S2.1

The anti-fibrotic effect of thymoquinone on thioacetamide-induced mouse hepatic fibrosis

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Aim: Thymoquinone (TQ) is the main active ingredient from the seeds of *Nigella sativa Linn*, which has been traditionally used in the Middle East and Southeast Asian countries to treat ailments including asthma, bronchitis, rheumatism, cancer and related inflammatory diseases. In the present study, we investigate the anti-fibrotic effect and the potential mechanisms of action of TQ against hepatic fibrosis *in vivo*. **Methods:** Liver fibrosis was induced by intraperitoneal injections of thioacetamide (TAA, 200 mg/kg) three weekly for 5 weeks in male kunming mice. The administration of TQ (20, 40 mg/kg) was started following TAA injections and was continued for 5 weeks to evaluate the protective effects by hematoxylin and eosin (H&E) staining and Masson staining. The protein and mRNA extracted from liver tissue were analyzed by Western blotting analysis and Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR). **Results:** Our data demonstrated that treated with TQ obviously improved tissue damage compared with TAA-only group, in that less inflammatory cell infiltration was observed in H&E and Masson staining results. TQ demonstrated great efficacy in curing hepatic fibrosis via attenuating the TAA-mediated α-smooth muscle actin (α-SMA) and Collagen-I. TQ significantly inhibited the expression of toll-like receptor 4 (TLR4), tissue inhibitor of metalloproteinase-1 (TIMP-1) and α-SMA by Western blotting analysis and RT-PCR. TQ also significantly inhibited phosphatidylinositol 3-kinase (PI3K) phosphorylation and the phosphorylation adenosine monophosphate-activated protein kinase (AMPK) and liver kinase B (LKB)-1. Furthermore, TQ significantly decreased the expression of interleukin (IL)-1b and IL-18 in TAA-induced mouse hepatic fibrosis. **Conclusion:** The potential anti-fibrosis mechanism of TQ might be associated with the depression of α-SMA and Collagen-I relating with hepatic fibrosis, down-regulating the accompanying inflammatory response in hepatic fibrosis. TQ suppressed PI3K signaling and activated LKB-1 signaling in TAA-induced mouse hepatic fibrosis, suggesting that TQ may be a potential candidate for the therapy of hepatic fibrosis.

Keywords: thymoquinone; thioacetamide; hepatic fibrosis

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S2.2

Panax notoginseng saponin reduces glucose/glucose oxidase-induced apoptosis and promotes mitochondrial biogenesis in H9c2 cells

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Aim: High blood glucose may auto-oxidize and generate free radicals, which are proposed to induce apoptosis in cardiac cells. Panax notoginseng saponin(PNS) has been shown to improve the diabetic cardiac symptoms. However, the underlying mechanisms have not been elucidated precisely. The aim of the present study was to investigate the cell apoptosis induced by glucose/glucose oxidase-dependent oxidative stress and the protective effect of PNS on H9c2 cardiac cells. **Methods:** Rat cardiomyoblast H9c2 cells were maintained in DMEM supplemented with 10% FBS. Cells were assigned to 3 groups: (1) control group (Con); (2) G/GO group: cells were stimulated with glucose (33 mmol/L) and GO (2 mU/mL) for 12 h; (3) PNS+G/GO group: cells were pretreated with PNS (2 g/L) for 6 h And followed by stimulation with glucose (33 mmol/L) and GO (2 mU/mL) for 12 h. Cell apoptosis was studied using flow cytometry. The generation of reactive oxygen species (ROS-super oxide anion and hydrogen peroxide) was measured by DCFH-DA assay, and the change in mitochondrial membrane potential (ΔΨm) was evaluated by JC-1. The mRNA levels of peroxisome-proliferator-activated receptor γ coactivator-1α (PGC-1α), nuclear respiratory factorl (NRF1), mitochondrial Transcription factor A(Tfam) and cytochrome c oxidase subunit I (COX I) were detected by Real-time quantitative PCR(Q-PCR). **Results:** The apoptotic ratio of control group was 8.18%±2.16%, that of model group was 48.20%±1.80%. And the apoptotic ratios of PNS group was 20.98%±5.20%. PNS could up-regulated the mRNA expression of PGC-1α, NRF1, Tfam and COXI (P<0.05). PNS also could protect mitochondrial membrane potential from depolarization by JC-1 staining. DCFH-DA staining demonstrated that PNS could significantly decrease intracellular ROS. **Conclusion:** It was indicated that PNS could reduced G/GO-induced H9c2 cardiomyocytes apoptosis, and promotes the mRNA expression levels of mitochondrial biogenesis genes.

Keywords: panax notoginseng saponin; glucose oxidase; apoptosis; mitochondrial biogenesis; H9c2 cell

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S2.3

Antifungal activity of ethanol-extraction from Flos Rosae Chinensis

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Aim: The present study was designed to investigate antifungal activities of the ethanol-extraction from Flos Rosae Chinensis(FRC) combined with Fluconazole against clinical isolates of Candida albicans resistant to fluconazole. **Methods:** The minimum inhibitory concentration (MIC) was determined using checkerboard microdilution assay. MIC80 was determined as the lowest concentration of the drugs (alone or in combination) that inhibited fungi growth by 80%, compared with that of drug-free wells. The time-kill curves and agar diffusion tests were further demonstrated for synergistic effect of FRC combined with fluconazole against clinical isolates of Candida albicans resistant to fluconazole. **Results:** Our study firstly found that FRC alone or in combination with fluconazole can effectively inhibit the growth of Candida albicans. FRC alone had efficient antifungal activities against Candida albicans within a MIC80 range of 20 to 40 μg/mL. It failed to enhance the effect of fluconazole against Candida albicans sensitive to fluconazole. But it could make Candida albicans resistant to fluconazole to be sensitive. The time-kill curves and agar diffusion test got the further proofs. **Conclusion:** FRC alone have certain degree of growth inhibition effect on Candida albicans. The treatment with Fluconazole combined with FRC may be an effective solution to overcome the resistance of Candida albicans.

Keywords: *Flos Rosae Chinensis;* ethanol-extraction; antifungal effect; *Candida albicans;* fluconazole resistance

Acknowledgements: This study was supported by the National Natural Science Foundation of China (grants 81173635 to Yuan-ying JIANG)

S2.4

Study on protein patterns of magosteen crude extract against *Plasmodium falciparum*

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Aim: Proteomic is the powerful tool for research and development of new antimalarial drug. In the present study, the protein patterns of *Plasmodium falcipaum* proteins between exposed and non-exposed to mangosteen crude extract and non exposed were compared by two-dimensional electrophoresis (2-DE). **Methods:** The parasites were culture and incubated with no drug or with mangosteen crude extracts for 12 h. Proteins were extracted and separated by their charges and sizes, the spot proteins were then analyzed by imaging software (PDQuest™; BioRad). **Results:** The protein expression patterns were compared for up- or down-regulation, more than 20 protein spots showed at least two-fold difference in density. And 10 selected spots were then picked and identified by mass spectrometry. **Conclusion:** These proteins may be drug targets of mangosteen action to *Plasmodium falciparum*.

Keywords: *Plasmodium falciparum*; two-dimensional electrophoresis (2-DE); mangosteen

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S2.5

Coniferyl ferulate, a strong inhibitor of Glutathione-S-transferase isolated from Radix *Angelicae sinensis*, to reverses multidrug resistance and down-regulation of P-glycoprotein

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Aim: Glutathione-S-transferase (GST) is key enzyme in multidrug resistance (MDR) of tumor. Inhibition of the expression or activity of GST has emerged as a promising therapeutic strategy for the reversal of MDR. Natural products might be important sources as potential chemosensitising agents with greater inhibition of the activity of GST. **Methods:** A high-throughput screening (HTS) model was established to screen for inhibitors of GST from natural Chinese herbs. The effect and mechanism of Coniferyl ferulate (CF), isolated from root of *Angelica sinensis* (Oliv.) Diels. (Radix *Angelicae sinensis*, RAS) on GST inhibition had been determined. Apoptosis analysis and a reverse transcription-polymerase chain reaction (RT-PCR) assay for P-gp/MDR1 expression in an adriamycin-resistant human endometrial cancer cell line were also utilised to evaluate the ability of CF to reverse MDR. **Results:** CF showed strong inhibition of human placental GST. Its 50% inhibition concentration (IC50) was 0.3 μmol/L, which was greater than an known GST p1-1 inhibitor, ethacrynic acid (EA), using the established HTS model. Kinetic analysis and computational docking were used to examine the mechanism of GST inhibition by CF. The results demonstrated the inhibition of GST activity by CF in a concentration-dependent manner and CF was likely to act as a reversible non-competitive inhibitor of GST. CF, in a concentration-dependent manner, significantly induced apoptosis in the B-MD-C1 (ADR+/+) cells, altered the cell cycle phase distribution and markely decreased the overexpression of P-gp with exposure to adriamycin. **Conclusion:** CF showed a potential MDR reveral effect for pharmaceutical use.

Keywords: coniferyl ferulate (CF); glutathione-S-transferase (GST); multidrug resistance

S2.6

Influences of Shuganjianpi formula on expressions of TGF-β/Smad signal pathway in hepatic stellate cell

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Aim: To observe the effect of Shuganjianpi formula(SGJP) about transforming growth factor(TGF)-β/Smad signaling transduction, explore the mechanism. **Methods:** HSC-T6 cell was used as *in vitro* model to evaluate the effect of SGJP on cell proliferation by MTT method, the synthesis of type I Collagen was measured by enzyme linked immunosorbentassay. Moreover, the expression of TGF-β_1, Smad2, Collagen-I mRNA and TβR-I, Smad7 protein were measured by semiquantitative RT-PCR and Western blot technology. **Results:** The results showed that SGJP could markedly inhibit the proliferation of HSC. In addition SGJP treatment could significantly down-regulate TGF-β_1, Smad2, Collagen-I mRNA, TβR-I protein, Collagen I content and up-regulate Smad7 protein expression. **Conclusion:** SGJP can resist hepatic fibrosis by accommodate TGF-β/Smad signal pathway, which may be its one of the mechanisms.

Keywords: shuganjianpi formula; liver fibrosis; hepatic stellate cell; TGF-β/Smad signal pathway

S2.7

Novel norepinephrine transporter inhibitors from a traditional Chinese medicine - *Polygala Tenuifolia*

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Aim: In central nervous system, norepinephrine (NE) was found to regulate mood, sleep and expression behaviors. There are many evidences supported that NE is involved in depression and the norepinephrine transporter (NET) is the target for anti-depressant medicine development due to the lower side effects and higher efficiency. Radix Polygalae is made of the dried root of *Polygala tenuifolia* (Polygalaceae) and used as the Chinese tranquilizers. Here, we want to investigate the role of the botanical extracts and its active compounds on NET inhibitors and their drugable potential on depression therapy. **Methods:** We clarified the binding affinity of different botanical fractions of NET, serotonin transporter (SERT) and dopamine transporter (DAT). We also used HEK293-NET, HEK293-SERT or HEK293-DAT cells to evaluate the inhibition of neurotransmitter uptake. The active fraction(s) is administered orally in depression-related animal models, including tetrabenazine-induced hypothermia, despair swimming and tail suspension. **Results:** In this study, we identified PDC-1421 served as a botanical selective NET inhibitor. The IC_{50} is 1.27±0.46 μg/mL in NET, while 60 and 200 folds to DAT and SERT in radioligand binding assay. For PDC-1421, the IC_{50} of NE uptake is 0.704±0.191 μg/mL in HEK293 cells, but the IC_{50} of dopamine and serotonin uptake are more than 100 μg/mL, respectively. Besides, PDC-1421 demonstrated a great response in tetrabenazine-induced hypothermia, despair swimming and tail suspension in mice. In addition, some compounds involving with polygalatenosides and polygalasaponins exhibit μmol/L level activity in the norepinephrine transporter binding assay. **Conclusion:** These results indicated that the product resulted from Radix Polygalae may be entitled to the botanical lead of depression and attention deficit hyperactivity disorder drug development.

Keywords: *Polygala tenuifolia*; norepinephrine transporter inhibitor; tetrabenazine-induced hypothermia; polygalatenoside; polygalasaponin

S2.8

Recent pharmacological progress of furanodiene

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Furanodiene (Fur) is a natural product isolated from*genus Gorgonia*, *smyrniumolusatrum*, *Commiphoraguidotti*, and especially rich in Chinese herb *CurcumaeRhizoma*. It is a heat-sensitive sesquiterpene identified dozens of year ago. In this review, we attempt to summarize the recent advances of its bioactivities. PublicationsonFurwere retrieved fromPubMed, CKNI, Elsevier, EBSCO, Spring Linker, and Wiley-Blackwell with the keyword "furanodiene". The informationwas systematically summarized andcarefully analyzed. Furshowed anti-inflammatory andhepatoprotective effects by improving 12-O-tetradecanoylphorbol-13-acetate(TPA)-induced ear edema and by protecting against D-GalN/tumor necrosis factor-α and D-GalN/lipopolysaccharide-induced liver injury in mice. Its anticancer effect both *in vitro* and *in vivo* is a recent research focus. Furinhibitedthe proliferation and growth of a series of cancer cell lines in a concentration dependent manner. Synergistic and enhanced anti-proliferative effect of Fur with paclitaxel and tamoxifenwas also observed. Furthermore, Furinduced cell cycle arrest at G_0/G_1 and G_2/M phases depending on the cell type and induced apoptosis in TNFR1-mediated extrinsic and mitochondria mediated intrinsic apoptotic pathways. In addition, Furtreatment resulted inendoplasmic reticulum stress and autophagy in lung cancer cells. It also demonstratedinhibitory effect on angiogenesis in a zebrafish model. Especially, it dramatically inhibited the tumor growth in a MCF7 tumor xenograft model. Current data suggested that Fur might be a potential anticancer lead compound deserving further investigation.

