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Hypolipidemic effects of quercetin and kaempferol in human hepatocellular carcinoma (HepG2) cells

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Abstract

High lipid levels are associated with the increase tendency of atherosclerosis formation. In the pathogenesis of atherosclerosis, increase in low density lipoprotein cholesterol (LDL-c) concentration has been identified as the main culprit in many cardiovascular disease (CVD) incidents. Both quercetin and kaempferol are flavonoids that most abundantly found in fruits and vegetables. Several studies have dictated that both compounds exhibit CVD protective effects through the regulation of lipid levels. In the present study, the hypolipidemic potential of quercetin and kaempferol through LDL-c uptake were tested on HepG2 cells. Cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in order to study the cytotoxicity effect quercetin and kaemperol on cell proliferation. The present study demonstrated that quercetin and kaempferol at low concentration of 15 µM, possess the highest hypolipidemic effects via LDL-c uptake in HepG2 cells ($p<0.05$). Interestingly, quercetin and kaempferol combination at 1:1 ratio possesses the best effect on LDL-c uptake in HepG2 cells as compared to other ratios. It is suggested that there is a possibility of synergistic effects of quercetin and kaempferol that enhance the LDL uptake more effectively than its single compounds alone. The decrease in cell viability was higher in mixture combinations of quercetin and kaemperol (1:1, 2:1 and 1:2) than to individually treated quercetin and kaempferol (1:0 and 0:1). Further studies should be conducted on primary human liver cells on the LDL uptake and cell viability to further justify the significance of both quercetin and kaempferol as lipid lowering agents as normal LDL-c uptake occurs in healthy cells, rather than a tumour cells like HepG2.

Introduction

In the past years, a diet rich in plant polyphenols has been shown to improve health and to decrease the incidence of cardiovascular disease (Schroeter *et al*., 2006; Perez-Vizcaino *et al*., 2009). Flavonoids constitute a large class of polyphenols found in plants. Extensive studies on quercetin and kaempferol has been done in the light of restricting cardiovascular diseases. Previous study reported that quercetin is also known for its strong anti-inflammatory capacities, whereby several in-vitro studies used various cell lines shown that quercetin is capable of inhibiting lipopolysacharide (LPS) -induced cytokine production (Boots *et al*., 2008). In addition, kaempferol shows the anti-atherosclerotic effect by modulating the gene and protein expression of inflammatory molecules in high cholesterol fed rabbits (Kong *et al*., 2013). Previous study observed that kaempferol treated rats had significant decreases in cholesterol, triglycerides and LDL-c which may be due to the reduction in the absorption of cholesterol. Therefore, both quercetin and kaempferol have the potential in reducing the risk of cardiovascular diseases; yet very few studies have investigated and demonstrated the mechanism involving LDL uptake of these flavonoids.

A study by Kim *et al.* (2008) reported that flavonoids which include kaempferol and quercetin exert inhibitory effects on the production of cholesterol in HepG2 and MCF-7. However, detailed mechanisms and kinetics of the inhibitory effects of the flavonoids are still unknown. Since there are several postulated mechanisms in reducing the risk of CVD via cholesterol lowering, LDL-c is one of the most of interest factor. Apparently, if the LDL-c uptake can be demonstrated significantly, it is then proposed that these flavonoids which present abundantly in our local fruits and medicinal plants can be commercially applied in CVD risk management, particularly at secondary prevention level. To date, there are no natural products or drugs that can be used to lower

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cholesterol through increasing LDL-c clearance in the liver (Ji *et al*., 2012). Thus, studies should be conducted on quercetin and kaempferol to further explore the potential of reducing cardiovascular risks through lipid lowering effect involving LDL-c uptake in the liver.

In the present research, quercetin and kaempferol were used to test their effects on the 25-hydroxycholesterol uptake in HepG2 cells. Different ratios were applied and the effects of treatment on cell viability were also investigated. These flavonoids have been studied widely in relation to decreasing the risks of CVD. Studies have shown that LDL-c is a strong risk factor for atherosclerotic events. Through the clearance of LDL-c, it is suggested to significantly reduce the LDL-c levels, hence, lowering the risk of CVD development.

