

School of Pharmaceutical Sciences, Jilin University, Jilin, P. R. China

Effects of *Acanthopanax senticosus* polysaccharide on the proliferation, apoptosis and cell cycle in human HepG2 cells

HAOTIAN WANG, BO SUN, ZHENG ZHANG, JINGLIN CHEN, QIANG HAO, YUXIN SUN, YUE YANG, ZENG WANG, JIN PEI

Received October 10, 2015, accepted November 6, 2015

Jin Pei, School of Pharmaceutical Sciences, Jilin University, Jilin, P. R. China
peijin@jlu.edu.cn

Pharmazie 71: 201–204 (2016)

doi: 10.1691/ph.2016.5807

One water-soluble polysaccharide (ASPS), with four molecular weight distributions of 7.4, 3.8, 4.5, 2.3×10^4 Da, was isolated from the root of *Acanthopanax senticosus* and the yield was 4.8% (w/w). ASPS was composed of arabinose (51.4%), glucose (24.5%), galactose (10.2%), xylose (5.7) and galacturonic acid (4.9%). Effects of ASPS on the proliferation, apoptosis and Wnt/ β -catenin signaling pathway were investigated in human hepatocellular carcinoma cell line HepG2 cells. The study showed that ASPS could inhibit the proliferation, increase the apoptosis rate in HepG2 cells; meanwhile, ASPS could increase the proportion of cells in G0/G1 phase, decrease the proportion of cells in S phase and G2/M phase, and elevate the expression level of β -catenin, C-myc and Cyclin D1 proteins in HepG2 cells. These results indicate that ASPS has a certain inhibition on the proliferation, can induce the apoptosis and G0/G1 phase arrest in HepG2 cells, and the mechanism may be related to the inhibition of ASPS on the activation of Wnt/ β -catenin pathway HepG2 cells.

1. Introduction

Liver cancer is a common clinical gastrointestinal cancer with a higher degree of malignancy and its mortality is in top three in the world (Ramesh 2014). In China, people who died of liver cancer have been more than a hundred thousand each year, accounting for about 45% of the total number of died people caused by liver cancer in the world (Gao et al. 2015; Jiang and Cao 2015). The etiology of liver cancer is complex, and virus infections such as hepatitis B and hepatitis C, obesity, chronic alcoholism and exposure to aflatoxin all can induce liver cancer (El-Serag and Rudolph, 2007). Current therapies for liver cancer include surgical resection, liver transplantation, local ablation, radioactive embolization, chemoembolization and molecular targeted therapy (Vivarelli et al. 2013). The surgical resection and liver transplantation are the primary therapies for patients, but the high cost of surgery and the unavailability of liver donors have limited the treatment of patients with liver cancer (Tsim et al. 2010); radiotherapy and chemotherapy are still the main therapies for patients who are in the late stage unwilling to accept the surgery or agree to receive postoperative treatment. Current chemotherapeutic drugs used for the treatment of liver cancer primarily include sorafenib, nilotinib, doxorubicin, lincitin etc. Although sorafenib, a molecular targeted agent, has shown a higher antitumor activity than that of other drugs in patients with advanced liver cancer, generally it can only prolong the survival for three months (Dizon and Kemeny 2002; Poon et al. 2002; Zhang et al. 2012); in addition, side effects of the chemotherapy are also an important cause to restrict its application in clinic. Traditional Chinese medicine is unique in the treatment of certain cancers, and at present, anticancer drugs made from plant extracts, accounting for more than 30% of total anticancer drugs, have become common anticancer drugs in clinic, such as ginseng saponin, camptothecin and paclitaxel (Ando et al. 2012; Bae et al. 2012; Minelli et al. 2012; Mochizuki et al. 1995). With the deepening of the research on the antitumor activity of Chinese herbal medicine, the extraction of antitumor active ingredients from Chinese herbal medicine and the research on their antitumor activities have become a new hotspot (Auyeung et al. 2009; Park et al. 2014).