Keywords: furanodiene; cancer; natural product
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S2.9

The inhibitory effect of iridoid glycoside extracted from Fructus Gardeniae on ion channel activity of influenza A virus M2 protein

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Aim: Iridoid glycoside is the main active of Fructus Gardeniae with antivirus and anti-inflammatory characteristics. The aim of this present study is to investigate the inhibitory effect of iridoid glycoside extracted from Fructus Gardeniae on ion channel activity of influenza A virus M2 protein. **Methods:** Western-blotting was applied to measure the M2 protein express in the lungs of mouse infected by influenza A/FM/1/47 virus. The intracellular pH and Ca^{2+} of MDCK cells were labeled by fluorescent probe SNARF-1/AM and fluo-3 respectively and the pHi and concentration of Ca^{2+} were measured by confocal laser scanning microscope. **Results:** Post 3 d of FM1 infection ($15LD_{50}$), the express of M2 protein in mouse lungs increased significantly. Treatment of mouse with irodoid glycoside (100, 50, 25 mg/mL ip for 3 d) reduced M2 protein express in mouse lungs with dose-effect relationship. *In vitro*, pHi (fluorescent intensity 580 nm/640 nm) decreased while concentration of Ca^{2+} increased significantly in MDCK cytoplasm post infection of 0.5, 1, and 1.5 h. Treatment of cultures with iridoid glycoside (20, 10, 5, 2.5 mg/mL for 1 h post infection) increased pHi and decreased concentration of Ca^{2+} in MDCK cytoplasm post influenza A virus infection. **Conclusion:** The current study presents

for the first time the potential of iridoid glycoside extracted from Fructus Gardeniae to inhibit the ion channel activity mediated by influenza A virus M2 protein.

Keywords: iridoid glycoside; Fructus Gardeniae; influenza A virus; M2 protein; pH

S2.10

Evaluation of hypersusceptibility reactions caused by Chaihu Zhusheye

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Aim: Hypersusceptibility reactions were reported caused by Chaihu Zhusheye (CHZSY) in clinical practice. In this study, anaphylaxis and anaphylactiod reaction tests of CHZSY *in vivo* and *in vitro* were investigated. **Methods:** Active systemic anaphylaxis (ASA) were primed and challenged by CHZSY in guinea pigs *in vivo*, and evaluation was taken according to the guidelines for ASA. Direct effects of CHZSY on with or without sensitized RBL-2H3 cells β-hexosaminidase release and rat peritoneal mast cells (RPMC) histamine release, and alteration of CD200R expression by CHZSY in mice basophils, were determined *in vitro*. **Results:** Although all guinea pigs developed anaphylaxis in positive control, no anaphylaxis reaction was found primed and challenged by CHZSY. β-hexosaminidase release in with or without sensitized RBL-2H3 cells, histamine release in RPMC and alteration of CD200R expression in mice basophils, showed no significant difference with negative control. **Conclusion:** No anaphylaxis or anaphylactiod reaction caused by CHZSY was found in this study. Hypersusceptibility reactions of CHZSY developed in clinical practice may relate to overdose, medication error.

Keywords: Chaihu Zhusheye; hypersusceptibility reactions; RBL-2H3 cells; RPMC; CD200R

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S2.11

Research development of traditional Chinese medicines in drug eluting stents

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Drug eluting stents (DESs) have revolutionized the interventional cardiology over the past decade since their arrival in 2002. Compared to bare metal stents that are deployed to keep the vessel open by mechanical force, DESs have an additional function of reducing restenosis by the action of the drug on the target site. This paper reviews the current status of the Traditional Chinese Medicines (TCMs) used as coating drug of coronary stent. Currently, paclitaxel is the most mature application TCM, such as Taxus Liberté DES of Boston Scientific Corporation abroad, YINYI micro-blind porous DES of Dalian domestic; Secondly, arsenic trioxide DES was approved by China Food and Drug Administration in 2012; Then, triptolide, ligustrazine, emodin, curcumin and puerarin are *in vitro* and *in vivo* studies; Finally, many other TCMs with inhibit the proliferation of vascular smooth muscle cells may be potential applications used in DES, such as breviscapine, resveratrol, ursolic acid, hydrastis, scutelloside, britannin, gambogicacid, etc. The TCMs with multiple pathways regulating effect of cardiovascular will be a research focus of new DES.

Keywords: drug eluting stent; traditional Chinese medicines; restenosis; thrombosis

S2. 12

Total glucosides of Danggui Buxue Tang ameliorates bleomycin-induced pulmonary fibrosis by inhibiting the TGF-β1/ Smad pathway and altering the MMP2/TIMP1 ratio

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Aim: Pulmonary fibrosis (PF) is a major cause of lung failure, but treatment remains ineffective. Our previous studies suggested that total glucosides of Danggui Buxue Tang (DBTG) had an inhibitory effect on bleomycin-induced lung fibrosis and its effect may be associated with the ability of DBTG to inhibit the synthesis of extracellular matrix and balance the MMPs/TIMPs system. **Methods:** In the present study, we further focused on the potential mechanisms of DBTG in the rat model of lung fibrosis induced by bleomycin and in TGF-β1-stimulated human fetal lung fibroblasts (HFL-1). **Results:** Treatment with DBTG significantly attenuated bleomycin-induced lung fibrosis and functional impairment in a dosage-dependent manner, including blockade of the differentiation of lung myofibroblast as determined by inhibiting alpha smooth muscle actin (a-SMA) and collagen-I expression. In addition, DBTG decreased the expression of TGF-β1, Smad3, and p-Smad3, which are all important members of the TGF-β/Smad signaling pathway. The anti-fibrosis activity and mechanisms of DBTG were further detected in vitro in HFL-1 cells treated with TGF-β1. The TIMP-1 protein levels were significantly decreased following treatment with DBTG while MMP-2 protein expression increased remarkably, thereby inhibiting TGF-β1-mediated extracellular matrixc (ECM) accumulation. **Conclusion:** Our study provides evidence that DBTG significantly ameliorated bleomycin-induced PF in rats via inhibiting the TGF-β/Smad signaling and altering the MMP2/TIMP1 ratio.

Keywords: pulmonary fibrosis; bleomycin; Danggui Buxue Tang; TGF-β; Smad

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S2.13

Efficacy of Chinese medicine ShuGanJieYu capsule for functional dyspepsia

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Aim: To assess the efficacy and safety of Chinese medicine ShuGanJieYu (SGJY) capsule for the treatment of functional dyspepsia (FD). **Methods:** Medline database, Cochrane Library, Chinese National Knowledge Infrastructure database, Chinese Biomedical Literature database, Wanfang database, and VIP database were strictly examined for relevant publications. Study selection, data extraction, quality assessment, and data analyses were conducted according to Cochrane Collaboration procedures. **Results:** Nine randomized controlled trials (RCTs) in total met inclusion criteria. All included trials had an unclear risk of bias. The summary odds ratio for symptom improvement was 3.57 (95% CI: 2.53–5.05), indicating FD patients with SGJY -mosapride combination treatment have over 3.5-fold higher probability of symptom relief compared with the patients with only mosapride treatment. The summary odds ratio for recurrence rate was 0.18 (95% CI: 0.09–0.37), indicating a significant decrease of recurrence rate of SGJY -mosapride combination treatment. **Conclusion:** SGJY-mosapride combination treatment might be an effective therapeutic regimen for FD patients. The benefit need to be confirmed in rigorously designed, multicentre, and large-scale trials.

Keywords: Shu Gan Jie Yu capsule; functional dyspepsia; meta-analysis

S2.14

The experimental study on model rats of fever due to yin-deficiency climacteric syndrome by Yang Ren Tiao Chong Soup

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Aim: To observe the effect of Yang Ren Tiao Chong Soup on model rats of fever due to yin-deficiency climacteric syndrome. **Methods:** The specific pathogen free (SPF) SD female rats were used as the experimental animal. Rat models of fever due to yin-deficiency climacteric syndrome were induced by removal of bilateral ovaries combination with perfusion of hot Chinese medicine. The changes of model rats' water intake, the temperature of anus, the rate of keratinocytes vaginal epithelial cells, the uterine coefficient and a variety of serum hormone level were all observed. **Results:** After Yang Ren Tiao Chong Soup treatment, the model rats' water intake was reduced and the temperature of anus was declined. The serum level of FSH, LH and PRL were decreased, but the level of E_2 was increased. The rate of keratinocytes vaginal epithelial cells was increased and the uterine coefficient was increased too. **Conclusion:** Yang Ren Tiao Chong Soup has a better regulation on neuroendocrine disorders of rats with fever due to yin-deficiency climacteric syndrome, which will provide theoretical basis for clinical treatment of climacteric syndrome by Yang Ren Tiao Chong Soup.

Keywords: Yang Ren Tiao Chong Soup; fever due to yin-deficiency; climacteric syndrome; hormone

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S2.15

Study on antibacterial effect of Tibetan medicine Qiweihonghuashusheng pills *in vitro*

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Aim: To investigate the antibacterial effect of Tibetan medicine Qiweihonghuashusheng pills in vitro and provide theoretical basis for clinical application. **Methods:** M-H agar well diffusion method was adopted to research the antibacterial effect of Tibetan medicine Qiweihonghuashusheng pills on three standard strains(Staphylococcus aureus ATCC25923, Escherichia coli ATCC25922, Salmonella CMCC50319) and 20 clinical isolates; the minimal inhibitory concentration (MIC) of sensitive strains were determined by the method of broth two-fold dilution. **Results:** Qiweihonghuashusheng pills had obvious antibacterial effect on Gram-positive bacteria *in vitro*, but weak inhibition on Gram-negative bacteria. The MIC to Staphylococcus aureus, Staphylococcus epidermidis and Staphylococcus huminis were between 6.25 mg/mL and 12.5 mg/mL. **Conclusion:** Qiweihonghuashusheng pills had significant antimicrobial effect on common gram-positive clinical isolates *in vitro*, which indicated high value in clinical application.

Keywords: Tibetan medicine; qiweihonghuashusheng pills; antibacterial effect *in vitro*

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S2.16

Paeoniflorin inhibit human fibroblast-like synoviocytes proliferation induced by PGE_2 via arresting EP2 receptor internalization

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Aim: This work was designed to investigate the potential molecular mechanism of Paeoniflorin (Pae) in inhibiting PGE_2 induced human fibroblast-like synoviocytes (hFLS) hyperplasy. **Methods:** Human FLSs were cultured with prostaglandin E_2 (PGE_2) in the presence of different concentrations of Pae. Synoviocyte proliferations were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl (MTT) assay. The expressions of EP2, Gαs, β-arrestin2, PKA in hFLS were measured by western blot or flow cytometry. Massage RNA change of EP2 and β-arrestin2 were detected by quantitative PCR. Cyclic adenosine monophosphate (cAMP) levels in synoviocytes were assessed by radioimmunoassay (RIA). **Results:** As we detected, Pae significantly inhibited hFLS proliferation induced by PGE_2 associating with an increase in cAMP levels. High levels of total EP2, β-arrestin2 and PKA expression and EP2 and β-arrestin2 mRNA in hFLS induced by PGE_2 were restored with different concentration of Pae administration. EP2 downregulated and β-arrestin2 upregulated in hFLS membrane after PGE_2 stimulation, however, both were improved by Pae in various degrees. **Conclusion:** Our findings suggest Pae inhibit human fibroblast-like synoviocytes proliferation induced by PGE_2 via arresting EP2 receptor internalization by inhibiting β-arrestin2 recruit to cell membrane.

Keywords: rheumatoid arthritis; synoviocytes; β-arrestin; receptor internalization; Paeoniflorin

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S2.17

Poria cocos extract inhibited the activation of hepatic stellate cells possibly via NFκB and PPARγ pathways

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Aim: Activation of hepatic stellate cells (HSCs) plays a key role in hepatic fibrosis and chronic liver diseases. In this study, we investigate if *Poria cocos* (a commonly used Chinese herb with anti-inflammatory activities) inhibits the NF-κB-related activation of hepatic stellate cells (HSCs). **Methods:** Two HSC lines, HSC-T6 (rat) and LX-2 (human), were used. These cells were pretreated with different subfractions extracted from *P cocos* and then stimulated with TNF-α. The roles of NF-κB and peroxisome proliferator-activated receptor gamma (PPARγ) activities were assessed using reporter gene assay. Nuclear translocation of NF-κB and expression of α-smooth muscle actin (α-SMA) were detected by Western blotting. NF-κB binding was evaluated by EMSA. RT-PCR was conducted to analyze the mRNA expressions. **Results:** Pretreatment of an ethyl acetate extract of *P cocos* (PC-EA, 3.125–25 g/mL) concentration-dependently suppressed TNF-α-stimulated NF-κB luciferase activities, nuclear translocation and DNA binding of NF-κB subcomponent p65, protein and mRNA expressions of α-SMA and *ICAM-1* transcription in both HSC-T6 and LX-2 cells. Whereas, PC-EA up-regulated TNF-α-suppressed PPARγ luciferase activities and *PPAR* transcription. No obvious cytotoxicity (MTT assay) of PC-EA was observed within the concentration. **Conclusion:** Our results suggest that an ethyl acetate extract of *P cocos* inhibited TNF-α stimulated HSC activation possibly through NF-κB and PPARγ modulation.

Keywords: Hepatic fibrosis; *Poria cocos*; hepatic stellate cell; NF-κB; PPARγ

S2.18

Evaluation of the anticataract effects of magnesium taurate *in vitro*

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Aim: Magnesium (Mg) is important in the lens epithelial Na-K-ATPase activity, which in turn maintains a low intracellular sodium and calcium and a high potassium. Mg also plays a role in preserving antioxidant activity, including reduced glutathione (GSH), superoxide dismutase (SOD) and catalase. Taurine also preserves lenticular redox status. Cataractous lenses have reduced taurine and Mg contents. The present study was done to evaluate the anticataract effects of Mg taurate (MgTt) *in vitro*. **Methods:** Lenses from normal *Sprague Dawley* rats were isolated and divided into 3 groups of 10 lenses each. The lenses in groups 1 and 2 were incubated for 48 hours in Dulbecco's Modified Eagle's Medium (DMEM) alone and in DMEM with 30 mmol/L galactose respectively, whereas group 3 lenses were incubated in DMEM with 30 mmol/L galactose and MgTt 1%. Post-incubation lenses were examined for opacity. Five lenses from each group were subjected to estimation of calcium-to-magnesium ratio (Ca/Mg) and five lenses to measure GSH, catalase and SOD activities. **Results:** In group 2, 50% lenses and in group 3, 10% lenses showed dense cortical opacity. The mean Ca/Mg in group 2 (1.75±0.08) was significantly higher compared to group 1 (1.51±0.14). Mean Ca/Mg in group 3, which was supplemented with MgTt, amounted to 1.64±0.03 and did not show differences from either group 1 or 2. Group 1 and 3 showed 1.53 and 1.4 times higher lens GSH contents as compared to group 2. The catalase activity was normalized in group 3 but was significantly lower in group 2 ($P<0.001$ versus group 1 and 3). The SOD was significantly higher in group 2 compared to group 1 ($P<0.05$). SOD activity in group 3 did not show significant difference from either group 1 or 2. **Conclusion:** This study demonstrated that MgTt delays the onset and progression of galactose-induced cataract *in vitro* by restoring the lens Ca/Mg and lens redox status.