Materials and Methods

Human Hepatocellular Carcinoma (HepG2) cells were obtained from the Institute of Marine Biotechnology (IMB) Laboratory, Universiti Malaysia Terengganu (UMT). Eagle's minimal essential medium (EMEM) supplement was from Nacalai Tesque, Japan. Trypan blue stain (0.4%) for use with Countess Automated Cell Counter (Cat. Num: T10282), trypsin-EDTA solution, cholesterol (>99%), fetal bovine serum (FBS), streptomycin/ penstrip, phosphate buffer solution tablets, MTT formazan, disinfecting solution, quercetin and kaempferol were all from Sigma Aldrich, Unites States of America. 25-hydroxycholesterol was from Cayman Chemical, US. Lipid depleted FBS was from Biowest and BODIPY® FL LDL was from Invitrogen, US.

Confluent cultures were seeded after 4 minutes exposure to trypsin. Cells were seeded at 1.0 X 10^4 cells per well per 100 µl culture medium in 96 well plate and grown until confluent. To study the effect of quercetin and keampferol on the LDL-c uptake, cells were incubated for 16 hours with different concentration of quercetin or kaempferol at 15, 30 and 45 µM in EMEM medium containing 0.05% lipid depleted FBS, 10 µg/ml cholesterol, 1 µg/ml 25-hydroxycholesterol.

To study the effect of quercetin and kaempferol mixture combinations at different ratio of quercetin:kaempferol (1:0, 0:1, 1:1, 2:1, 1:2) and incubated for 16 hours. All groups of cells were in quadruplets. Varioskan fluorescent plate reader was used to read the plate at 490 and 515 nm (Ji *et al.*, 2012). Cell viability was examined using MTT cell assay. Cell of density of 1.0 x 10^4 per well was seeded in a 96 well plate and grown to confluency. Ratio concentrations of quercetin: kaempferol (1:0, 0:1, 1:1, 1:2 and 2:1) was prepared and administered to cells. Cells were incubated for 16 hours at 37°C with 5% CO_2.

Data was expressed as mean and standard error of mean (SEM), of multiple determinations performed using quercetin and kaempferol derived from different concentrations with SPSS software version 16.0. One-way ANOVA was conducted and multiple comparison analysis was carried out using Tukey post-hoc test. In all analysis, if $p < 0.05$, the difference was considered significant.

Results and Discussion

Figure 1 shows both quercetin and kaempferol at 15 µM exhibit the highest LDL uptake by 158.4% and 58.5%, respectively. The trend observed shows better and higher LDL uptake with lower concentration ($p<0.05$). In general, quercetin demonstrated higher LDL uptake as compared to kaempferol. Hence, on the basis of these findings, quercetin and kaempferol concentration at 15 µM was selected as the optimized condition that maximize the LDL-c uptake in HepG2 cells.

Previously, Moon *et al.* (2012) reported that quercetin strongly upregulated the LDL receptor (LDLR) gene expression by increasing the clearance of circulating LDL-c levels from the blood. Thus, this study strongly relates to the present study as it supports the high potential in quercetin which exerts hypolipidemic effect through the clearance of LDL. Furthermore, based on a recent study by Mbikay *et al.* (2014), at low micromolar concentrations (2-10 µM), quercetin-3-glucoside (Q3G) increased LDLR expression, reduced proprotein convertase subtilisin/ kexin type 9 (PCSK9) secretion and stimulated LDL uptake in Huh7 human hepatocytes culture. Comparing to Q3G, LDLR regulation has reportedly been achieved in HepG2 cells treated with quercetin aglycoside (quercetin) at 75 µM (Moon *et al*., 2012). Therefore, greater efficiency of Q3G could be explained by more active receptor-mediated uptake by cells. Even though LDLR up regulation was reportedly to have been achieved at 75 µM, it is assumed that even at lower quercetin levels of 15 µM, there is a significant effect on the LDL uptake in HepG2 cells. It is proposed in the present study that due to the effect of quercetin on the LDLR up regulation expression, it could have increased the clearance of LDL cholesterol in HepG2 cells via LDL uptake. Hence, quercetin can be seen to have an inhibitory effect on LDL which may contribute to