Acanthopanax senticosi, a Chinese herbal medicine, is the root of *Araliaceae acanthopanax* plant, and its main active ingredients are glycosides and flavonoids. Their activities include antitumor efficacy (Yoon et al. 2004), antioxidation (Kim et al. 2010; Lee et al. 2004), antifatigue efficacy (Nishibe et al. 1990), neural protection and repair (Bai et al. 2011; Fujikawa et al. 2005; Tohda et al. 2008), improvement of immunity (Lin et al. 2008; Zhang et al. 1990), and many others. *Acanthopanax senticosus* polysaccharide (ASPS) is one of active ingredients extracted from the root of *Acanthopanax senticosus*, with activities including immunomodulatory (Fang et al. 1985; Smalinskiene et al. 2014), antioxidative (Chen et al. 2011), antiradiation (Li and Zhou 2007) and hypoglycemic effects (Fu et al. 2012). It has been considered a potential for the application in clinic and has attracted much attention of scholars. In view of the limited number of studies on the antitumor activity of ASPS currently, human HepG2 cells were used as the study object to observe the effect of ASPS on the proliferation and apoptosis of HepG2 cells, and at the same time, in consideration that Wnt/ β -catenin signaling pathway may mediate a variety of malignant behaviors of tumors, the effect of ASPS on Wnt/ β -catenin signaling pathway in HepG2 cells was further analyzed in this study.

2. Investigations and results

2.1. Molecular weight and monosaccharide composition

The crude polysaccharide isolated from *Acanthopanax senticosus* using a hot water extract method was a beige powder and the yield was 4.8% (w/w). ASPS showed four molecular weight distributions, and the molecular weight distribution of ASPS is shown in Table 1.

According to GC analysis, the sugar composition of ASPS was identified and quantified. The results demonstrated that arabinose appeared to be the predominant monosaccharide (51.4 %), followed by glucose (24.5 %), galactose (10.2 %), xylose (5.7 %) and galacturonic acid (4.9 %).

Table 1: Molecular weight distribution of the polysaccharides extracted from *Acanthopanax senticosus*

Polysaccharides	Molecular weight distribution (10 ⁴ Da)			
	Main distribution		Minor distribution	
ASPS	7.4	3.8	4.5	2.3

2.2. Effects of ASPS on the proliferation in HepG2 cells

HepG2 cells treated with 0, 10, 20, 40 and 80 mg/L of ASPS, respectively, were detected at 24, 48, 72 and 96 h with CCK-8 kit. ASPS showed a significant inhibitory effect on the proliferation in HepG2 cells, the proliferation inhibition rate increased with the extension of ASPS duration and the increase in ASPS concentration, and the overall inhibitory effect was in a dose- and time-dependent manner, indicating that ASPS has a certain inhibitory effect on the proliferation in HepG2 cells (Fig. 1).

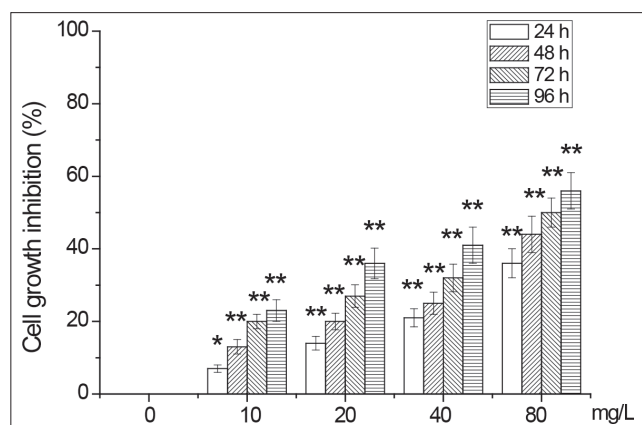


Fig. 1: Effects of ASPS at different concentrations on the proliferation in HepG2 cells. HepG2 cells were treated with 0, 10, 20, 40 and 80 mg/L of ASPS for 24, 48, 72 and 96 h. The proliferation inhibition rate was measured by MTT assay. Each value is presented as mean $\pm$ SD (n=3). *: $P < 0.05$ compared with 0 mg/L ASPS, **: $P < 0.01$ compared with 0 mg/L ASPS.