Keywords: cataract; *in vitro* study; Mg taurate; magnesium; calcium; glutathione; catalase; superoxide dismutase

S2.19

Effect of Panax notoginseng saponins on cytokines in liver fibrosis rats

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Aim: Objectives: To observe the effects of Panax notoginseng saponins(PNS) on cytokines production in liver fibrosis rats, explore the mechanism. **Methods:** The rats were randomly divided into six groups, including normal group, liver fibrosis model group, PNS (50, 100, 200 mg/kg) treated groups and Col group. Rat liver fibrosis was induced by CCl_4 twice a week for 18 weeks. PNS were used daily via lavage at 9th week for 10 weeks. The interleukin (IL)-1, IL-6, IL-10, tumor necrosis factor (TNF)-α level in serum were determinate by radioimmunity. Liver samples were taken to examine the degree of liver fibrosis by HE and stained by

immuninochemistry of nuclear factor(NF)-κB, transforming growth factor (TGF)-β. Moreover TGF-β, TNF-αmRNA expression was detected by RT-PCR technology. **Results:** As compared with the fibrotic model group, PNS (100, 200 mg/kg) not only significantly reduced histopathological change, but also effectively prevent the level of IL-1, IL-6, NF-κB, TNF-α, TGF-β, and step up level of IL-10. **Conclusion:** PNS has protective effect on liver fibrosis by regulating cytokine network.

Keywords: PNS; liver fibrosis; cytokines; rat

S2.20

Effects of Liuwei Dihuang decoction and its compatible prescriptions on urine metabolites of cortisone acetate induced kidney-Yin deficiency mouse model

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Aim: To investigate the effects of Liuwei Dihuang decoction (LW) and its San-bu (three tonics) and San-xie (three eliminators) components on urine metabolites of kidney-Yin deficiency mouse model induced by cortisone acetate. **Methods:** The kidney-Yin deficiency mouse model was induced by glucocorticosteroid. The methodology of the metabonomic approach consisted of high-resolution ^{1}H nuclear magnetic resonance (NMR) spectroscopy and multivariate statistical technique for the establishment of urine metabolic patterns of the treatment mice. In the study, 24h urine was collected pre-dose and at d 7 post-dose after mice were injected with cortisone acetate at 25 mg/kg. The acquired data were transferred into Simca-P 10.0 software to be processed using pattern recognition analysis. **Results:** The results showed that the urine metabolic patterns of kidney-Yin deficiency mouse model and control group were significantly different. Differential metabolites observed between model and control group included ten increased metabolites, such as acetate, succinate, citrate, etc and six decreased metabolites, such as leucine, phenylalanine, tyrosine, *etc*. Oral administration of LW, San-bu and San-xie affected urine metabolic patterns of kidney-Yin deficiency mouse model in different levels. LW, San-bu and San-xie acted on twelve, fourteen and twelve metabolites respectively. There were similar and differential metabolites that LW, San-bu and San-xie acted on. LW acted on hippurate, taurine, citrate specially, while San-bu on alanine. The differential metabolites that San-bu and San-xie acted on are alanine, dimethylamine and citrate. **Conclusion:** Kidney-Yin deficiency mouse model showed metabolism disturbances of glycose, energy and amino acid. LW, San-bu and San-xie improved metabolism disturbances of Kidney-Yin deficiency mouse model and the actions of them were different.

Keywords: Liuwei Dihuang decoction; Kidney-Yin deficiency mouse model; Metabolite

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S2.21

Endothelium-dependent vasorelaxation induced by an ethanol extract of *Rubus chingii* Hu (Rosaceae)

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Aim: *Rubus chingii* Hu (RC) is an important traditional Chinese medicine. It has been shown to have antioxidant and antiinflammatory effects. The present study was performed to evaluate the vascular effects of ethanol extract of RC (ERC) in rats. **Methods:** ERC was examined for their vascular relaxant effects in phenylephrine-precontracted aortic rings. **Results:** ERC induced vasorelaxation in a concentration-dependent manner. Endothelium-denudation abolished the EEH-induced vasorelaxation. Pretreatment of the endothelium-intact aortic rings with L-NAME and ODQ significantly inhibited the ERC-induced vasorelaxation. Extracellular Ca^{2+} depletion and treatments with thapsigargin and Gd^{3+}, modulators of the store-operated Ca^{2+} entry (SOCE), significantly attenuated the ERC-induced vasorelaxation. Wortmannin, an inhibitor of Akt, markedly attenuated the ERC-induced vasorelaxation. **Conclusion:** The present study demonstrates that ERC induces endothelium-dependent vasorelaxation via Akt- and SOCE-eNOS-NO-cGMP signaling.

Keywords: *Rubus chingii* Hu; Akt; eNOS; nitric oxide; Ca^{2+}

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S2.22

Effects and the mechanism of lignans from *Eucommia ulmoides* on rat mesangial cell proliferation induced by Ang II

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Aim: To study the effects of lignans from *Eucommia ulmoides* on the proliferation in rat mesangial cell induced by Ang II, providing theoretical basis for the application of lignans from *Eucommia ulmoides* in renoprotection. **Methods:** Rat glomerular mesangial cell (HBZY-1) were cultured in vitro and divided into 5 groups: control group, Ang II group (10^{-8} mol/L Ang II), low concentration lignans group (10^{-8} mol/L Ang II+20 mg/L lignans), middle concentration lignans group (10^{-8} mol/L Ang II+40 mg/L lignans), high concentration lignans group (10^{-8} mol/L Ang II+80 mg/L lignans). The proliferation of cell was assessed by MTT after 24, 36, and 48 h treatment. The cell cycle and cell apoptosis were determined by flow cytometre. The mRNA and protein level of P21, P27, Bax, Bcl-2, and AR in HBZY-1 were determined by RT-qPCR and Western Blot. The activity of AR was detected by UV determination. **Results:** The cellular proliferation induced by Ang II was significantly suppressed by *Eucommia* lignans. In addition, Treatment with *Eucommia* lignans increased both the mRNA and protein levels of P21, P27 and Bax except Bcl-2. Meanwhile, the mRNA, protein expression and activity of AR was decreased by *Eucommia* lignans. **Conclusion:** The proliferation of HBZY-1 was inhibited by lignans via regulating cell cycle and cell apoptosis. To the mechanism, AR potentially play a key role in this process.

Keywords: Ang II; celluar proliferation; cell cycle; cell apoptosis; lignans from *Eucommia ulmoides*; aldose reductase

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S2.23

Protective effect of FLC against LPS/ D-GalN-induced liver injury in mice

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Aim: The aim of the the present study was to investigate the effects of FLC, a plant-derived compound, on the acute liver injury in mice induced by LPS/ D-GalN. **Methods:** A mixture of LPS/D-GalN was administered intraperitoneally to prepare an animal model of acute liver injury. FLC (10, 20, and 40 mg/kg) were orally administered 0.5 h after LPS/D-GalN injected in mice. The survival rate was measured in 48 h after LPS/GalN injection. Blood and liver tissue samples were collected 6h after LPS/GalN injection. The levels of serum AST, ALT, IFN-γ, and TNF-α were measured. Liver tissue pathology were observed by HE staining. **Results:** In the model group, only less than 20% animals survived 48h after the LPS/D-GalN injection. The serum levels of AST, ALT, TNF-α, and IFN-γ increased 6h after LPS/GalN administration, and histologic changes in the model group indicated hepatic cell damage. Treatment of the mice with FLC (20 and 40 mg/kg) improved the survival rate markedly ($P<0.05$ and $P<0.01$ respectively) and could reduce the serum AST, ALT, TNF-α, and IFN-γ levels. **Conclusion:** FLC attenuated the LPS/GalN-induced hepatic damage. This study indicated that FLC may be clinically useful in patients with acute liver injury.

Keywords: FLC; LPS/D-GalN; survival rate; AST; ALT

S2.24

Effects of total saponins of chaenomeles speciosa on the number and degranulation ratio of mast cells and expression of tryptase in synovium of rats with adjuvant arthritis

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Aim: To observe the effects of total saponins of Chaenomeles speciosa (TSCS) on synovial pathology,synovial mast cell degranulation and tryptase expression and to investigate the relationship between the functions of mast cells and effects of TSCS on adjuvant arthritis (AA) in rats. **Methods:** Male Wistar rats were randomly divided into normal control group,model group and TSCS group (200 mg/kg), *n*=15 in each group. AA was induced by injection of 0.1 mL Freund's complete adjuvant in left hind limb footpad. Normal control group and model group received saline treatment,while rats in the TSCS group were treated once a day for 21 d. The body weight and paw volume of the rats were measured every 6 d. Synovial tissues of the right hind ankles were sampled and stained with HE for observing synovial pathology to evaluate the effects of TSCS on AA, then stained with TB for observing the number and degranulation ratio of synovial mast cells and finally detected by immunohistochemical staining method to investigate the expression of tryptase in synovium. **Results:** TSCS increased significantly the body weight of AA rats, while decreased obviously the paw volume. TSCS significantly inhibited inflammatory cell infiltration, synovial cell hyperplasia, and synovial fibroplasia in synovium of AA rats. TSCS could significantly diminish the numbers of total and degranulated mast cells in AA rats, TSCS decreased the expression of tryptase in synovium. Analyzed by Spearman's bivariate correlation, the number of mast cells and degranulation ratio of mast cells were positively correlated with the pathological scores. **Conclusion:** TSCS can improve pathological condition of inflammatory synovium in AA rats by inhibiting the function of synovial mast cells, which may play an important underlying role in the immunoregulation of TSCS on AA.

Keywords: total saponins of Chaenomeles speciosa; adjuvant arthritis; mast cells; degranulation; tryptase
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S2.25

The anti-influenza A virus effect of theaflavin derivatives

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Aim: To study the anti-influenza A virus effect of a commercially available natural product preparation with high content (>85%) of theaflavin derivatives which contained TF1, TF2A, TF2B, and TF3, designated as TFmix. **Methods:** Inhibition of viral-entry by TFmix was determined with luciferase asssy in MDCK cells infected with A/Thailand/Kan353/2004 H5N1 pseudovirus. Hemagglutination inhibition assay and neuraminidase inhibition assay were further used to investigate the mechanism for its anti-influenza activity. Anti-influenza activity of TFmix was further confirmed in MDCK cells infected with influenza A virus FM_1 strain. Cytotoxicity was determined by MTT assay. **Results:** TFmix significantly inhibited entry of H5N1 pseudovirus into MDCK cells with IC_{50} of 151.88±18.95 μg/mL. It had no effect on HA1 submit. TFmix also significantly inhibited activity of neuraminidase with IC_{50} of 129.09±1.33 μg/mL. TFmix showed a significant anti-influenza viral effect on FM_1 in MDCK cells. TFmix showed low cytotoxicity on MDCK cells with CC_{50} of 879.89±4.54 μg/mL. **Conclusion:** TFmix can inhibit influenza A virus *in vitro*. TFmix can also inhibit neuramidinase activity to some extent.

Keywords: Theaflavin derivatives (TFmix); influenza A virus; H5N1 subtype; H1N1 subtype; hemagglutinin; neuraminidase

S2.26

Research of pharmacodynamics and mechanism of ChaiQingxiaopi capsule on hyperplasia of mammary glands

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Aim: To study the therapentic effect of ChaiQingxiaopi capsule (CQC) on the hyperplasia of mammary glands (HMG) in rats and analyze the possible mechanism in treating HMG. **Methods:** (1) Seventy rats were divided into control, model, tamoxifen, Xiaoru Sanjie capsule, low-, mid-, and high-dose CQC (0.433, 0.855, and 1.712 g/kg) groups. After 5 weeks treatment, we measured the height and diameter of nipple, then determined viscera indexes and the levels of estrogen (E_2), progestogen (P), prolactin (PRL), testosterone (T), superoxide dismutase (SOD) and malondialdehyde(MDA) in serum; and the pathologic changes in morphology of mammary glands were observed; (2) The acute blood stasis rat model was established with subcutaneous injection of 0.1% adrenaline hydrochloride and ice water bath . After 1 week treatment, blood was taken from the abdominal artery, and then its blood viscosity and plasma viscosity were measured by blood rheometer; (3) The expression of ERa and PR in mammary gland epithelial cells was analyzed by immuno-histochemistry. **Results:** CQC could remarkably reduce the diameter and height of the nipple, increase the index of thymus and spleen, reduced the index of the uterus and ovaries. It could also increase E_2, P, PRL, and decrease T level in serum, and increase SOD activity and decrease MDA(P<0.05 or P<0.01); CQC can effectively improve the blood viscosity and increase the anti-stress ability (P<0.05 or P<0.01); (3) The more the hyperplasia of mammary glands is, the contents of ER and PRis likely high, CQC group can significantly reduce the mammary gland epithelial cells of the ERa and PR expression (P<0.01). **Conclusion:** CQC has significant therapeutic effect on HMG, it can adjust the sex hormones levels in serum; improve the immunity, oxidative stress and blood rheology; and reduce the expression of ER alpha and PR in mammary gland epithelial cells. Its mechanism may be associated with CQC has a certain effect of phytoestrogen, on pituitary-the hypothalamus- gonadal axis has certain influence, by lowering the content of ER alpha in mammary glands or reduce mammary glands sensitivity to the ER alpha, the race for competitive estrogen receptors.

