tagonists. **METHODS** To detect inhibition of adenylate cyclase up on RXFP3 activation by human relaxin-3 (H3 relaxin), HEK-RXFP3, CHO-RXFP3, SN56 and GT1-7 cells were plated in poly-L-lysine coated 24 well plates. The following day, the cells were starved in serum free cell culture media for 6 h. Next, cells were treated in triplicate with serum free media (control) and H3 relaxin for 5−15 min. Then, the cells were treated with serum free media with DMSO (control) or forskolin (5−100 μmol·L^{-1}) for 15 min at 37℃ with 5%CO_2. At the end of the incubation, cell culture media was discarded and the cells were lysed with 0.1 mol·L^{-1} HCl. The cAMP concentration in each lysate was detected by ELISA (Cayman Chemicals). The data from three experiments were analysed using one way ANOVA followed by Bonferroni post hoc test or Dunnett's *post hoc* test. **RESULTS** In CHO-RXFP3 and HEK-RXFP3 cells, 10 nmol·L^{-1} of H3 relaxin was able to significantly inhibit the forskolin (5 μmol·L^{-1}) induced cAMP levels (P<0.05). In SN56 neuronal like cell line endogenously expressing RXFP3, 100 nmol·L^{-1} H3 relaxin was able to significantly reduce forskolin (3 μmol·L^{-1}) induced cAMP (P<0.05). However, in wild type HEK293T and CHO-K1 cells, 10 nmol·L^{-1} H3 relaxin was not able to significantly reduce the forskolin 22(5 μmol·L^{-1}) induced cAMP levels. In GT1−7 mouse hypothalamic cells endogenously expressing RXFP3,100 nmol·L^{-1} H3 relaxin and 5 or 3 μmol·L^{-1} forskolin, was able toa significantly increase cAMP levels (P<0.05). **CONCLUSION** Inhibition of forskolin induced cAMP assay can be used to detect $G_{i/o}$ mediated cAMP inhibition related signaling events due to RXFP3 activation by its agonists in CHO-RXFP3, HEK-RXFP3 and SN56 cell lines.

Key words: neuropeptide; relaxin-3; adenylate cyclise; forskolin

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Effect of osthole, a *Fructus Cnidii*-derivednature coumarin, on osteogenesis and bone healing in mice

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Abstract: **OBJECTIVE** To investigate the effect of osthole, a natural coumarin isolated from traditional Chinese medicine *Fructus Cnidii*, on osteogenesis *in vitro* and bone fracture healing *in vivo*. **METHODS** Primary bone marrow mesenchymal stem cells (MSCs) were isolated from 6-week C57/B6 mice, and osteogenic differentiation was assessed by alkaline phosphate (ALP) activity and calcium nodule formation. Adult (12-week) C57 mice were subjected to mid-shaft osteotomy on femur. The mice were oral administrated with osthole (5, 20 or 50 mg·kg^{-1}) or vehicle solvent daily from post-operational week 1. Radiographic imaging, real time molecular imaging, micro computed tomography (μCT) and histology analysis were performed to evaluate the healing progress. **RESULTS** Results showed that osthole promoted osteogenesis of bone marrow MSCs by enhancing ALP activity and mineralization dose dependently in the range of 1−100 μmol·L^{-1}. Plain radiographs showed that administration of osthole at 20 and 50 mg·kg^{-1} significantly accelerated fracture healing by reducing the period of reparative phase. Further investigation with μCT and histology showed that osthole-treated group had high proportion of newly-formed woven bone and smaller cartilage island compare to control group at week 2; and treatment group had completed endochondral ossification and started remodeling phase at week 3. Molecular imaging of near-infrared (NIR) fluorescent labeled palmidronate depositing on newly formed bone suggested that osthole treatment (20 mg·kg^{-1}) augmented callus mineralization process at both postoperative week 2 and week 3 by 80.72% and 25.95% respectively. **CONCLUSION** Osthole demonstrates significant osteopromotive effect *in vitro* and anabolic effect on bone formation in fracture repair, which makes it a potential agent for bone regeneration and against osteoporosis.

Key words: osthole; mesenchymal stem cells; osteogenic differentiation; fracture healing;

endochondral ossification

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Usage of fresh medicinal plants and users′ perceptions in Singapore

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Abstract: **OBJECTIVE** To gather information regarding the usage of fresh medicinal plants and users′ perceptions via face-to-face interviews. To date, there is no published report of firsthand account of their usage in Singapore. Such important information may be permanently lost if not properly collated in time. **METHODS** Information on demographic data, plant-use methods and perception of usage was collated. Participants were recruited via the local newspapers, by word of mouth etc. The survey protocol was approved by the NUS Institutional Review Board. Fresh plant samples/photographs were obtained from the users and voucher specimens were kept. **RESULTS** Two hundredusers who have used a total of 103 species of fresh medicinal plants anytime in the last 5 years participated in the survey. The five most commonly used plants were *Clinacanthusnutans* (34 users), *Strobilanthescrispus* (31 users), *Pereskiableo* (25 users), *Aloe vera* (18 users), and *Zingiberofficinale* (16 users). The top 3 most commonly cited medical conditions were diseases of respiratory system (50 users), neoplasm (29 users) anddiseases of circulatory system (20 users). A total of 173 users (86.5%) did not consult any healthcare professional for advice about plant usage, and only one user consulted the pharmacist. Some of the common reasons given for using fresh medicinal plants were recommendation by others (150, 75.0%), efficacy (137, 68.5%), and safety (117, 58.5%). Most users (170, 85.0%) were satisfied or highly satisfied with the outcome of plants used. **CONCLUSION** Two hundred users of fresh medicinal plants have been successfully interviewed and the information documented systematically in a database. The results suggest that fresh medicinal plants have a role to play in healthcare in modern society. The information collated will serve as a useful resource for identifying promising plants for future drug discovery efforts.

Key words: survey; fresh medicinal plants; usage; perceptions

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Preparation of hypocrellin B nanoparticlesfor photodynamic therapy

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Abstract: **OBJECTIVE** To prepare hypocrellin B-loaded apoferritin nanoparticles (HB-AFT NPs) for photodynamic therapy on tumor. **METHODS** HB-AFT NPs were prepared by taking advantage of the reversibleunfolding and refolding character of apoferritin in different pH environments. The photophysical and photobiological properties of hypocrellin B-loaded apoferritin were measured. **RESULETS** HB molecules were successfully encapsulated within apoferritincavity. HB-AFT-NPs exhibited higher ROS productionthan free HB. Additionally, phototoxicity of HB-AFT NPs to MDA-MB-231 cells was significantly improved as compared to free HB. **CONCLUSION** Together these results demonstrate that hypocrellin