2.3. Effects of ASPS on the apoptosis in HepG2 cells

HepG2 cells were treated with 0, 10, 20, 40 and 80 mg/L of ASPS, respectively, and 48 h later they were detected with Annexin-FITC/PI double staining. It was found that early, late and total apoptosis rates of HepG2 cells treated with 10, 20, 40 and 80 mg/L ASPS all were higher than those of HepG2 cells treated with 0 mg/L of ASPS, indicating that ASPS can induce the apoptosis of HepG2 cells (Fig. 2).

2.4. Effects of ASPS on the cell cycle in HepG2 cells

HepG2 cells were treated with 0, 10, 20, 40 and 80 mg/L of ASPS, and 48 h later a flow cytometry was used to detect their cell cycles. The results showed that compared with those treated with 0 mg/L of ASPS, the proportion of G₀/G₁ phase cells increased, and that of both S phase and G₂/M phase cells decreased in HepG2 cells treated with 10, 20, 40 and 80 mg/L of ASPS, with a dose-dependent manner (Table 2).

2.5. Effects of ASPS on Wnt/ β -catenin signaling pathway in HepG2 cells

HepG2 cells were treated with 0, 10, 20, 40 and 80 mg/L of ASPS, and 48 h later they were detected by western blotting analysis. The results showed that compared with those treated with 0 mg/L ASPS, β -catenin, C-myc and Cyclin D1 protein levels were lower in HepG2 cells treated with 10, 20, 40 and 80 mg/L of ASPS, indi-

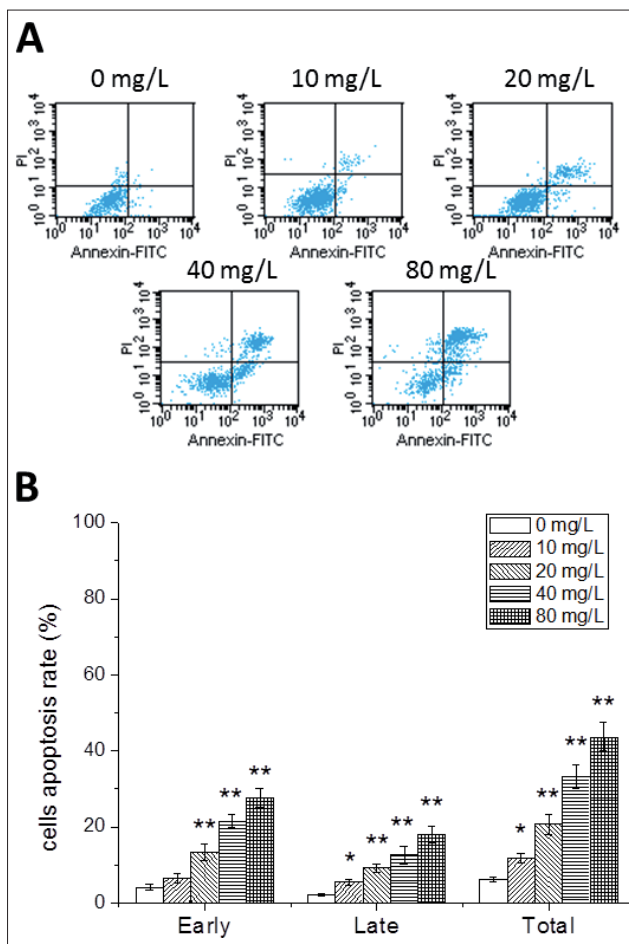


Fig. 2 Effects of ASPS at different concentrations on the apoptotic rate in HepG2 cells. (A) flow cytometric analysis; (B) cell apoptosis rate. HepG2 cells were treated with 0, 10, 20, 40 and 80 mg/L of ASPS for 48 h, respectively. Then they were stained with FITC-conjugated Annexin V and PI for flow cytometric analysis. Each value is presented as mean $\pm$ SD (n=3). *: $P < 0.05$ compared with 0 mg/L ASPS, **: $P < 0.01$ compared with 0 mg/L ASPS.