Keywords: ChaiQingxiaopi capsule (CQC); hyperplasia of mammary glands (HMG); pharmacodynamics; mechanism

S2.27

Effect of baicalin on phosphorylation of TBC1D1 in skeletal muscle

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Aim: Baicalin can improve insulin sensitivity. but little is known about the underlying mechanism. Whether baicalin prevent abnormal glucose tolerance in high fat diet mice. whether baicalin regulate insulin signaling pathway in skeletal muscle. **Methods:** Divide mice into 4 groups randomly: Normal control group; High-fat model group; Baicalin (300 mg/kg) intervention group and Rosiglitazone (3mg/kg) intervention group in high-fat diet model. Detect blood glucose and blood lipid after eating normal feed and high fat feed respectively for 6 weeks. Dissect and detect expression of Akt and its substrate TBC1D1 in soleus muscle by Western blot. **Results:** Fasting blood glucose and blood lipid in high-fat diet group was obviously decreased after baicalin intervention(P<0.05).The blood glucose also decrease significantly in 0.5, 1, and 2 h after intraperitoneal injection of glucose (P<0.05),while the expression of phosphorylated Akt and TBC1D1 increased to the level close to normal control group. **Conclusion:** Abnormal glucose tolerance exists in high-fat diet mice, Baicalin can improve abnormal glucose tolerance in high-fat diet mice. One of the mechanisms may improve insulin resistance by increasing the expression of phosphorylated Akt and phosphorylated TBC1D1 in skeletal muscle.

Keywords: baicalin; glucose tolerance; skeletal muscle; Akt; TBC1D1

S2.28

The fatigue-resisting action of *Pholidota chinensis lindl*

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Aim: The effects of extractive isolated from *Pholidota chinensis lindl* were observed on oxygen-deficient endurance and exercise tolerance in mice. **Methods:** The survival times and gasping duration in the models of hypoxia were detected after administration. In the study of extract on the ability of hypoxia endurance at normal pressure, mice were divided by normal saline, extracts (5, 10 g/kg, and hydrochloric propranolol. The study of the antagonism of sodium nitrite, mice were randomly divided into normal saline, hydrochloric propranolol, extract (5 and 10 g/kg). The study on exercise tolerance, mice were randomly selected into normal saline, extract (5 and 10 g/kg). **Results:** In the study of extract on the ability of hypoxia endurance at normal pressure. The survival time at normal pressure was prolonged by extracts in a dose-dependent manner. In the study protective effects of extract on survival time after peritoneal injection $NaNO_2$ in mice. The

survival time was prolonged by extracts in a dose-dependent manner from 18.10±1.85 min for normal saline to 27.70±4.50 min (5 g/kg) , 39.00±4.16 (10 g/kg) min, respectively showed significant prolongation of survival times after peritoneal injection $NaNO_2$ in mice. In the test on exercise tolerance in mice: The survival time of the swimming was prolonged by extracts in a dose-dependent manner from9.57±6.27 min for normal saline to 14.40±2.76 min (5 g/kg) and 25.10+3.21 (10 g/kg) min,respectively. **Conclusion:** The effects of extractive isolated from *Pholidota chinensis lindl* enhanced the ability of the fatigue-resisting in a dose-dependent manner.

Keywords: pholidota chinensis lindl; fatigue-resisting; oxygen-deficient

S2.29

Neuroprotective effect of Danqimingmusan extract on rat retinal damage induced by NMDA

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Aim: We investigated the neuroprotective effect of Danqimingmusan extract (DE) using a rat model of retinal damage induced by N-methyl-D-aspartate (NMDA) in the present study. **Methods:** Twenty Sprague-Dawley rats were divided into five groups: the NMDA group and DE groups received intravitreal injection of NMDA (2 μL, 40 nmol/L); rats in DE groups were orally given different dosages of DE (3.903, 7.805, and 15.61 g/kg, bw) 7days before NMDA treatment; rats in the control and NMDA group received oral saline. **Results:** The percentage of neuron numbers in retinal ganglion cell layer greatly increased in a dose-dependent manner (69%±6%, 89%±7% and 103%±7% of control) after the 7 days treatment with DE in comparison to NMDA group (51%±6% of control, $P<0.05$). In addition, the oral administration of 7.805 g/kg DE significantly increased SOD activity and decreased MDA level in the serum as compared with NMDA group ($P<0.001$). **Conclusion:** These findings indicated that DE can reduce the NMDA-induced lipid peroxidation of rat retina by improving SOD activity and inhibiting MDA production, and which may stop the loss of retina ganglion cells and repair the damaged retina.

Keywords: Danqimingmusan extract; NMDA; SOD; MDA; Retina

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S2.30

Protective effects of the extracts from *Rhizoma Imperatae* on adriamycin-induced renal damage in rats

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Aim: *Rhizoma Imperatae* was traditionally used as diuretic and anti-inflammatory agents in China. In this study, the protective effects of *Rhizoma Imperatae* extracts on adriamycin-induced renal damage in rats were evaluated. **Methods:** The extract was extracted from *Rhizoma Imperatae* with 70% EtOH, and partitioned with petroleum ether, ethyl acetate, *n*-butanol to petroleum ether fraction (PEF), ethyl acetate fraction (EAF), *n*-butanol fraction (BF), and aqueous fraction (AF). Renal damage in rats were induced by adriamycin (ADR), and treated with PEF, EAF, BF, AF, and prednisolone acetate for 8 weeks. Body weights, organ weights, 24-h urinary volume (UV), 24-h urinary protein (UPR) and serum biochemical parameters and morphological changes were measured. **Results:** Body weights of ADR rats treated with extracts were partly recovered. The UPR levels of EAF and AF were reduced at week 6th ($P<0.05$), those of BF were reduced at week 7th ($P<0.01$). Alb and TP levels of EAF were higher than those of ADR group ($P<0.05$). Comparing with ADR group, treated groups had lower ($P<0.05$) TG or TC levels and similar SCr or BUN levels. The pathomorphological changes of renal tissue in treated group were evidently alleviated. **Conclusion:** *Rhizoma Imperatae* extracts possess certain protective effects in adriamycin induced nephropathy rats, among which ethyl acetate extract showed significant activity.

Keywords: *Rhizoma Imperatae*; adriamycin induced nephropathy rats; glomerulonephritis

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S2.31

Hepatoprotective effect of the flavonoid fraction isolated from the flower of *Inula britannica* against D-Galactosamine-induced hepatic injury

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Aim: The aim of this study was to investigate the mechanism and the protective effect of *Inula britannica* flower flavonoids (IBFF) on antioxidants and the inhibition of inflammation in liver injury. **Methods:** IBFF 125, 250, and 500 mg/kg was administered orally once a day for consecutive 7 d. D-Galactosamine (D-Gal) was used to induce liver injury at 850 mg/kg by intraperitoneal injection in mice. The alanine aminotransferase (ALT), aspartate aminotransferase (AST), malonaldehyde (MDA) and the antioxidant enzymes such as superoxide dismutases (SOD), glutathione peroxidase (GSH-PX) and catalase (CAT) were determined. Furthermore, the levels of the tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were measured by ELISA kits and their mRNA were analysed by RT-PCR method. **Results:** The results showed that ALT, AST and MDA were increased as well as TNF-α, iNOS, COX-2 and their mRNA levels in the liver, while SOD, CAT, and GSH-PX activities were decreased in the D-Gal-treated as compared with the control. However, the administration of IBFF to D-Gal-induced hepatic injury mice reversed the increases in ALT, AST, and MDA, and the decreases in SOD, GSH-PX and CAT. IBFF attenuated the D-Gal-induced increases in TNF-α, iNOS, and COX-2 and their mRNA levels in the liver. **Conclusion:** The results suggested that the protective effect of IBFF against D-Gal-induced hepatic injury maybe associated with its antioxidative and anti-inflammatory activities.

Keywords: IBFF; acute liver injury; D-Gal; antioxidation; anti-inflammation

S2.32

Modulation of endothelial progenitor cell number and function with *Panax notoginseng* saponins

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Aim: Endothelial dysfunction is pivotal in atherosclerosis. Endothelial progenitor cells (EPCs) play a key role in the homeostasis of damaged vascular repair. Previous findings from our laboratory have shown the benefits of Panax notoginseng saponins (PNS) on atherosclerosis. The present research was designed to explore the effect of PNS on EPCs number, functionality and the underling mechanisms. **Methods:** EPCs were isolated from mice bone marrow and treated with different concentrations (50, 100, and 150 μg/mL) of PNS for 48 h. The number of EPCs and colony forming units (CFU) were counted. The proliferation, migration and adhesion were evaluated using the cell counting kit-8 (CCK-8), Transwell assay and adhesion assay, respectively. The mRNA and protein expression levels of cell-derived factor 1α (SDF-1α) and its receptor CXCR4 were determined by real-time PCR and Western blot. **Results:** PNS significantly increased the number of EPCs and CFU in a concentration-dependent manner. In addition, PNS (100 and 150 μg/mL) treatment significantly enhanced the proliferation, migration and adhesion of EPCs *in vitro*, the increased mRNA and protein expression levels of SDF-1α and CXCR4 were detected as well. **Conclusion:** Our findings suggest that PNS are effective in improving EPC number and functionality *in vitro*. SDF-1α-CXCR4 interactions and the possible modulatory role of PNS may contribute to increased EPC number and functionality.

Keywords: endothelial progenitor cell; Panax notoginseng saponins; SDF-1α; CXCR4

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S2.33

Comparison of toxic reaction of Zhuanggu-guanjie pill in different model rats of osteoarthritis

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Aim: Zhuanggu-guanjie pill (ZGGJP) is a kind of effective chinese patent drug to osteoarthritis(OA) with kidney deficiency (KDOA), but certain side effects occur in clinic. This study was to determine whether ZGGJP induced different toxicity in rats with KDOA compared to those in normal rats. **Methods:** Rat model with KDOA was established by oophorectomy and anterior cruciate ligament transection and partial medial meniscectomy in the right knee joint after 4 weeks. Six groups were included as follows: sham operation (CON), OA, KDOA, ZGGJP for sham operation (CON-T), ZGGJP for OA (OA-T), ZGGJP for KDOA (KDOA-T). ZGGJP was orally administrated as 1.11g/kg/d for 30 days after OA operation. Finally, livers were harvested for organ coefficients, histopathologic and antioxidant indexes, serum for related biochemical indexes. **Results:** Organ coefficients in CON-T increased apparently and histopathologic obvious changes in livers. Aspartate aminotransferase, alkaline phosphatase andγ-glutamyltransferase levels were increased significantly in KDOA-T, albumin level in KDOA and KDOA-T declined markedly, alanine aminotransferase level in CON-T had obvious changes. ZGGJP reduced superoxide dismutase level but dramatically increased malondialdehyde level. **Conclusion:** Traditional Chinese medicines needed to be applied properly according to syndrome differentiation for fewer side effects.

Keywords: Zhuanggu-guanjie pill; osteoarthritis; kidney deficiency; liver toxicity
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S2.34

Ferulic acid protects endothelial cells from radiation induced oxidative stress through Nrf2-ARE pathway.

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Aim: Ferulic acid (FA), a phenolic phytochemical, effectively scavenges superoxide anion radical and inhibits the lipid peroxidation. The mechanism of FA of protecting human umbilical vein endothelial cells (HUVECs) from radiation induced oxidative stress was investigated in the present study. **Methods:** The influence of FA on expression of antioxidant factors (glutamate-cysteine ligase catalytic subunit (GCLC), glutamate-cysteine ligase regulatory subunit (GCLM), NADPH quinone oxidoreductase-1 (NQO1) and heme oxygenase-1 (HO-1) was observed on both protein and genetic level by means of biological methods such as Western blot and RT-PCR. The upstream signaling pathway involved in FA mediated Nrf2 activation was determined by signaling inhibitors. **Results:** FA significantly increased the transcription of antioxidant related genes such as GCLC, GCLM, NQO1, and HO-1 mRNA in radiated cells, and these are involved in a significant increase the intracellular GSH content and the expression of NAPDH. FA evidently promoted nuclear factor erythroid 2-related factor 2 (Nrf2) translocation into nuclei and increased the intracellular GSH and NADPH levels in radiated cells. Phosphatidylinositol 3-kinase (PI3K) and extracellular signal regulated kinase (ERK) pathways were associated with FA-induced Nrf2 activation. **Conclusion:** The present study demonstrated that FA-induced Nrf2 activation is the major regulatory pathway of cytoprotective gene expression against oxidative stress via PI3K and ERK signaling pathways.

Keywords: Ferulic acid; Radiation; Nuclear factor erythroid 2-related factor 2; Oxidation
Acknowledgements: This work was supported by the National Natural Science Foundation of China (81073028 and 81130067).

S2.35

Protective Effect of gypenosides against fatty liver disease induced by high fat and cholesterol diet and alcohol in rats

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Aim: The protective effects of gypenosides from *Gynostemma pentaphyllum* throughout fatty liver disease (FLD) were examined in rats treated with high fat and cholesterol diet and alcohol. **Methods:** Male SD rats were divided into control, model, lovastatin, silymarin and gypenosides 15, 30, 60 mg/kg groups. Rats of the latter 6 groups were fed high fat and cholesterol diet and administed alcohol intragastricly once a day. **Results:** After 10 weeks, Comparing with model group, gypenosides groups hepatic index were significantly ameliorated; serum triglyceride (TG), total cholesterol (TC), free fatty acid (FFA), high density lipoprotein cholesterol (HDL-C), malondialdehyde (MDA) contents were significantly decreased; superoxidedismutase (SOD) activity in both serum and hepatic tissue were obviously increased; serum low density lipoprotein cholesterol (LDL-C) content was increased; serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) activity was decreased; hepatocyte apoptosis were decreased significantly; level of peroxisome proliferator-activated receptor α (PPAR-α) content and mRNA expression in hepatic tissue were significantly increased; hepatic steatosis and mitochondrial damage were improved in different degrees in pathological slices. **Conclusion:** These results suggested that gypenosides could prophylaxis liver fatty degeneration in fatty liver disease rats through modulating lipid metabolism, ameliorating liver function, and reducing oxidative stress.

Keywords: fatty liver disease; gypenosides; lipid metabolism; PPAR-α1

S2.36

Combination of catechin and epicatechin gallate from Fructus crataegi potentiates β-lactam antibiotics against *methicillin- resistant Staphylococcus aureus* (MRSA) *in vitro* and *in vivo*

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Fructus crataegi (hawthorn) is the common name of all plant species in the genus Crataegus of the Rosaceae family. In the present study, three monomers of (+)-catechin (C), (–)-epicatechin gallate (ECg) and (–)-epigallocatechin (EGC) were isolated from the hawthorn under the guide of antibacterial sensitization activity. The bioactivity of the composite fraction in enhancing the antibacterial effect of oxacillin against MRSA was greater than that of the individual monomer of the hawthorn extract *in vitro*. Diluted 2-fold and checkerboard method were used to analyze antibacterial activity and screen for the combination and proportion of monomers with the best bioactivity. The result showed that C (128 mg/L) combined with ECg (16 mg/L) had the greatest effect and the combination also reduced the bacterial load in blood of septic mice challenged with a sublethal dose of MRSA, increased daunomycin accumulation within MRSA and down-regulated the mRNA expression of *norA*, *norC*, and *abcA*, three important efflux pumps of MRSA. In summary, C and ECg enhanced the antibacterial effect of β-lactam antibiotics against MRSA *in vitro* and *in vivo*, which might be related to the increased accumulation of antibiotics within MRSA via suppression of important efflux pumps' gene expression.