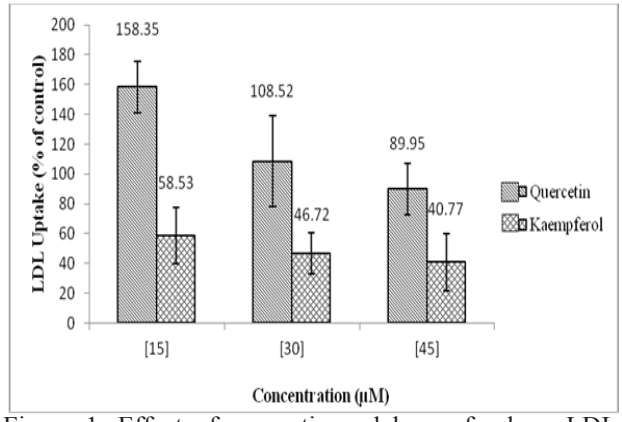

Figure 1. Effect of quercetin and kaempferol on LDL uptake in HepG2 cells

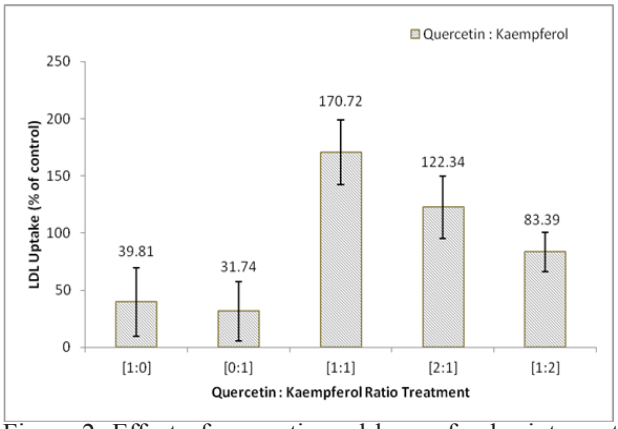

Figure 2. Effect of quercetin and kaempferol mixture at different ratios on LDL uptake in HepG2 cells

a protective flavonoid against CHD.

The effect of kaempferol at 15-45 µM on the LDL uptake in HepG2 cells is similar to quercetin, but the latter exhibited to a lower extend. A study by Kim *et al*. (2008) demonstrated that kaempferol exhibit the highest cholesterol lowering effects at a high concentration of 350 µM in HepG2 cells. However, a study by Berger *et al.* (2013) reported that kaempferol mediated a prominent reduction of cell viability and proliferation rate. In addition, toxicity assays revealed signs of relevant cellular toxicity in primary human hepatocytes only starting at 50 µM. Therefore, even though high concentrations of kaempferol exhibit the most effective cholesterol lowering effects but negatively affects the cell viability, thus, it might not be logically applicable. The present study suggest kaempferol even at low concentration exhibited significant hypochelesterolemia effects in HepG2 cells via LDL uptake.

Figure 2 shows that quercetin: kaempferol ratio of 1:1 gave the highest LDL uptake expression by 170.7%, followed by 2:1 and 1:2 at 122.3% and 83.4% respectively. In addition, quercetin: kaempferol ratio of 1:1 is approximately 4 times more effective in LDL uptake as compared to both individually treated quercetin and kaempferol. Hence, it is deduced that there might be a significant synergistic effect of quercetin and kaempferol used in the combination ratio of 1:1.

Saw *et al.* (2014) showed that combinations of quercetin and kaempferol at much lower concentrations (1.56 and 3.13 µM) detected synergism in H_2O_2-induced ROS assay and ARE luciferase reporter gene assay and also provided mechanistic insight into the synergistic activities. In another study, Ackland *et al*. (2005) utilized human gut (HuTu-80 and Caco-2) and breast cancer cells (PMC42) to show the synergistic effect of quercetin and kaempferol in reducing cell proliferation. In addition, Han *et al*. (2011) found that quercetin and kaempferol were synergetic with adriamycin (ADR) to enhance the antitumor efficiency of ADR, which indicate that quercetin and kaempferol have the ability to increase the sensitivity to chemotherapeutic agents such as ADR in addition to the inhibition of tumor cell growth. Therefore, studies have shown that there has been synergistic effect between quercetin and kaempferol but none so far on the LDL uptake in HepG2 cells.

Harris *et al*. (2013) demonstrated that treatment with EPA+DHA appears to lower patient triglycerides more effectively whereas EPA alone did not. In another point of view, the combinations of similar compounds such as flavonoids or omega 3 fatty acids may give a stronger and more powerful effect. Various studies have proved that quercetin and kaempferol have positive effects against CVD; hence, the combination of both is suggested to elevate and amplify the protective effects.

Based on the present study, it is proposed that quercetin and kaempferol combination at 1:1 ratio possesses the best synergistic effect on hypolipidemic potential through LDL uptake in HepG2 cells. The present study is the first documenting research proposing the possibility of synergistic effects of quercetin and kaempferol in LDL uptake. More studies should be conducted to verify the possibilities and the mechanism behind it.

