P-029

Ethanolic extract of broccoli leaves exerts anti-diabetic effect by activating the insulin signaling pathway in human HepG2 cells and 3T3-L1 adipocytes

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Since diabetic mellitus is one of the common metabolic disorders, many studies are focused on the development of anti-diabetic medicine. The present study focused on the analysis of anti-diabetic activity of broccoli leaves extract (BLE) in human HepG2 cells and mouse 3T3-L1 adipocytes. The BLE was prepared according to pulsed electric field extraction technology and, MTT assay was performed to evaluate the cytotoxicity of BLE on HepG2 cells and 3T3-L1 adipocyte. Based on the MTT assay, BLE did not affect cell viability up to 1,250 μg/ml in HepG2 cells and up to 2,500 μg/ml in 3T3 cell. The effect of BLE on the insulin signaling pathway in HepG2 cells and 3T3-L1 adipocytes was investigated using western blot analysis. Phosphorylation levels of both glycogen synthase kinase-3β (GSK3 β) and protein kinase B (AKT) were increased, and the phosphorylation level of glycogen synthase (GYS2) was decreased in HepG2 cells by BLE dose dependently. In addition, BLE increased the phosphorylation level of AMP-activated protein kinase (AMPK) in HepG2 cells. Furthermore, BLE also increased the phosphorylation levels of both GSK3 β and AKT proteins in 3T3-L1 adipocytes incubated in inflammatory media (L/CM). Overall results suggest that BLE has potential anti-diabetic effect, and by product of broccoli can be developed as a useful therapeutic agent in the future.

Keywords: Broccoli leaves extract, Anti-diabetic, Insulin signaling, HepG2 cell, 3T3-L1 adipocyte

P-030

Anti-oxidant and anti - inflammatory effects of ethanolic extract of broccoli leaves in LPS-stimulated RAW 264.7 cells

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Broccoli (*Brassica oleracea var. italica*) is an edible green plant belongs to the cruciferous vegetable family which is a rich source of glucosinolates, flavonoids, vitamins and mineral nutrients. In the present study, we investigated the anti-oxidant and anti-inflammatory activities of broccoli leaves extract (BLE). Broccoli leaves were extracted with 80% ethanol using pulsed electric field technology (PEF), and free radical scavenging activity of the extract was determined based on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. BLE showed anti-oxidant activity by increasing the DPPH radical scavenging ability in a dose dependent manner with the IC50 value of 3,234 μg/ml. Cell viability was measured by the MTT assay, and had no significant cytotoxicity up to 1,500 μg/ml BLE concentration in Raw 264.7 cells. RAW 264.7 cells were treated with different concentrations of BLE to investigate the anti-inflammatory activity. Production of nitric oxide, and pro-inflammatory cytokines including interleukin (IL)-1β, interleukin (IL)-6, and tumor necrosis factor (TNF-α) in LPS stimulated RAW 264.7 cells were decreased by 50%, 39%, 64%, 68% respectively at the BLE concentration of 1,250 μg/ml. Based on the western blot analysis, BLE reduced the expression of both cyclooxygenase-2 (COX-2) and inducible nitric oxide (iNOS) protein levels in a does dependent manner. Overall results suggest that BLE has anti-inflammatory and anti-oxidant properties, and investigation of these biological functions in the by-products of broccoli might be valuable for the development of health foods.

Keywords: Broccoli leaves extract, Anti-oxidant activity, Anti-inflammatory activity, RAW 264.7 cells, DPPH

P-031

Prevalence and phylogenetic analysis of avian haemosporidia in Chonbuk Province, Republlic of Korea from 2016 to 2017

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Avian blood parasites, including genera *Plasmodium* and *Haemoproteus* are found worldwide,however, only limited information is available in Republic of Korea (ROK). In this study, the prevalence of genera *Plasmodium* and *Haemoproteus* and its phylogenetic characteristics were investigated in wild birds in ROK. Blood samples were collected from 240 wild birds of 27 species in the Chonbuk province, ROK from 2016 to 2017. While 77 (32.1%) were positive for avian haemosporidia on microscopic examination of blood smears, 135 (56.3%) were positive on polymerase chain reaction (PCR) targeting the cytochrome *b* gene. By direct sequencing of PCR amplicons, 129 (95.6%) were identified as *Haemoproteus* species and 6 (4.4%) as *Plasmodium* species. Phylogenetic analysis using the cytochrome *b* gene revealed that resident and migrant birds have very similar genetic lineages of both parasites in ROK, suggesting the possibility that migrant birds may act as a mediator for the parasite among Asian countries. The study was supported by the Korea Ministry of Environment (MOE) as Public Technology Program based on Environmental Policy (No. 2016000210002).

P-032

Treatment ovalicin to stimulated DH82 cells alleviated pruritus and inflammation by inhibition of IL-31 signaling and ROS.

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Pruritus is cutaneous disease accompanied with secondary infection and worsen life quality. However, only steroidal drug is used even, that have a severe side effect. Therefore, we need to development of non-steroidal pruritus drug. Ovalicin is a compound of extracted from *Cordyceps militar* is using for dandruff, seborrheic dermatitis treatment and agricultural insect pests. Several research reported ovalicin has a potential to inhibition of itching behavior, however, precise mechanism and not proved in canine cell lines. Activated IL-31 receptors binding with IL-31 expressed TRPV1 and released histamine for arising scratching behavior. After LPS treatment for 24 hours, DH82 cells were increased immune response. However, ovalicin treatment to stimulated DH82 cells by LPS was significantly decreased IL-4, IFN-γ and IL-31. Moreover, dimer form of IL-31 receptors, IL-31RA and OSMR, and pruritus targeted genes, TRPV1 and histamine R2 were significantly decreased after ovalicin treatment by inhibition of intracellular calcium influx. Furthermore, ovalicin treatment had an anti-inflammatory effect by ROS decreasing. Ovalicin treatment reduced to inflammation related molecules, COX-II, NF-kB and NOS. Therefore, this study proved the ovalicin is a potential to pruritus and inflammation drug through inhibition of IL-31 signaling mechanism and ROS.