Keywords: Fructus crataegi; (+)-catechin; (–)-epicatechin gallate; synergistic effect; MRSA; drug accumulation

S2.37

The effects of *Vaccaria Segetalis* extract on inhibiting angiogenesis *in vivo* and its underlying mechanism

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Aim: To study the effects of *Vaccaria Segetalis* extract (VSET) on inhibiting angiogenesis *in vivo* and its underlying mechanism. **Methods:** Matrigel implant model induced by bFGF was established. Masson and CD31-FITC staining detected the angiogenesis with the treatment of VSET. Immunohistochemistry was applied to measure expression of pAKT and pERK. Otherwise, mice hepatic carcinoma cells (H22) model was established to detect antiangiogenic and anti-tumor activity. **Results:** Quantitative analysis of the microvascular areas in Matrigel implant indicated that VSET could inhibit angiogenesis and reduce the expression of CD31-FITC at the concentration of 2.5 and 5 mg/kg. The content of pAKT and pERK were decreased with the treatment of VSET. VSET given by daily oral administration significantly inhibited tumor-induced angiogenesis and tumor growth in an intradermal inoculation mice model. **Conclusion:** VSET possessed anti-angiogenesis effects *in vivo*.

Keywords: VSET; angiogenesis; *in vivo*; pAKT; pERK

S2.38

Effects of salidroside on mitochondria biogenesis in human liver and fibroblast cells

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Aim: Mitochondria biogenesis plays an important role in the antioxidant effects of many natural compounds. We aim to explore the possible role of salidroside in simulating the biogenesis of mitochondria in human liver cells L02 and fibroblast cells IMR90. **Methods:** Different doses of salidroside (1, 5, and 25 μmol/L) were administrated to L02 and IMR90. The biogenesis of mitochondria was measured by NAO staining and flow cytometry. The level of regulatory proteins and mRNA were detected by Western blot and RT-PCR, respectively. Reactive oxygen species (ROS) level was determined by H_2DCFDA probe assay. Resveratrol was used as a positive control. **Results:** Mitochondria biogenesis and the expression level of regulatory proteins (PGC-1α and p-AMPK) were significantly increased in both cells treated with salidroside dose-dependently at 48h, and ROS level was decreased at the same time. PGC-1α mRNA expression was significantly increased by the compound as well. **Conclusion:** Salidroside promotes the biogenesis of mitochondria, which may be accountable for its anti-oxidant ability.

Keywords: salidroside; mitochondria; biogenesis; anti-oxidant ability

S2.39

Effects of Chinese herbal prescription Yinqiaosan on activation of the influenza-virus-induced PKC signal cascade in mice

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Aim: Protein Kinase C (PKC) signal transduction system plays an important role in the interaction of two complex systems, the virus and the host. In this study, we examined the effects of Chinese herbal prescription Yinqiaosan (YQS) on PKC transduction in influenza virus infected mice. **Methods:** Nose dropping with influenza A virus strain PR/8/34 to infect mice. Water extract of YQS was administered orally for 4 d. Lung tissue was collected at d 1, 3, 5, and 7 after infection. Lung index was used to evaluate antiviral effect of YQS. Used Real-time PCR technique to observe the dynamic expression HA-mRNA of and PKCβII. Used Western blotting to observe the dynamic expression of Receptor for Activated C Kinase1 (RACK1) and influenza virus matrix M1 protein. **Results:** Compared with model group, lung index was lower in high and middle dose group at d 5 (P<0.05). Both the levels of HA-mRNA and PKCβII increased at d 1 after infection, and reached the maximum value at d 3, and then decreased gradually after d 5. In high and middle dose group, the expressions of HA-mRNA, PKCβII, RACK1, and M1 were obviously lower than that in model group at the same time point (P<0.05, P<0.01). **Conclusion:** These results suggest that YQS has antiviral effect on influenza A virus infection, and PKC signal transduction pathway may play an important role in mechanism of drug action.

Keywords: Chinese herbal prescription; influenza; signal transduction pathway
Acknowledgements: This research was financially supported by the National Natural Science Foundation of China (81102871).

S2.40

Vascular protective and bone sparing effects of *Pueraria mirifica* in ovariectomized rats

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Aim: The tuberous roots of *Pueraria mirifica* has been used in Thai traditional medicine for aged women and men as a rejuvenating herb to promote youthfulness. The crude drug of *P mirifica* was proven to be relatively safe and effective in the treatment of menopausal symptoms. In this study, the vascular protective and bone sparing effect of a Thai traditional drug containing *P mirifica* were investigated in ovariectomized rats. **Methods:** Twenty four female Wister albino rats were undergone bilateral ovariectomy and divided into 3 groups with 8 animals in each group. The OVX rats in group 1, 2, and 3 were orally administered with drug powder containing *P mirifica* 100 mg/kg per day (OVX+*P mirifica*), subcutaneously injected of estradiol valerate 300 μg/kg/week (OVX+estrogen) and fed with distilled water (OVX control), respectively. Group 4 (n=8) received sham operation and treated with distilled water (sham control). All of them were treated for 42 consecutive days. At the end of experiment, blood sample was obtained by cardiac puncture for determination of lipid parameters, nitric oxide and alkaline phosphatase. Descending thoracic aortas were isolated for vascular function measurement. The right femurs were collected and assayed for dry weights, ash weights and calcium contents. The left femurs and thoracic aortas were prepared for histopathological study. **Results:** Acetylcholine-mediated endothelium-dependent relaxation was significantly impaired in OVX group (P<0.05) but restored in the OVX+*P mirifica* and OVX+estrogen groups. Microscopic examinations of thoracic aorta in the OVX+*P mirifica* and OVX+estrogen groups showed the lesser degree of medial smooth muscle and endothelial cell degenerations. Compared to the OVX control group (P<0.05), treatment with *P mirifica* and estrogen resulted in significantly increased in NO production, relative dry weight and ash weight of the right femurs. Decreasing of bone degenerative found in microscopic examinations was accompanied by a significant decrease in alkaline phosphatase level. **Conclusion:** *P mirifica* preserves endothelial vasodilator function and bone structure in ovariectomized rats.

Keywords: *P mirifica*; ovariectomized rats; thoracic aorta; femur bone

S2.41

Study on antibacterial effect of Tibetan medicine Renqingmangjue *in vitro*

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Aim: To investigate the antibacterial effect of Tibetan medicine Renqingmangjue *in vitro* and provide theoretical basis for clinical application. **Methods:** Agar well diffusion method was adopted to research the antibacterial effect of Renqingmangjue on four standard strains (Staphylococcus aureus ATCC25923, Escherichia coli ATCC25922, Salmonella CMCC50319, Streptococcus hemolytic-β CMCC32210) and 20 clinical isolates; the minimal inhibitory concentration (MIC) of sensitive strains were determined by the method of broth two-fold dilution. **Results:** Renqingmangjue had obvious antibacterial effect on Gram-positive bacteria *in vitro*, but weak inhibition on Gram-negative bacteria. The MIC to Streptococcus hemolytic-β, Staphylococcus aureus, Staphylococcus epidermidis and Staphylococcus huminis were all 12.5 mg/mL.

Conclusion: Renqingmangjue had significant antimicrobial effect on common gram-positive clinical isolates *in vitro*, which indicated high value in clinical application.

Keywords: Tibetan medicine; renqingmangjue; antibacterial effect; *in vitro*
Acknowledgements: This study was supported by 2011 Natural Science Fund Project of Tibet science and technology department

S2.42

Cucurbitacin E exhibits inhibitory effects on CYP2C, 3A activities in rat and human liver *in vitro*

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Aim: This study investigated the effects of cucurbitacin E (CuE), a tetracyclic triterpenes compound from Traditional Chinese medicine, on the cytochrome P450 (CYP) 1A2 (phenacetin-O-deethylase), 2C (tolbutamide 4-hydroxylase), 2E1 (chlorzoxazone 6β-hydroxylase), 3A (testosterone 6-hydroxylase) activities *in vitro* using rat and human liver microsomes. **Methods:** Pooled rat/human liver microsomes were employed to incubate with the substrates of CYP isoforms *in vitro*. HPLC-DAD was used to measure the model substrates and metabolites, respectively. **Results:** CuE exhibited no inhibition on CYP1A2, 2E1 activities, but a competitive CYP3A4 inhibitor (K_i=2.38 μmol/L) and a medium inhibitor of CYP2C11 (K_i=26.8 μmol/L). In addition, CuE was observed to cause rat CYP3A2 inactivation in the time, concentration-, and NADPH-dependent mode, which was predicted to be the feature of mechanism-based inhibition. **Conclusion:** CuE inhibits CYP2C, 3A activities in rat and human liver microsomes *in vitro* with different mode of inhibition. Given that CYP2C9 and CYP3A4 are responsible for the metabolism of a large number of clinical drugs, the potential herb-drug interactions of CuE with drugs which are the substrates of these CYPs may be careful monitored.

Keywords: cucurbitacin E; CYP2C; CYP3A; herb-drug interactions
Acknowledgements: This work was supported by the Science and Technology Commission of Shanghai Municipality (11DZ2260300 and 12XD1406100).

S2.43

Pharmacological and toxicological profiles of a standardized extract of *Centella asiatica* ECa 233

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A standardized extract of *Centella asiatica* namely ECa 233, defined as white to off-white extracted powder of *Centella asiatica* containing triterpenoid glycosides not less than 80% and the ratio of madecassoside and asiaticoside content should be within 1.50±0.50, was established by a group of researchers at The Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. ECa 233 has been shown to ameliorate learning and memory deficit induced by either transient bilateral occlusion of common carotid arteries or by an intracerebroventricular injection of beta-amyloid peptide (25–35). Orally given ECa 233 significantly improved performances of cognitive deficit mice in Morris Water Maze and step-down tests. Recently, anxiolytic effects of ECa 233, assessed by an elevated plus maze and dark-light box, was demonstrated in both non-stressed mice subjected to acute stress and chronic stress induced by immobilization. In addition to positive pharmacological effects observed, ECa 233 demonstrated very favorable safety profiles in acute as well as sub-chronic toxicity testing. No lethality was observed in mice receiving orally given ECa 233 at the dose of 10g/kg (bw). Oral administration of ECa 233 in the dose range of 10–1000 mg/kg (bw) for 90 d did not produce significant alteration in terms of body weight, blood chemistry, clinical chemistry and histopathology. Moreover, total hepatic CYP content and activities of some phase I and phase II drug metabolizing enzymes were unaffected by ECa 233.

S2.44

Anti-malarial drug artesunate attenuates rhinovirus-induced asthma exacerbation

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Aim: Rhinovirus (RV)-induced asthma exacerbation has put a great burden on the healthcare management of asthmatics. The aim of this study was to investigate the effects of anti-malarial drug artesunate in the treatment of rhinovirus-induced asthma exacerbation. **Methods:** We established a RV-induced asthma exacerbation mouse model by sensitizing and challenging mice with house dust mite (HDM) or saline, followed by infection with RV-1B, a minor group RV which binds to mouse airway epithelial cells, or UV-inactivated RV. Artesunate was given by intraperitoneal route both before and after RV infection. Vehicle control mice were injected with 6% DMSO. Bronchoalveolar lavage fluid and lung homogenates were examined for inflammatory cells infiltration, and Th2 cytokine protein production and mRNA expressions. Airway hyper-responsiveness was measured using direct airway resistance analysis. **Results:** Compared to HDM/UV-inactivated RV-treated mice, HDM/RV-treated mice developed significant increases in airway infiltration of neutrophils, eosinophils and lymphocytes. The HDM/RV-treated group also produced higher protein levels of IL-4, IL-5, IL-13. Besides, the HDM/RV-treated group generated higher mRNA levels of eotaxin-1 and MUC5AC. As compared with DMSO, artesunate significantly inhibited RV-induced exacerbation in terms of total inflammatory cell, eosinophil and lymphocyte counts recovered in bronchoalveolar lavage fluid. We will further investigate the effects of artesunate on the secretion of Th2 cytokines, inflammatory mediators mRNA expression and airway hyper-responsiveness. **Conclusion:** Artesunate attenuated the exacerbation by RV on house dust mite-induced asthma, and our findings implicate a potential value of artesunate in the treatment of RV-induced asthma exacerbation.

Keywords: rhinovirus (RV); asthma exacerbation; house dust mite (HDM); artesunate

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S2.45

Plasma metabonomics study on the effect and mechanism of Bu-zhong-yi-qi-tang in spleen-deficiency rats

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Aim: Bu-zhong-yi-qi-tang (HET) is a traditional Chinese medicine used for the treatment of spleen-deficiency in clinic. However, the mechanism responsible for its actions has not been investigated experimentally. The present study aimed to investigate the pharmacological effects and mechanism of HET by using spleen-deficiency rat model. **Methods:** Spleen-deficiency was established by oral administration 10 g/kg body weight of Radix Rhei, starving and heavy loaded swimming. HET at three doses (5, 10, 20 g/kg body weight) were administered by gavage after 14 days modeling, body behaviour and weight were examined once a week during the whole test then the tissues collected to measure the viscera indexes. Plasma was collected for NMR analysis then combined multiple statistical analysis method to evaluate the treatment of HET and discover differentiating metabolites. **Results:** Obvious separation trend between control and model group in PCA score plot which indicated that spleen-deficiency model was successfully duplicated. Lipid, isoleucine, 3-hydroxy butyrate, Oxidation trimethylamine (+), β-glucose and α-glucose were identified as biomarkers in rats plasma through PLS-DA score plot. Compared with control group, level of lipid, isoleucine, 3-hydroxy butyrate increased significantly, oxidation trimethylamine (+), β-glucose and α-glucose descended significantly in model group while those were much closer to normal in treatment group. **Conclusion:** These results indicated that HET had the certain therapeutic effect on spleen-deficiency rats and the six metabolites are expected to be the potential biological markers for spleen-deficiency symptoms.