In Figure 3, the decrease of cell viability is higher in mixture combinations of quercetin and kaemperol (1:1, 2:1 and 1:2) compared to individually treated quercetin and kaempferol (1:0 and 0:1) with the highest decrease in cell viability at -39.9% followed by -36.6% and -35.5% in ratios 2:1, 1:2 and 1:1, respectively. A study conducted by Ackland *et al.* (2005) revealed that the combination of quercetin and kaempferol is more effective in reducing cell growth in human intestinal lines HuTu-80 and Caco-

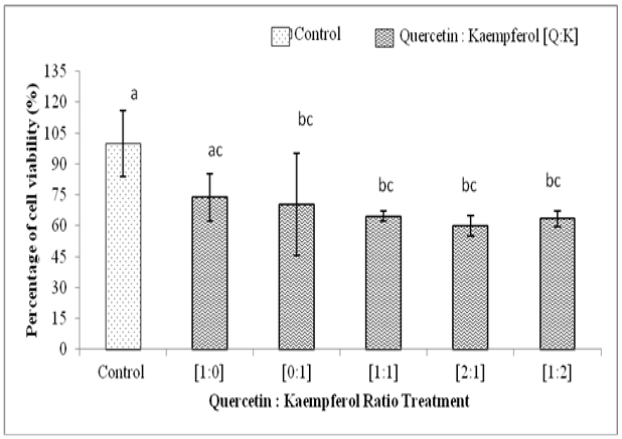

Figure 3. Comparative effect of quercetin and kaempferol mixture at different ratios on HepG2 cell viability (%)

2 and in the PMC42 breast carcinoma cell line than is either quercetin or kaempferol applied alone. Another study by Jaramillo-Carmona *et al*. (2014) also demonstrated that the combination of quercetin and kaempferol exhibited a greater cytotoxic efficacy (IC_{50}) than did either quercetin or kaempferol alone. The interaction of quercetin and kaempferol at a 1Q:1K ratio for 48 hours had a slightly greater but still significant effect on HCT-116 cell death than quercetin or kaempferol alone. However, when cells were exposed at 2Q:1K ratio, cell death increased acutely. When the cells were incubated with a combination in a 1Q:2K ratio, a significant increase in cell death was caused compared to the flavonoids alone, but it was lower than the result of treatment with the ratio of 2Q:1K. These results were similar to the present study's trend and support the fact that lower cell viability was achieved with quercetin and kaempferol combinations compared to their single flavonoids alone. However, even though the present results show that Q:K ratios at 1:1, 2:1 and 1:2 had lower cell viability compared to ratios 1:0 and 0:1, the effect was not significant ($p>0.05$).

In another study by Mylonis *et al.* (2010), it was demonstrated that exposure of Huh7 cells to 10 μM kaempferol caused significant reduction of their viability, which was remarkably more evident under hypoxic conditions. This finding shows that kaempferol even at low concentrations have been shown to reduce cell viability. As for quercetin, concentration levels above 50 μM show great increase in cell death in HepG2 cells incubated for 18 hours (Ramos *et al*., 2005). Based on this study, due to the same culture cells (HepG2 cells) and also almost similar incubation period (16 hours), concentrations below 50 μM were selected for the present research.

Conclusion

To date, the present research was the first study reporting the hypolipidemic effect of quercetin and kaempferol via LDL-c uptake in HepG2 cells. The favorable potential is observed the best at the lowest concentration, i.e. 15 μM, indicating that the effect is not at a dose-dependent manner. It was also found that quercetin is more potent than kaempferol at similar concentration. The present study suggests that part of the mechanism involved in lipid lowering effects of these flavonoids is via LDL-c uptake or clearance which acts as one of the most important factor in CVD risk modulation. In conclusion, there might be some possible synergestic effects based on the highest LDL-c uptake at the 1:1 ratio mixture combination and thus further studies are warranted in understanding the complete picture of these flavonoids action.

References

Ackland, M.L., Van de, W. S. and Jones, R. 2005. Synergistic antiproliferative action of the flavonols quercetin and kaempferol in cultured human cancer cell lines. In Vivo 19 1): 69-76.

Berger, A., Venturelli, S., Kallnischkies, M., Bocker, A., Busch, C., Weiland, T., Noor, S., Leischner, C., Weiss, T.S., Laue,r U.M., Bischoff, S.C. and Bitzer, M. 2013. Kaempferol, a new nutrition-derived pan-inhibitor of human histone deacetylases. The Journal of Nutritional Biochemistry 24(6): 977-985.