Table 2: Effects of ASPS at different concentrations on cells cycle in HepG2 cells

Concentration (mg/L)	G ₀ /G ₁	S	G ₂ /M
0	40.26 $\pm$ 3.15	37.42 $\pm$ 2.62	22.43 $\pm$ 2.10
10	45.63 $\pm$ 2.63*	34.41 $\pm$ 3.54*	18.71 $\pm$ 0.98*
20	52.82 $\pm$ 3.06**	30.65 $\pm$ 2.28*	11.65 $\pm$ 1.24**
40	59.33 $\pm$ 4.22**	26.38 $\pm$ 2.71**	8.23 $\pm$ 2.66**
80	64.38 $\pm$ 3.37**	22.45 $\pm$ 1.66**	6.59 $\pm$ 1.02**

Each value is presented as mean $\pm$ SD (n=3). *: $P < 0.05$ compared with 0 mg/L ASPS, **: $P < 0.01$ compared with 0 mg/L ASPS.

cating that ASPS has a inhibitory effect on Wnt/ β -catenin pathway in HepG2 cells (Fig. 3).

2.6. Conclusion

In this study, ASPS was isolated from the root of *Acanthopanax senticosus* and ASPS showed a certain inhibitory effect on the proliferation, can induce apoptosis and G₀/G₁ phase arrest in HepG2 cells, and the mechanism may be related to the inhibition of ASPS on the activation of the Wnt/ β -catenin pathway.

3. Discussion

Some natural plant polysaccharides have been verified to have a good antitumor activity due to their inhibition on the cell prolifer-

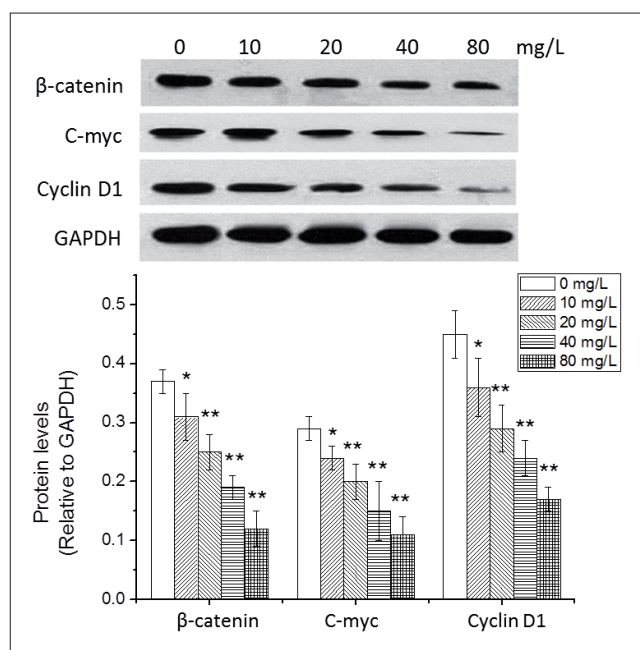


Fig. 3: Effects of ASPS at different concentrations on the Wnt/ β -catenin signaling pathway in HepG2 cells. HepG2 cells were treated with 0, 10, 20, 40 and 80 mg/L of ASPS for 48 h. Then the expressions of β -catenin, C-myc and Cyclin D1 were evaluated by western blot assay. Each value is presented as mean $\pm$ SD (n=3). *: $P < 0.05$ compared with 0 mg/L ASPS, **: $P < 0.01$ compared with 0 mg/L ASPS.

ation and inducing apoptosis, such as wolfberry polysaccharides (Gao et al. 2014; Shen and Du 2012), *Codonopsis pilosula* polysaccharide (Xin et al. 2012; Xu et al. 2012), safflower polysaccharides (Luo et al. 2015) and *Salvia miltiorrhiza* polysaccharide (Wang et al. 2014), etc. The excessive proliferation is one of the basic characteristics of malignant tumors and the use of an effective means to inhibit the proliferation of malignant tumors should be a feasible measure to control the growth of them (Kuo et al. 2009). In this study, ASPS was used to treat HepG2 cells and ASPS showed a significant inhibitory effect on HepG2 cells in a dose- and time-dependent manner. It is well known that the decrease in apoptosis is related to the occurrence and development of malignant tumors. Our study demonstrated that after treatment with ASPS, the early, late and total apoptosis rates increased in HepG2 cells treated with different concentrations of ASPS, indicating that ASPS can induce the apoptosis in HepG2 cells.