Keywords: spleen-deficiency; HET; metabonomics; Traditional Chinese Medicine; NMR

S2.46

Ganoderma lucidum polysaccharide accelerates refractory wound healing by inhibition of mitochondrial oxidative stress in diabetes

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Aim: Impaired wound healing in diabetic patients is a serious complication with limited treatment regimens. Gene therapy of mitochondrial antioxidant enzyme manganese superoxide dismutase (MnSOD) was able to restore diabetic wound healing with suppression of $O_2^{\cdot-}$. The present study was designed to determine protective effects of *Ganoderma lucidum* polysaccharide (*Gl*-PS), as a potent inducer of MnSOD, on diabetic wound healing. **Method and results:** Streptozotocin-induced diabetic mice with full-thickness excisional wounds were administered with *Gl*-PS (10, 50, or 250 mg/kg/d, ig). *Gl*-PS dose-dependently rescued the delay of wound closure and increased the mean perfusion rate around the wound in diabetic mice. Diabetic conditions markly increased mitochondrial $O_2^{\cdot-}$ production and nitrotyrosine formation in wound tissues, which were normalized with *Gl*-PS treatment. In diabetic wound tissues, the protein level of MnSOD was unchanged whereas MnSOD activity was inhibited and its nitration was potentiated; *Gl*-PS administration suppressed MnSOD nitration and increased MnSOD and glutathione peroxidase (GPx) activities. Moreover, *Gl*-PS attenuated the redox enzyme p66Shc expression and phosphorylation in diabetic mice skin. **Conclusion:** *Gl*-PS rescued the delayed wound healing and improved wound angiogenesis in diabetic mice, at least in part, by suppression of mitochondrial oxidative stress.

Keywords: oxidative stress; mitochondria; diabetes; manganese superoxide dismutase; p66Shc; nitrotyrosine

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S2.47

Hepatoprotective effect of oleanolic acid on *D*-galactosamine-induced acute liver injury in mice

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Aim: To study the protective effect of oleanolic acid (OA) on D-galactosamine-induced acute liver injury in mice. **Methods:** Male mice were pretreated with OA (200 μmol/kg, dissolved in vegetable oil, *po*) twice per day for 3 d. One hour after the last dose, mice were intoxicated with of *D*-glactosamine (800 mg/kg, ip). Eight hours later, blood and livers were collected. Liver injury was evaluated by serum alanine aminotransferase (ALT), aspirate aminotransferase (AST) activities and by histopathology, and the expression of genes related to toxicity was determined by real-time RT-PCR. **Results:** OA pretreatment protected against *D*-galactosamine-induced hepatoxicity, as evidenced by reduced serum ALT and AST activities, decreased hepatic thiobarbituric acid reactive substances, and by improved liver histopathology. To examine the mechanism of the protection, the toxicity-related gene expressions were examined. OA attenuated D-galactosamine-induced Chop10 and Gadd45, markers of endoplasmic reticulum stress; OA also decreased the

expression of pro-inflammatory markers such as early growth response protein-1 (Egr1), mouse keratinocyte-derived chemokine (mKC), and tumor necrosis factor-α (TNF-α). **Conclusion:** OA is effective in protecting against Dgalactosamine-induced acute liver injury. The mechanisms of the protection appear to be due, at least part, to attenuated ER stress, and decreased inflammation and oxidative stress.

Keywords: oleanolic acid; D-galactosamine; hepatotoxicity; gene expression

S2.48

The influence of insular cortex on motivational aversion associated with morphine withdrawal and the inhibition effect of pseudoginsenoside-F11 on motivational aversion

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Aim: Negative affective state is thought to be an important factor that triggers relapse. In this study, the role of insular cortex (IC) on the conditioned place aversion (CPA) in mice was investigated and the inhibition effect of pseudoginsenoside-F11 (PF11), an ocotillol type saponin existed in American ginseng, on CPA and the possible mechanism were also explored. **Methods:** The influences of IC lesion by microinjection kainic acid (KA) and NMDA receptor in IC by microinjection NMDA receptor antagonist (AP-V) on CPA were investigated. The expression of FosB/ΔFosB was detected by immunohistochemisty. The effects of PF11 on CPA and the expression of FosB/ΔFosB in IC were also explored. **Results:** KA and AP-V injected into IC inhibited CPA. The expression of FosB/ΔFosB in IC was increased after the conditioning session of CPA. PF11 inhibited the acquisition of CPA, facilitated the extinction of CPA. The increased expression of FosB/ΔFosB in IC was also inhibited by PF11. **Conclusion:** IC was an important brain area in regulating motivational aversion. PF11 could improve the motivational aversion through inhibiting the expression of FosB/ΔFosB in IC.

Keywords: morphine; insular cortex; PF11; conditioned place aversion (CPA)

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S2.49

Salvianolic acid B prevents arsenic trioxide-induced cardiotoxicity *in vivo* and enhances its anticancer activity *in vitro*

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Aim: Clinical attempts to reduce the cardiotoxicity of arsenic trioxide (ATO) without compromising its anticancer activities remain to be an unresolved issue. In this study, we determined whether Salvianolic acid B (Sal B) can protect against ATO-induced cardiac toxicity *in vivo* and increase the toxicity of ATO toward cancer cells. **Methods:** Combination treatment of Sal B and ATO was investigated using BALB/c mice and human hepatoma (HepG2) cells and human cervical cancer (HeLa) cells. **Results:** The results showed that the combination treatment significantly improved the ATO-induced loss of cardiac function, attenuated damage of cardiomyocytic structure, and suppressed the ATO-induced release of cardiac enzymes into serum in BALB/c mouse models. The expression levels of Bcl-2 and p-Akt in the mice treated with ATO alone were reduced, whereas those in the mice given the combination treatment were similar to those in the control mice. Moreover, the combination treatment significantly enhanced the ATO-induced cytotoxicity and apoptosis of HepG2 cells and HeLa cells. Increases in apoptotic marker cleaved poly (ADP-ribose) polymerase and decreases in procaspase-3 expressions were observed through Western blot. **Conclusion:** These observations indicate that the combination treatment of Sal B and ATO is potentially applicable for treating cancer with reduced cardiotoxic side effects.

Keywords: salvianolic acid B; arsenic trioxide; cardioprotection; anticancer activity

S2.50

Progress on toxicologic researches of triptolide: target, mechanism and detoxication

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Triptolide ($C_{20}H_{24}O_6$, TP), a diterpene triepoxide, is a major active component of extracts derived from the medicinal plant Tripterygium wilfordii Hook F (TWHF). Recent studies have proved its effectiveness on tumor and some auto-immune disorders such as rheumatoid arthritis. However, it also causes obvious toxicity on reproductive system, liver and kidney, which become huge barrier for its further development and clinical application. In order to reduce its toxicity, many researches have explored its pharmacokinetics, biologic targets and pathways. Molecular structure modification and different administration route are also investigated to minimize its side effects. Therefore, this review is aimed to summarize the progress on toxicologictoxic researches of triptolide through searching and sorting the literatures about triptolide in recent two decades. Its toxic dosage, administration route, possible toxic molecular targets and mechanisms, as well as detoxication method like chemical modification and novel drug delivery systems are reviewed. Although great progresses have been made, its toxic mechanism is still not totally elucidated, which need further studies.

Keywords: triptolide; toxicity; molecular target and mechanism; detoxication

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S2.51

The protective effect of glycyrrhizin on LCA induced liver injury and its relation with PXR

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Aim: Licorice (LE) was reported hepatoprotective under several models in vivo. Glycyrrhizin is the major bioactive component isolated from LE herb. In the present study the effect of glycyrrhizin on the CYP3A expression mediated by the Pregnant X Receptor and its hepatoprotective effect were investigated. **Methods:** Glycyrrhizin 50, 100, 250, 500 mmol/L were added in HepG2 cells for 12 h respectively. RT-PCR and Western blot were used to determine CYP3A4 mRNA and protein levels in HepG2 cells treated with glycyrrhizin. The ability of glycyrrhizin to transactivate PXR was determined by reporter assay and EMSA. The liver injury models in mice were caued by LCA. The livers of the treated mice were sampled and evaluated by his topathology and quantitative measures of the liver markers ALTand AST. LCA concentration in serum was determined by UPLC-TOF/MS. **Results:** Treatment of HepG2 cells with glycyrrhizin resulted in marked induction both CYP3A4 mRNA and protein levels. The transcriptional activation of the CYP3A4 gene by glycyrrhizin was PXR-dependent. Co-administration of glycyrrhizin resulted in decreases in plasma ALT and AST activities, multifocal necrosis amounts and serum LCA level as compared with the results to the group treated with LCA alone. **Conclusion:** The induction of the hepatic CYP3A4 by glycyrrhizin is mediated through the activation of PXR. PXR-mediated effects on CYP3A and CYP7A may contribute to the hepatoprotection of glycyrrhizin against LCA-induced liver injury.

Keywords: glycyrrhizin; Licorice; cytochrome 3A; pregnane X receptor

S2.52

Molecular mechanism of the inhibition effect of cepharanthine hydrochloride in HepG2.2.15 cells

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Aim: Cepharanthine hydrochloride, a bisbenzyl isoquinoline alkaloid, which is extracted from the roots of stephania cepharantha and then salificatied with hydrochloric acid. Our previous studies have showed that CH could inhibited the replication of Hepatitis B Virus (HBV). This thesis mainly aims to study the anti-HBV mechanism of CH in HepG2.2.15 cells and its effects on expression levels of NF-κB, further explores their connection. **Methods:** HepG2.2.15 cells were treated with different concentrations of CH and Lamivudine for different times. Flow

cytometry methods was then used to detect apoptosis rates. The expression levels of X gene and X protein were detected respectively by RT-PCR and ELISA. NF-κB activity is detected by detecting the expression of P65 and protein using Western blot. **Results:** After be treated with CH, the expression levels of X gene and X protein in HepG2.2.15 cells decreased to some extent .CH could remarkably increased apoptosis rates of HepG2.2.15 cells, and the suppression was in a dose dependent manner. CH increased the expression level of IκBα and inhibited nuclear translocation of p65 from the cytoplasm, thus inhibited the activity of NF-κB. **Conclusion:** CH inhibits the expression levels of X gene and X protein in HepG2.2.15 cells, which may be the mechanism of its inhibition on virus replication. The up-regulation of IκBα and inhibition of NF-κB activity may be the most important mechanism of its apoptosis induction and HBV replication inhibition.

Keywords: Cepharanthine Hydrochloride; NF-κB; Hepatitis B Virus; apoptosis

S2.53

Ganoderiol A-enriched extract suppresses migration and adhesion via inhibiting FAK-SRC-paxillin cascade pathway in MDA-MB-231 cells

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Aim: This study aims to test the effects and mechanisms of anti-migration, anti-adhesion and anti-invasion of ganoderiol A-enriched extract (GAEE) isolated from *Ganoderma lucidum*, a famous traditional Chinese medicine. **Methods:** MTT assay, flow cytometry, and Hoechst-33342 staining were performed to detect the cell proliferation, cells in different phases or apoptosis, respectively. Wound-healing assay and Transwell assay were employed to investigate the cell migration and invasion, while cell adhesion was tested via observing the cellular morphology stained with crystal violet. The expression of proteins was detected by Western blot analysis and immunofluorescence. **Results:** GAEE, mainly containing ganoderiol A, dihydrogenated ganoderiol A and ganoderiol A isomer, inhibited cell adhesion and migration at the non-toxic doses in breast cancer MDA-MB-231 cells. Further study showed that GAEE decreased the active forms of FAK and disrupted the interaction between FAK and SRC, which leads to the deactivating of paxillin. Moreover, GAEE also down-regulated the expressions of RhoA, Rac1, and Cdc42 which are vital for cell movement. **Conclusion:** GAEE suppresses cell migration via FAK-SRC-paxillin signaling pathway in MDA-MB-231 cells.

Keywords: ganoderiol A; migration; FAK; paxillin; MDA-MB-231

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S2.54

Protective effects of *in-vitro* cultured Calculus bovis on intrahepatic cholestasis induced by α-naphthylisothiocyanate in rats

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Aim: *In-vitro* cultured Calculus bovis (ICCB) is an artificial substitute of natural Calculus bovis, which has been widely used for sedation, relieving fever and hepatic disease since ancient times. The effect of ICCB on α-naphthylisothiocyanate (ANIT)-induced intrahepatic cholestasis was investigated in this study. **Methods:** ICCB (50, 100, 200 mg/kg) was intragastrically (ig) given to experimental rats for seven consecutive days. At the 5th day, the animals were treated with ANIT which brings about liver injury and intrahepatic cholestasis. Bile flow was measured every 30 min for 2 h. Histopathological change in the liver was estimated by hematoxylin and eosin staining. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkalinephosphatase (ALP) and total bilirubin (TBIL) in the serum were analyzed using biochemical methods. Malondialdehyde (MDA) and superoxide dismutase (SOD) activity in the liver were also determined by biochemical assay. **Results:** Hepatocyte necrosis, degenerative changes and severe interlobular duct epithelial damage were observed in ANIT-induced cholestatic rats, which were partly relieved by ICCB. ICCB enhanced markedly bile flow in cholestatic rats. Meanwhile, ICCB decreased dose-dependently serum ALT, AST, ALP, and TBIL levels in cholestatic rats. Moreover, cholestatic rats displayed a significant increase in MDA level and decrease in SOD activity, which were alleviated by ICCB.

Conclusion: ICCB has protective effects on ANIT-induced intrahepatic cholestasis.