Boots, A.W., Haenen, G.R. and Bast, A. 2008. Health effects of quercetin: from antioxidant to nutraceutical. European Journal of Pharmacology 585(2-3): 325-337.

Han, Y., Cao, L., Wu, J., Hao, H., Shi, Y., Xu, G. and Ren, H. 2011. The effects of quercetin and kaempferol on multidrug resistance and the expression of related genes in human erythroleukemic K562/A cells. African Journal of Biotechnology 10(62): 13399-13406.

Harris, W.S., Dayspring, T.D. and Moran, T.J. 2013. Omega-3 fatty acids and cardiovascular disease: new developments and applications. Postgraduate Medicine 125(6): 100-113.

Jaramillo-Carmona, S., Lopez, S., Abia, R., Rodriguez-Arcos, R., Jimene, A., Giullen, R. and Muriana, F.J.G. 2014. Combination of quercetin and kaempferol enhances in vitro cytotoxicity on human colon cancer (HCT-116) cells. Records of Natural Products 8(3): 262-271.

Ji, X., Rivers, L., Zielinski, Z., Xu, M., MacDougall, E., Stephen, J., Zhang, S., Wang, Y., Chapman, R.G., Keddy, P., Robertson, G.S., Kirby, C.W., Embleton, J., Worrall, K., Murphy, A.M., Dekoeyer, D.L., Tai, H.H., Yu L., Charter, E. and Zhang, J-Z. 2012. Quantitative analysis of phenolic components and glycoalkaloids from 20 potato clones and in vitro evaluation of

antioxidant, cholesterol uptake, and neuroprotective activities. Food Chemistry 133(4): 1177-1187.

Kim, K.T., Yeo, E.J., Moon, S.H., Cho, S.G., Han, Y.S., Nah, S.Y. and Paik, H.D. 2008. Inhibitory effects of naringenin, kaempferol and apigenin on cholesterol biosynthesis in HepG2 and MCF-7 cells. Food Science and Biotechnology 17(6): 1361-1364.

Kong, L., Luo, C., Li, X., Zhou, Y. and He, H. 2013. The anti-inflammatory effect of kaempferol on early atherosclerosis in high cholesterol fed rabbits. Lipids in Health and Disease 12: 115-126.

Mbikay, M., Sirois, F., Simoes, S., Mayne, J. and Chretie, M. 2014. Quercetin-3-glucoside increases low-density lipoprotein receptor (LDLR) expression, attenuates proprotein convertase subtilisin/kexin 9 (PCSK9) secretion, and stimulates LDL uptake by Huh7 human hepatocytes in culture. Federation of European Biochemical Societies Open Bio 4: 755-762.

Moon, J., Lee, S.M., Do, H.J., Cho, Y., Chung, J.H. and Shin, M.J. 2012. Quercetin up-regulates LDL receptor expression in HepG2 cells. Phytotherapy Research: 26(11): 1688-1694.

Mylonis, I., Lakka, A., Tsakalof, A. and Simos, G. 2010. The dietary flavonoid kaempferol effectively inhibits HIF-1 activity and hepatoma cancer cell viability under hypoxic conditions. Biochemical and Biophysical Research Communications 398(1): 74-78.

Perez-Vizcaino, F., Duarte, J., Jimenez, R., Santos-Buelga, C. and Osuna, A. 2009. Antihypertensive effects of the flavonoid quercetin. Pharmacological Reports 61(1): 67-75.

Ramos, S., Alia, M., Bravo, L. and Goya, L. 2005. Comparative effects of food-derived polyphenols on the viability and apoptosis of a human hepatoma cell line (HepG2). Journal of Agricultural and Food Chemistry 53(4): 1271-1280.

Saw, C.L., Guo, Y., Yang, A.Y., Paredes-Gonzalez, X., Ramirez, C., Pung, D. and Kong, A.N. 2014. The berry constituents quercetin, kaempferol, and pterostilbene synergistically attenuate reactive oxygen species: involvement of the Nrf2-ARE signaling pathway. Food and Chemical Toxicology 72: 303-311.

Schroeter, H., Heiss, C., Balzer, J., Kleinbongard, P., Keen, C.L., Hollenberg, N.K., Sies, H., Kwik-Uribe, C., Schmitz, H.H. and Kelm, M. 2006. Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proceedings of the National Academy of Sciences of the United States of America, p. 1024-1029. United State: National Academy of Sciences.