

CRS) and aspirin (4h ASP) induced ulceration. The ulcer index and % protection was calculated. The gastric juice collected from 4h PL rats was studied for the effects on gastric volume, pH, free acid, total acid-pepsin output, DNA gastric juice cell shedding and mucosal cell proliferation. Biochemical estimation such as histamine content, mucus content and H⁺K⁺ATPase inhibitory activity was also evaluated. The effect of APF on stomach histopathology was also studied. Statistical analysis was done by one way ANOVA followed by Dunnett's post hoc test for multiple comparisons against control group. The difference was considered to be significant when P < 0.05. Oral treatment of APF (200 and 400 mg/kg, p.o.) for 7 days showed significant protection against acute gastric ulcer induced by 4h PL

and 95% EtOH, but was found to have no effect on CRS and ASP-induced gastric ulceration. The gastric studies showed that treatment with APF significantly increased the gastric juice pH, decrease the gastric volume and total acid-pepsin output and significantly reduced the gastric juice cell shedding. A significant decrease in histamine content and a reduction in free phosphate ions responsible for H⁺K⁺ATPase inhibitory activity were also observed. In conclusion, the results of the present study indicate that the gastroprotective activity of APF contributes mainly to the peripheral effects on the gastric secretion and the probable mechanism underlying behind such activity might be both due to the possible inhibition on the histaminic (H₂) receptor and H⁺K⁺ATPase pump.

INVESTIGATION OF THREE TRITERPENE ACIDS AGAINST CANCER CELL PROLIFERATION ACTIVITY

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The object of this study was to investigate the inhibitory effect of betulinic acid, oleanolic acid and ursolic acid on human colon cancer cell proliferation activity. Triterpene acids are members of the terpene family and are widely distributed in food plants. A rosehip fraction containing a mixture of betulinic acid, oleanolic acid and ursolic acid displayed inhibitory effect against the release of pro-inflammatory cytokine interleukin-6 with the combination of oleanolic acid/betulinic acid and ursolic acid/betulinic acid displaying significant synergistic inhibitory effects (1). Our results showed that these three compounds also displayed good inhibitory effect against cancer cell proliferation activity at the concentration of 10 µg/ml. both betulinic acid and ursolic acid displayed strong inhibition against HT-29 (88% and 79%, respectively) and SW-480 (76% and 82%, respectively), but a moderate to weak inhibition against Caco-2 (19% and 22%, respectively). Oleanolic acid displayed only moderate inhibition against Caco-2 (49%), very weak inhibition against HT-29 (7%) and

no inhibition against SW-480. No synergistic effects were observed between combinations of the triterpene acids. We only found an additive effect in the combination of oleanolic acid/ursolic acid and only against proliferation of HT-29. Antagonistic effects between the pure compounds were observed when betulinic acid was one of the components in the combination. Although betulinic acid and ursolic acid showed good inhibitory effects against all three cancer cell lines, their combination displayed antagonistic effects and showed poor inhibition against cancer cell proliferations. However, these triterpenic acids and their combination effects should be further investigated in different *in vitro* or *in vivo* experiment to confirm their bioactivities and mechanism.

Reference: (1) Saaby L., Moesby L., Hansen E.W., Christensen S.B. (2011). Isolation of immunomodulatory triterpene acids from a standardized rose hip powder (*Rosa canina* L.). *Phytotherapy Research*, 25, 195–201.

DECURSIN AND RELATED COMPOUNDS INHIBIT VASCULAR ENDOTHELIAL MIGRATION BY SPHINGOSINE KINASE INHIBITION

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The pyranocoumarin compound decursin isolated from the herb, *Angelica gigas*, are known to possess potent anti-inflammatory activities. However,

little is known about their anti-angiogenic activity. Here, we investigated the anti-angiogenic effects of decursin by the changes of sphingolipid metabolites

and related enzymes activities. Cell migration was measured by Boyden chamber. S1P and Sphk-1,2 was quantitatively measured both by HPLC-fluorescence detection after OPA derivatization and by LC-MS/MS system. HDAC activity assay kit (Cayman Co) was used. Sphk mRNA level was measured by qRT-PCR using $\Delta\Delta C_t$ method. Antibodies for PKC isoform was obtained from Cell Science Co. and detected using western blot. Decursin inhibited sphingosine kinase (Sphk) induced angiogenic processes *in vitro*, including cell proliferation, migration of human umbilical vein endothelial cells (HUVEC). Interestingly, Sphk-1 activity was significantly decreased by 43% compared to control, while Sphk-II activity was 1.6 fold increased by decursin treatment. The S1P in cells and in cultured media was decreased dose-dependently by decursin,

indicating that the reduced synthesis of angiogenic lipid mediator S1P which binding to S1P receptors (S1PRs) is essential to transmit S1P-S1PR axis signaling for angiogenesis. Decursin specifically reduced Rac-1. The increased Sphk-II activity and thus S1P production in nuclear fraction blocked histone deacetylase (HDAC) activity. Indeed, the mRNA expression of Sphk-II was 3-fold increased by decursin. Sphingosine level was also increased in 2-fold. Decursin treatment enhanced the migration of Sphk-1 and PKC α into nuclear membrane. The expression of PKC α and PKC η (eta) were decreased while other PKC isoforms were not changed. Our data suggests that decursin have a potent anti-angiogenic property via S1PR-S1P axis by regulating both SPHK-I and -II activities and thus reducing S1P synthesis and release.

ISOLATION OF GELATIN HYDROLYSATES EFFECTIVE ON BONE FORMATION AND RESORPTION

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Gelatin is a mixture of polypeptides obtained by partial hydrolysis of collagens extracted from connective tissues of animals. The aim of this study was to provide the gelatin hydrolysate (GH) which is effective on bone formation. Porcine skin gelatin was hydrolyzed by manipulating two reaction parameters; enzyme combination and reaction time. Four combinations of enzymes (alcalase+protamex, protamex+flavourzyme, flavourzyme+alcalase and alcalase+protamex+flavourzyme) and 3 reaction times (4, 12, and 24 h) were examined. The resultant 12 GHs were fractionated into 3 ranges of molecular weight (total, >3kDa, and <3kDa), and 36 various GH were obtained. An *in vitro* study on bone formation was carried out in osteoblast-like MG63 cell proliferation and opti-

mal hydrolysis condition for the maximal bone formation activity was selected. The enzyme combination of protamex+flavourzyme provided the GH with the highest activity. Higher bone formation activity was observed in small molecular weight (<3kDa) GH at reaction time of 4 hour. The effects of selected GH on bone resorption were determined in bone marrow-derived osteoclasts cells driven by RANKL and M-CSF. GH suppressed the formation of TRAP-positive osteoclasts. Furthermore, RANKL induced TRAP activity was greatly inhibited by GH treatment. The effects on bone formation and resorption were not observed with gelatin treatment. These results suggest that GH isolated in this study is a promising agent for the prevention and treatment of bone loss.

INTESTINAL ABSORPTION AND PRESYSTEMIC ELIMINATION OF VARIOUS CHEMICAL CONSTITUENTS PRESENT IN GBE50 EXTRACT, A STANDARDIZED EXTRACT OF GINKGO BILOBA LEAVES

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The nature and level of systemic exposure to the active herbal constituents will profoundly affect their effects at action sites, which is fundamental in understanding their roles in the overall beneficial effects of an

herbal medicine. The objective of this study is to gain a full picture of the systemic exposure to various putatively active ginkgo constituents after p. o. administration of GBE50 extract, a standardized extract of Ginkgo biloba