Keywords: *In-vitro* cultured Calculus bovis (ICCB); Intrahepatic cholestasis; ANIT; Oxidative stress

S2.55

Paeoniflorin attenuates bleomycin-induced pulmonary fibrosis in mice

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Aim: To investigate the effects and underlying mechanisms of paeoniflorin (PAE) on experimental pulmonary fibrosis (PF) in mice. **Methods:** PF model was established in mice by an intratracheal instillation of bleomycin (BLM). Histopathological changes were evaluated by H&E stain and Masson's trichrome stain. Changes in cytokines or proteins were measured by commercial kits or Western blot. The mRNA expressions of MMP-1 and TIMP-1 were detected by RT-PCR. **Results:** PAE (50 mg/kg) prolonged the survival periods and attenuated histopathological changes in lung tissues of PF mice. It also decreased the contents of hydroxyproline, type I collagen and α-SMA. PAE down-regulated the levels of TGF-β1, Smad4 and the phosphorylations of Smad2/3, while up-regulated the level of Smad7 and content of IFN-γ in lung tissues. But, it slightly affected mRNA expressions of MMP-1 and TIMP-1. **Conclusion:** PAE attenuates PF by suppressing type I collagen synthesis *via* inhibiting the activation of TGF-β/Smad pathway and increasing the expression of IFN-γ.

Keywords: paeoniflorin; pulmonary fibrosis; type I collagen; TGF-β/Smad pathway

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S2.56

Hepatoprotetive effects of curcumin against alcohol-induced liver injury via activation of Nrf2/ARE pathway

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Aim: Curcumin, a natural polyphenol in the spice turmeric, exhibits a good antioxidant property. NF-E2-Related Factor-2 (Nrf2) is a transcription factor that plays a crucial role in the cellular protection against oxidative stress. The present study was to investigate the protective effects of curcumin on alcohol-induced hepatic injury and its underlying mechanism. **Methods:** Mice were intragastrically treated with alcohol (56% alcohol at 10 mL/kg)) and curcumin (150 mg/kg and 300 mg/kg) once a day, respectively, until sacrificed at 8 weeks for histopathological analysis, serum aminotransferase evaluation, hepatic lipid peroxidation assay and mechanistic evaluation. **Results:** Curcumin significantly protects the liver from injury by reducing the activities of serum aspartate aminotransferase and alanine aminotransferase, and by improving the histological architecture of the liver. Similarly, curcumin attenuates oxidative stress by increasing the content of hepatic glutathione and superoxide dismutase, leading to the reduction in the level of lipid hydroperoxide. In addition, mRNA and protein analysis substantiated that curcumin treatment notably promotes the liver Nrf2 nuclear translocation and its downstream antioxidant enzyme genes including HO-1 and NQO-1 in alcohol-treated mice. **Conclusion:** Our data clearly demonstrate that curcumin has prophylactic effect on alcohol-induced liver injury that is highly associated with its effect of enhancing the Nrf2 antioxidant signaling pathway.

S2.57

Natural polysaccharides protect kidney from renal ischemia-reperfusion injury

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Aim: The objective of the present studies was to discover and evaluate natural polysaccharides that could prevent and attenuate renal ischemia-reperfusion (IR) injury. **Methods:** Mouse renal IR model was used in this study. Male mice were

subjected to right renal ischemia for 30 min and reperfusion for 24 h, or to a sham operation with left kidney removed. Kidneys undergone IR showed characteristic morphological changes, such as tubular dilatation, and brush border loss. Low-molecular-weight fucoidan (LMWF) or ganoderma lucidum polysaccharide peptide (GLPP) was intraperitoneally injected from 7 d before renal IR until sacrifice. HK-2 cells (human kidney proximal tubular cells) were used to study the mechanism in which LMWF and GLPP protect kidney from renal ischemia-reperfusion injury. **Results:** Experimental results showed that LMWF and GLPP significantly corrected the renal dysfunction and the abnormal levels of MPO, MDA and SOD induced by renal IR. LMWF also inhibited the activation of MAPK pathways, which consequently resulted in a significant decrease in the release of cytochrome c from mitochondria, ratios of Bax/Bcl-2 and cleaved caspase-3/procaspase-3, and phosphorylation of p53. LMWF alleviated hypoxia-reoxygenation or $CoCl_2$ induced cell viability loss and $\Delta\Psi m$ dissipation in HK2 cells, which indicates LMWF may result in an inhibition of the apoptosis pathway through reducing activity of MAPK pathways in a dose-dependent manner. **Conclusion:** Our *in vivo* and *in vitro* studies show that LMWF and GLPP ameliorate acute renal IR injury via regulating intracellular signaling pathways. The data provide evidence that some natural polysaccharides may serve as a potential therapeutic agent for acute renal IR injury.

Keywords: ischemia-reperfusion; fucoidan; ganoderma lucidum; drug discovery

S2.58

The role of Heijiangdan Ointment intervention on oxidative stress during radiation dermatitis induced by ^{60}Co γ-ray in mice

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Aim: To investigate the role of Heijiangdan Ointment intervention on oxidative stress in ^{60}Co γ-ray radiation injured dermatitis tissue in mice. **Methods:** Female Wistar mice with 4 grade radiation dermatitis induced by ^{60}Co γ-ray were randomly divided into 4 groups (n=12) : model group(treated with saline), Heijiangdan Ointment group, recombinant human epidermal growth factor (rhEGF) group, and Trolox group. On d 11 and 21, 6 mice in each group were chosen. The histological and collagen changes in ulceration were observed by staining of HE, Victoria blue's+Ponceau's and Sirius red. The levels of superoxide dismutase (SOD), maleic dialdehyde (MDA) and lactate dehydrogenase (LDH) were detected by using spectrophotometer. The fibroblast mitochondria were observed by transmission electron microscope. **Results:** Ond 6, 11, 16, and 21, the area of skin ulcer in Heijiangdan Ointment group was smaller than Trolox group and model group, larger than rhEGF group (P<0.05). After 21 days' treatment, the inflammatory cells in wound tissue in Heijiangdan Ointment group was less than model group, while elastic fibers, collagen fibers and collagen type I and III were more than model group. On d 11 and 21, there was a significant difference between Heijiangdan Ointment group and model group (P<0.05) on SOD activity, MDA level and LDH activity. **Conclusion:** Heijiangdan Ointment can relieve the injury of oxidative stress, increase the antioxidant activity, reduced fibroblast mitochondrial damage, promoting mitochondrial repair in mice.

Keywords: radiation dermatitis; oxidative stress; Heijiangdan Ointment; jiedu-huoxue; traditional Chinese medicine external treatment

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S2.59

Tanshinol protects against hydrogen peroxide-induced oxidative stress via regulating Wnt/FoxO pathway in osteoblastic differentiation of C2C12 cells

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Aim: The "Aging and Oxidative Stress" theory is recently thought as a new mechanism of the pathogenesis related to osteoporosis. In our previous study, Tanshinol isolated from *Salvia miltiorrhiza* Bge (Danshen) showed effectively activities to prevent or reverse bone loss and up-regulate Wnt signaling in excess Glucocorticoid (GC) (a factor induced oxidative stress) induced osteoporosis or GC-treated rat marrow stromal cell (rMSC). In this study, the direct effect of Tanshinol against hydrogen peroxide (H_2O_2)-induced oxidative stress and the decline of osteoblastic differentiation and the underlying mechanisms *in vitro* were investigated. **Methods:** C2C12 cells were exposed to hydrogen peroxide (H_2O_2) with or without Tanshinol (0.1-10 μmol/L), so we observed changes in cell viability, redox status, and osteoblastic differentiation through MTT assay, flow cytometry and biomarkers analysis respectively. Furthermore, we monitored the FoxO and Wnt signaling pathway by the use of dual-luciferase assay, and qRT-PCR and/or Western blotting. **Results:** Tashinol (0.1–10 μmol/L) significantly reduced the accumulation of ROS and prevented the decrease of cell viability. Moreover, Tashinol strongly prevented the decline of C2C12 cells on differentiation to osteoblasts in the presence of hydrogen peroxide (H_2O_2). Further mechanistic studies revealed that Tanshinol treatment prevented the arrest in G0 phase of cell cycle induced and inhibited apoptosis via limiting caspase-3 activation by H_2O_2. Our results showed that Tashinol suppressed the stimulation of FoxO target genes via abrogating oxidative stress and promotes Wnt/Tcf mediated transcription through eliminating the cross talk between FoxO3a and β-Catenin. **Conclusion:** we demonstrated that Tanshinol shuts off the FoxO switch through removing ROS and attenuating oxidative stress and turns on TCF switch that is achieved by increasing the interacting between β-catenin and TCF transcription factors in osteoblastic differentiation of pluripotent C2C12 cells.

Keywords: tanshinol; oxidative stress; osteoporosis; Wnt; FoxO; hydrogen peroxide; C2C12 cells

S2.60

ShengFu oil enhances skin regeneration and scarless repair of full-thickness scald in rabbits

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Aim: ShengFu oil (SFO), a herbal preparation, is widely used to treat various burns and scalds. It is therefore urgent to research the mechanism of the skin regeneration and scarless repair of full-thickness scalds by SFO. **Methods:** Full-thickness scald wounds with area of 6 cm^2 were reproduced on both sides of the back in 18 experimental rabbits by water vapor. These rabbits were respectively treated with sesame oil, SFO, and mupirocin ointment in dose of 0.15 mL/cm^2, 2–3 times per day. All the rabbits were sacrificed on post scald day 45, and wound tissues were subjected with HE staining. The protein expressions of TGF-β_1, βFGF, and VEGF were observed. **Results:** The wound healing quality, healing time and healing rates of rabbits in SFO group were better, shorter and higher than that in the other two groups, respectively. The structure of wound tissues in SFO group was in much better integrity than that in the other two groups, including regenerated hair follicles in the corium layer and regularly arranged collagen fibers. The protein expressions of TGF-β_1, βFGF, and VEGF in SFO group were all higher than those in the other two groups. **Conclusion:** SFO can up-regulate the protein expressions of TGF-β_1, βFGF, and VEGF, induce vascular regeneration, promote wound healing, and shorten wound healing time.

Keywords: burns; ShengFu oil; skin regeneration; scarless repair

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S2.61

Protective effect of 3'-daidzein sulfonate sodium on mice hepatic injury induced by CCl_4

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Aim: To explore the protective effects of 3'-daidzein sulfonate sodium(DSS)on chronic hepatic injury induced by carbon tetrachloride(CCl_4) in mice. **Methods:** Healthy Kunming male mice, weighting (21±3) g, 12 per group were randomized 5 groups: control group, model group, bifendate(DDB) positive control group (2.5 mg/kg), DSS low and high dose groups (0.1 mg/mL, 0.3 mg/kg). The chronic hepatic injury mice were administrated 10% CCl_4 dissolved in plant oil solution by intraperitoneal injection twice a week. At the same time, the different group mice were treated with normal saline, DDB (2.5 mg/kg) and DSS (0.1 mg/mL, 0.3 mg/kg) respectively, by intragastric administration once a day and continued for 6 weeks. After the last administration, the mice blood and liver were taken. Flow cytometry was used to detect T lymphocyte subsets, enzymes analysis technique was used to observe the liver function and Western blot method was used to detect the content of IL-1β, IL-6, and TNF-α in liver. **Results:** In chronic hepatic injury

mice, the activities of ALT and AST were increased obviously, $CD3^+$ cell population were elevated, $CD8^+$ T lymphocyte subsets ratio was reduced, and $CD4^+/CD8^+$ ratio was enhanced. After treated with DSS these changes were reversed. **Conclusion:** DSS plays a protective role in chronic hepatic injury mice induced by CCl_4, and its mechanism probably result of DSS modulating the immunologic function.

Keywords: 3′-daidzein sulfonate sodium; chronic liver injury; carbon tetrachloride
Acknowledgements: This work was supported by the National Natural Science Foundation of China (30760284 and 81160399).

S2.62

Protective effects of total flavones from *Epimedium* against oxidative stress-triggered damage and spermatogenic cell death in experimental cryptorchid mouse testis

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Aim: Although previous studies have shown that germ cell apoptosis in testes is an essential feature of cryptorchidism, the underlying molecular mechanism remains to be determined. Total flavonoids of *Epimedium* (TFE), an antioxidant on pharmacological activity, has obvious protective effect on male spermatogenesis. Nevertheless there is no relevant reports about protection of TFE on cryptorchidism induced spermatogenesis impairment, and yet the possible protective mechanisms of TFE against cryptorchid testis injury remains unknown. So in this project we investigate the protective effect of TFE on oxidative stress, apoptosis, and cell proliferation in the mouse ce testis after experimental cryptorchidism. **Methods:** Adult Balb/c male mice were equally divided into 4 groups (sham, model, model+l-TFE, model+h-TFE). Cryptorchid model was operated on to induce experimental bilateral cryptorchidism via an abdominal incision and suturing of the testis to the lateral abdominal wall. The mice in model+l-TFE group were given TFE 100 mg/kg per d and 200 mg/kg per d in model+h-TFE group for 14 days after the operation. At the end of the experiment, all animals were sacrificed. Testis tissues were weighed and collected for histopathologic and biochemical analysis. **Results:** The TFE-treated mice showed a remarkably improved histologic appearance. The testis weights was improved by administration of TFE compared with the cryptorchid group (P<0.01). TFE treated on experimental mice remarkably improved sperm count (P<0.01). TFE treatment significantly decreased the elevated tissue MDA levels (P<0.01) and increased contents of tissue total antioxidant capacity, SOD (P<0.05) and GSH-Px (P<0.01) enzyme activities in testes. Our data indicate a significant reduction in the activity of in situ identification of apoptosis using TUNEL and there was a rise in the expression of proliferating cell nuclear antigen (PCNA) in testis tissues of TFE-treated mice in the cryptorchid group. **Conclusion:** These results suggest that administration of TFE is a potentially beneficial agent to reduce testicular damage in cryptorchid mice by decreasing oxidative stress.

Keywords: cryptorchidism; oxidative stress; total flavones of *Epimedium*
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S2.63

Osteogenic effects of the flavanes from green tea polyphenols

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Aim: Anti-oxidative stress therapy is a new way for prevention and treatment of osteoporosis. In previous work, five flavanes (+)-catechin (1), (–)-epiafzelechin (2), (–)-catechin (3), (–)-epicatechin (4) and (–)-afzelechin (5) were isolated from the green tea polyphenols. This study aims to evaluate the osteogenic effects and antioxidative activities of these 5 flavanes *in vitro*. **Methods:** Proliferation rate, alkaline phosphates activity (ALP), hydroxyproline concentration, and the formation of mineralized bone nodules were the functional factors used for measuring the osteogenic effect of flavanes on proliferation, differentiation and mineralization in osteoblasts. The antioxidative activities of these flavanes were determined by protecting against H_2O_2-induced apoptosis in C2C12 myogenic cells. **Results:** The results of proliferation rate, ALP, hydroxyproline concentration, and the formation of mineralized bone nodules in osteoblasts demonstrated that the activities of 4 were the strongest and 1 showed the lowest. Moreover, the does of these flavanes, which protect more than 50% C2C12 cells against H_2O_2-induced apoptosis, are 350.60 μg/mL (1), 14.50 μg/mL (2), 45.63 μg/mL (3), 6.25 μg/mL (4), and 200.45 μg/mL (5). **Conclusion:** We found the activities of these five flavanes in promoting osteblastic proliferation and differentiation are positively correlated with their antioxidantive activities. It indicated that the osteogenic effect of this kind flavane was related with anti-oxidative stress leading to an anabolic effect on osteoblast bone formation.

