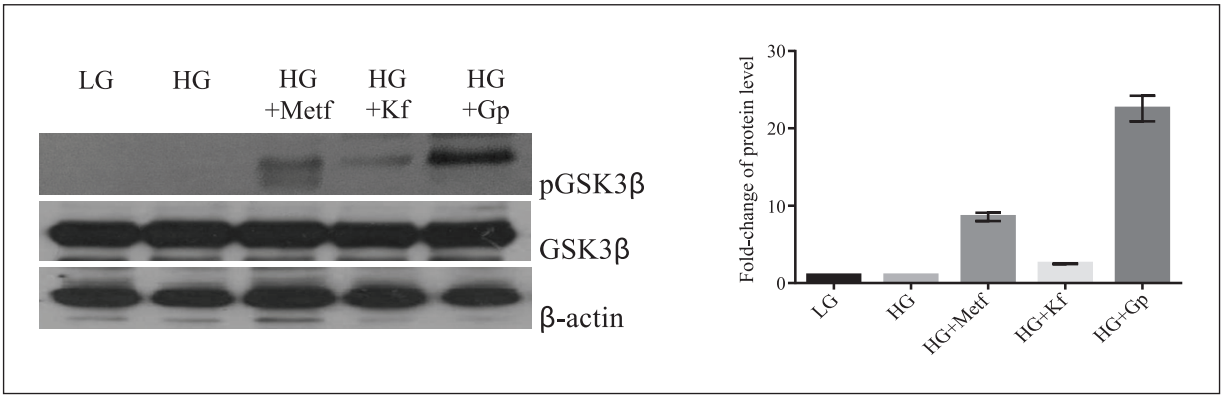

Fig. 2. Fold-change of GSK3β phosphorylation levels in low glucose (LG) or high glucose (HG) HepG2 cells with incubation of metformin (Metf), *G. procumbens* (Gp) or kaempferol (Kf). Each sample was extracted 24 hours after respective treatment. Levels of phospho-GSK3β (Ser9) were normalised to total levels of GSK3β. Data presented as mean ± S.D.

aqueous extract of *G. procumbens* resulted in increased glucose uptake as well as increased phosphorylation of GSK3β. Similarly, treatment with kaempferol also resulted in improved glucose uptake and increased levels of pGSK3β.

It is noteworthy that phytochemical analysis of *G. procumbens* extracts identified rutin (quercetin-3-O-rutinoside), quercetin (kaempferol-3-o-rutinoside), astragalin (kaempferol-3-glucoside) and kaempferol as components (Hassan *et al*., 2010). These phytoconstituents have been implicated in the blood glucose-lowering activity of the plant and reported to mimic or improve insulin action at the cellular level (Hassan *et al*., 2010). In addition, kaempferol 3-neohesperidoside has been reported to show insulin-like properties in glucose lowering in rat soleus muscle via a mechanism involving inhibition of GSK3β through PI3K-GSK3 pathway (Cazarolli *et al*., 2009). To our knowledge, the anti-hyperglycaemic effect of kaempferol in HepG2 cells observed in the present study has not been reported previously. Our findings suggest that the biochemical basis of the anti-hyperglycaemic effect observed in *G. procumbens* aqueous extract is in part attributed to inhibition of GSK3β elicited by kaempferol. In conclusion, the present study provides scientific evidence for the documented traditional use of *G. procumbens* to treat diabetes. Our findings offer a possible explanation for the many insulinomimetic effects of *G. procumbens* reported by previous investigators. As such, GSK3, as an important component in insulin signaling, is a plausible drug target for development of therapeutics against diabetes.

ACKNOWLEDGEMENTS

This work was supported by research grants from the Institute of Pharmaceuticals and Nutraceuticals Malaysia, Ministry of Science, Technology and Innovation (09-05-IFN-BPH-001).

REFERENCES

Algariri, K., Kuong, Y.M., Atangwho, I.J., Asmawi, M.Z., Sadikun, A., Murugaiyah, V. & Ismail, N. 2013. Hypoglycemic and anti-hyperglycemic study of *Gynura procumbens* leaf extracts. *Asian Pacific Journal of Tropical Biomedicine* **3(5):** 974-981.

Cazarolli, L.H., Folador, P., Pizzolatti, M.G. & Mena Barreto Silva, F.R. 2009. Signaling pathways of kaempferol-3-neohesperidoside in glycogen synthesis in rat soleus muscle. *Biochimie* **91(7):** 843-849.

Choi, E.M. 2011. Kaempferol protects MC3T3-E1 cells through antioxidant effect and regulation of mitochondrial function. *Food and Chemical Toxicology* **49(8):** 1800-1805.

Chong, C.J., Lee, H.W., Halimah, A.S., Jalifah, L., Jualang, A.G., Lee, P.C., Embi, N. & Hasidah, M.S. 2012. Hypoglycemic effects of *Gynura procumbens* fraction on streptozotocin-induced diabetic rats involved phosphorylation of GSK3β (Ser-9) in liver. *Sains Malaysiana* **41(8):** 969-975.

Cross, D.A., Alessi, D.R., Cohen, P., Andjelkovich, M. & Hemmings, B.A. 1995. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* **378(6559):** 785-789.

Embi, N., Rylatt, D.B. & Cohen, P. 1980. Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *European Journal of Biochemistry* **107(2):** 519-527.

Gao, C., Hölscher, C., Liu, Y. & Li, L. 2011. GSK3: a key target for the development of novel treatments for type 2 diabetes mellitus and Alzheimer disease. *Annual Reviews Neuroscience* **23(1):** 1-11.

Hassan, Z., Yam, M.F., Mariam, A. & Ahmad Pauzi, M.Y. 2010. Antidiabetic properties and mechanism of action of *Gynura procumbens* water extract in streptozotocin-induced diabetic rats. *Molecules* **15(12):** 9008-9023.

Henriksen, E.J. & Dokken, B.B. 2006. Role of glycogen synthase kinase-3 in insulin resistance and type 2 diabetes. *Current Drug Targets* **7(11):** 1435-1441.

Hoe, S.Z., Lee, C.N., Mok, S.L., Kamaruddin, M.Y. & Lam, S.K. 2011. *Gynura procumbens* Merr. decreases blood pressure in rats by vasodilatation via inhibition of calcium channels. *Clinics* **66(1):** 143-150.

Iskander, M.N., Song, Y., Coupar, I.M. & Jiratchariyakul, W., 2002. Antiinflammatory screening of the medicinal plant *Gynura procumbens*. *Plant Foods for Human Nutrition* **57(3-4):** 233-244.

Meijer, L., Flajolet, M. & Greengard, P. 2004. Pharmacological inhibitors of glycogen synthase kinase 3. *Trends in Pharmacological Sciences* **25(9):** 471-480.

Perry, L.M. 1980. *Medical Plants of East and Southeast Asia: Attributed Properties and Uses.* Cambridge: The MIT Press. p. 632.

Wagman, A.S., Johnson, K.W. & Bussiere, D.E. 2004. Discovery and development of GSK3 inhibitors for the treatment of type 2 diabetes. *Current Pharmaceutical Design* **10(10):** 1105-1137.

Wang, H., Kumar, A., Lamont, R.J. & Scott, D.A. 2014. GSK3β and the control of infectious bacterial diseases. *Trends in Microbiology* **22(4):** 208-217.

Zhang, Y. & Liu, D. 2011. Flavonol kaempferol improves chronic hyperglycemia-impaired pancreatic beta-cell viability and insulin secretory function. *European Journal of Pharmacology* **670(1):** 325-332.