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S2.64

Effects of total lignans from Du-Zhong (*Eucommia ulmoides* Oliv) cortex prevent bone loss *in vivo* and *in vitro*

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Our previous study demonstrated that the potential protective effects of Du-Zhong on ovariectomy (OVX) induced osteoporosis in rats. The present study systematically investigate the *in vivo* and *in vitro* effect of total lignans (TL) extracted from Du-Zhong cortex on bone formation using ovariectomy rat model and primary cultures of rats osteoblasts. Eighty 3-month-old female Sprague-Dawley rats were used and randomly assigned into sham-operated group (SHAM) and five OVX subgroups, ie OVX with vehicle (OVX); OVX with 17alpha-ethinylestradiol (E_2, 25 μg/kg per day); OVX with TL of graded doses (20, 40, or 80 mg/kg per day). The treatment began 4 weeks after the surgery and lasted for 16 weeks. *In vitro* experiments were performed to determine the potential molecular mechanisms of the anti-osteoporotic effect of TL. Treatment with TL significantly prevent OVX-induced decrease in biomechanical quality of femur such as maximum stress and Young's modulus. The mechanical changes were associated with the prevention of a further BMD decrease or even with some improvements in microarchitecture. TL inhibited BMD decrease in the femur caused by OVX, which was accompanied by a significant decrease in skeletal remodeling, as was evidenced by the decreased levels of the bone turnover markers. μCT analysis of the femoral metaphysis showed prevent the deterioration of trabecular microarchitecture. TL induced primary osteoblastic cell proliferation and differentiation, inhibition of osteoclastogenesis through an increase in osteoprotegrin (OPG) and a decrease in NF-κB ligand (RANKL) expression *in vitro*. We concluded that TL treatment can effectively suppress the loss of bone mass induced by OVX and *in vitro* evidence suggests this could be through actions on both osteoblasts and osteoclasts.

Keywords: osteoblast; osteoclast; osteoporosis; ovariectomy; Du-Zhong (*Eucommia ulmoides* Oliv) total saponins

S2.65

Protective mechanisms of phenolic alkaloids from *Menispermum dauricum* rhizome on oxygen-glucose deprivation/ reoxygenation injury *in vitro*

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Aim: Our previous studies have reported that the phenolic alkaloids of *Menispermum dauricum* rhizome (PAM) have protective effects against ischemic brain injury. In the present study, the underlying mechanisms of PAM against oxygen-glucose deprivation injury *in vitro* were investigated. **Methods:** Oxygen-glucose deprivation/reoxygenation (OGD/R) in primary rat cortical cultures and brain slices were used to mimic ischemia-reperfusion injury respectively. Different PAM dosage groups were added at the beginning of the OGD and lasted till the end of reoxygenation period. Mitochondrial membrane potential (MMP) in rat cortical cultures were examined by using flow cytometry. Infarction of brain slice was assessed by staining with 2,3,5-triphenyltetrazolium chloride following reoxygenation for 4 h. The expression of glial glutamate transporter-1 (GLT-1) and excitatory amino acid carrier 1(EAAC1) of cortex and hippocampus were observed by Western blot method. **Results:** Our results showed that PAM (0.3, 3, and 30

μg/mL) protected rat brain slices against OGD/R induced toxicity. OGD/R significantly decreased the expression of GLT-1 and increased the expression of EAAC1 in cortical and hippocampus slices. Although PAM had no significant effect on the expression of EAAC1 in hippocampus slices, but PAM markedly prevented the effects induced by OGD/R on expression of GLT-1 and EAAC1 in cortical slices. Under OGD/R conditions, the level of MMP was markedly decreased in rat cortical cultures. PAM (0.1, 1, and 10 μg/mL) were obviously increased the level of MMP. **Conclusion:** PAM can alleviate rat brain slices injury induced by OGD/R. The mechanisms were likely due to regulating the expression of GLT-1 and EAAC1. PAM also stabilized the level of MMP under OGD/R condition in rat cortical cultures.

Keywords: phenolic alkaloids of *Menispermum dauricum* rhizome; oxygen glucose deprivation; excitatory amino acid transporter; mitochondrial membrane potential

S2.66

Salvianolic acid A inhibits vascular smooth muscle cells proliferation via cAMP/PKA/CREB cascade

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Aim: cAMP/PKA/cAMP response element binding protein (CREB) signaling cascade negatively regulates PDGF-BB induced smooth muscle cell (SMC) proliferation, which plays a crucial role in the pathogenesis of neointimal formation and restenosis after angioplasty. Studies have shown that restenosis after angioplasty may be alleviated by polyphenols. Salvianolic A (SalA) is kind of polyphenols extracted from salvia. In this report, we investigate whether cAMP/PKA/CREB cascade exerts an action on the anti-proliferative effects of SalA on SMCs. The underlying signal transduction pathway was also investigated. **Methods:** Two kinds of SMCs, including Human aortic smooth muscle cells(HASMCs) and primary rat vascular smooth muscle cells (RASMCs), were pretreated with SalA (0.01 to 0.1 μmol/L) for 2h, then stimulated by serum (10%) for 24 h. After these steps, several experiments were done: MTT and BrdU incorperation assay were used to measure the anti-proliferation rate. The influence of SalA on cell cycle was tested by flow cytometry. The activation of cAMP/PKA/CREB signal pathway was measured by cAMP-ELISA kit and Western blotting. **Results:** Our results told that SalA (0.01 to 0.1μmol/L) could inhibit PDGF-BB induced SMCs proliferation. The increased cell population of G_0/G_1 phase was corresponding to the concentration of SalA. Experiments also show the concentration of cAMP increased and activity of PKA and CREB were upregulated by SalA. **Conclusion:** The studies suggest that SalA inhibits PDGF-BB induced SMC proliferation via the activation of cAMP/ PKA/CREB signaling cascade. Therefore, SalA could be a promising treatment of stent restenosis and other cardiovascular diseases.

Keywords: salvianolic A; vascular smooth muscle cell; proliferation; vascular remodeling.

S2.67

Polyporus umbellatus (Pers.) Fries: traditional uses, phytochemistry, pharmacology, pharmacokinetics and quality control

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Aim: *Polyporus umbellatus* (Pers) Fries (Polyporaceae), a widely used traditional Chinese medicine, was known as *Zhu Ling*, *Grifola umbellata* and *Dendropolyporus umbellatus*. It was taken to treat the edema, scanty urine, vaginal discharge, urinary dysfunction, as well as jaundice and diarrhea. The aim of this review is to provide comprehensive information on the traditional uses, propagation, phytochemistry, pharmacology, pharmacokinetics and quality control of *P umbellatus* to explore their therapeutic potential and future research opportunities. **Methods:** All the available information on *P umbellatus* was collected via electronic search (using Web of Science, Pubmed, Science Direct, Splinker and Google Scholar) and a library search. **Results:** Phytochemical research on *P umbellatus* has led to the isolation of steroids, anthraquinones, alkaloids, triterpenoids and nucleosides. Crude extracts, fractions and isolated components of *P umbellatus* showed a wide spectrum of pharmacological activities like diuretic, nephroprotective, anticancer, immuno-enhancing, hepatoprotective, anti-inflammatory and antioxidative activities. **Conclusion:** *P umbellatus* has been widely used medicinal resources for centuries without any toxicities or adverse effects. Various studies have provided evidence for its traditional uses. However, there is a need to search for individual secondary metabolites responsible for these actions and study their mode of actions, bioavailability and physiological pathways in sufficient detail. The promising results should be further substantiated by clinical trials.

Keywords: *Polyporus umbellatus*; *Grifola umbellata*; phytochemistry; diuretic; nephroprotective; anticancer; pharmacokinetics

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S2.68

Oligomer procyanidins (F2) isolated from grape seeds inhibits HIF-1 pathway in U251 and Hep3B cells

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Aim: Oligomer procyanidins (F2, degree of polymerization 2–15), a natural fraction isolated from grape seeds, was demonstrated to inhibit the multiple kinds of tumor cells growth and migration in our previous study. Grape seed extract (GSE) was reported to inhibit HIF-1 pathway and its target. In this study, the effect of F2 on HIF-1 activity and the underlying mechanism were investigated. **Methods:** The HIF-1 responsive U251 and Hep3B cells were selected in the present study. Cells were incubated in hypoxic chamber to induce HIF-1α expression. Luciferase assay was used to test HIF-1 activity. Western blot and PCR were used to examine the expressions of HIF-1. **Results:** F2 markedly inhibited HIF-1α expression in both cancer cells under normoxic and hypoxic conditions. F2 decreased hypoxia-induced HIF-1 target genes-VEGF mRNA and proteins expression. However, HIF-1a mRNA was unchanged by F2 treatment, suggesting that suppression of HIF-1a protein expression does not involve down-regulation of its transcription. F2 significantly inhibited hypoxia-induced PI3K/AKT and MAPK/ERK1/2 signaling pathway, which involve in the HIF-1α translation. These results suggest that F2 decreased the HIF-1α accumulation probably via inhibiting HIF-1α translation. **Conclusion:** These data suggest that F2 is a potent HIF-1 inhibitor and its potential as a cancer therapeutic agent warrants further study.

Keywords: HIF-1; F2; protein translation; angiogenesis; in vasion

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S2.69

Effects of Jin Chai antiviral capsule *in vivo* approach of influenza virus infection through inhibition of virus—induced NF-κB activation

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Aim: To investigate the treatment of Jin Chai antiviral capsule for influenza virus PR8/34 (H1N1) infection. **Methods:** The model of pneumonia was established by dropping influenza virus into the nose of normal mice, using specific IKK inhibitiors at non-toxic concentrations, this study analysed the expression of NF-κB, virus load, andcytokine in mouse model at d 1, 3, 5, and 7 day after affected. **Results:** The immunohistochemical assay showed that NF-κB pathway were activated upon PR8/34 (H1N1). Real-time PCR assay showed that both IKK inhibitiors at non-toxic concentrations and Jin Chai antiviral capsule dose groups leaded to decrease in signalling virus load and reduced cytokine (ELISA). Jin Chai antiviral capsule can decrease the expression of NF-κB. IKKalpha large dose groups are significantly decrease the expression of NF-κB compared with model group at each time point ($P<0.05$, $P<0.01$); IKKalpha middle dose groups are significantly decrease the expression of NF-κB compared with model group at the 3th day and the 5th day ($P<0.05$); IKKalpha small dose groups are significantly decrease the expression of NF-κB compared with model group at the 3th day

(P<0.05). Specific IKK inhibitors used in non-toxic concentration, also were given the Jin Chai antiviral capsulel (large dose groups), showed that there was no obvious difference between the IKK inhibitiors group and IKK inhibitiors+Jin Chai antiviral capsule group. **Conclusion:** Effects of Jin Chai antiviral capsule approach of influenza virus infection through inhibition of virus-induced NF-κB activation

Keywords: Jin Chai antiviral capsule; influenza virus H1N1; NF-κB pathway

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S2.70

Primary study of naringin and naringenin on homocysteine of hyperlipidemia model rats

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Aim: To study the effect of naringin and naringenin on serum total homocysteine (tHcy) in hyperlipidemia model rats. **Methods:** The hyperlipidemia model rats were made by high lipid emulsion ig. The rats were divided into 7 groups, they were normal feed group, high lipid controls, high and low naringin and naringenin groups, respectively. They had been treated by high lipid emulsion ig for 4 weeks to set up hyperlipidemia model rats except the normal feed group fed by normal feed. Then, vehicle (0.5% CMC) or different dosages of naringin or naringenin had been given to the rats for 3 weeks. The bloods were collected and centrifuged to obtain sera. The serum total homocysteine (tHcy), triglyceride (TG), cholesterol (Ch), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) were measured automatically. **Results:** The lipids were higher in hyperlipidemia model rats than those in normal feed rats. Both naringin and naringenin displayed little effect on lipids in these dosages. Low naringenin and naringin groups decreased tHcy significantly (P<0.01 and 0.05, respectively) while others showed no significant changes. tHcy and lipids displayed no significant correlation by correlation testing. **Conclusion:** Naringin and naringenin had no significant effect on lipids in hyperlipidemia model rats, they might affect the concentration of tHcy in low dose but further study was necessary to confirm that.

Keywords: naringin; naringenin; homocysteine; hyperlipidemia model rats

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S2.71

Repair effect of Sanchi-loaded electrospun artificial skin substitute on damaged skin in rats

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Aim: Artificial skin substitute made of biomaterials and traditional Chinese medicine without living cells is promising for damaged skin repair. The kind of skin substitute possesses excellent histocompatibility without any risk of virus or immunogenicity. The objective of this study was to evaluate the repair effect of a novel Sanchi (*Panax Notoginseng* (Burk) F H Chen)-loaded electrospun artificial skin substitute (SASS) on damaged skin in rats. **Methods:** SASS was made of polylactic acid, gelatin and *Panax Notoginseng* by electrospinning method. Animal model of skin damage was established. Wound of 3 cm×3 cm in the back of each SD rat was formed under anesthesia. After model establishment, the rats were randomized into two groups. In one group the damaged skin was covered with SASS and in the other group with petrolatum gauze (PG). The wound healing was observed for 8 weeks. The tissue sample was drawn for histological examination on 2nd, 4th and 8th week. **Results:** The results showed that all rats in both groups grew well, but the resistance to infection and prevention wound bleeding in SASS group were better than in PG cover group. Histological observation indicated that SASS could improve much tissue regeneration than PG. SASS material is soft and easy to cut, has a good ability of anti-tearing and adhesion to wound. **Conclusion:** Compared with PG, SASS is an excellent artificial substitute for skin damage repair.

Keywords: artificial skin; electrospun; regenerative medicine; skin damage repair; *Panax Notoginseng*