In addition to their effects on proliferation and apoptosis, chemotherapeutic drugs may exert their antitumor effects by affecting the cell cycle (Zhao et al. 2014). It was found in this study that the treatment with ASPS could increase the proportion of cells in the G_0/G_1 phase and reduce the proportion of those in S, G_2/M phases, suggesting that the treatment with ASPS could induce the cell cycle arrest in the G_0/G_1 phase. It is hypothesized that ASPS may inhibit the growth of HepG2 cells by affecting the cell cycle, primarily in the G_0/G_1 phase. These results suggest that ASPS should have a comprehensive antitumor effect on the proliferation, the apoptosis and the cell cycle in HepG2 cells.

Of many intracellular signaling pathways, Wnt/ β -catenin signaling pathway can play an important role in the proliferation, apoptosis and cell cycle of cells, and the abnormality in its level is related to the occurrence and development of tumors (Stewart et al. 2014). Previous studies indicate that the pathway is activated in liver cancer cells and the inhibition on the activation of this pathway should be a feasible measure to treat liver cancer and β -catenin is a member of Wnt/ β -catenin pathway (Lachenmayer et al. 2012; Zhang et al. 2012). For this reason, this study investigated the effect of ASPS on β -catenin, and meanwhile, in view of the fact that C-myc and cyclin D1 should be the downstream proteins in the pathway (Xu et al. 2015), the effect of ASPS on levels of these two

proteins was further investigated. The results showed that after the treatment with ASPS for 48 h, levels of β -catenin, C-myc, cyclin D1 proteins were decreased, suggesting that ASPS could inhibit the Wnt/ β -catenin pathway. Currently, to explore an anticancer strategy through the level of intracellular signaling pathways has become a hot topic, especially aiming at the Wnt/ β -catenin signaling pathway to study or design an anticancer drug has been advocated, which may indicate a better prospect in the application of ASPS.

4. Experimental

4.1. Reagents

Acanthopanax senticosus was purchased from a local market in Changchun city of China. HepG2 cell line was purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). Cell counting kit was purchased from Biyuntian Biotechnology Co. (Jiangsu, China). Newborn calf serum and RPMI-1640 culture medium were purchased from Hyclone Co. (UT, USA). Annexin V-FITC/PI double staining apoptosis detection kit was purchased from Baosai Biotechnology Co. (Beijing, China). β -catenin (SC-5208), Cyclin D1 (sc-718) and C-myc (sc-764) were purchased from Santa Cruz Biotechnology, Inc. (TX, USA).

4.2. Extraction of the polysaccharides

Acanthopanax senticosus was dried at 60 °C in an oven and ground to obtain a fine powder, then weighed (50 g each) and extracted with 20 volumes of distilled water at 100 °C for 2 h. The suspension was centrifuged (3500 rpm, 10 min), and the supernatant was concentrated under vacuum. The protein in the product of condensation was deproteinized using the Sevag reagent (Sun et al. 2008). After removal of the Sevag reagent, 4 volumes of anhydrous ethanol were added to this concentrate, and the mixture was stirred at room temperature for 20 min and then left at 4 °C overnight. The precipitate was collected by centrifugation (3500 rpm, 10 min), subsequently washed three times with anhydrous ethanol, acetone and ether, respectively, then dissolved in distilled water and dialyzed in dialysis bag against distilled water at room temperature for three successive days. The retained fraction was recovered, concentrated under vacuum and lyophilized to obtain the crude water soluble polysaccharides, which was named as ASPS.

4.3. Molecular weight determination

Molecular weight of ASPS was determined by HPSEC. The sample of polysaccharide fractions was dissolved in distilled water, applied to a HPLC system of Shimadzu equipped with a TSK-GEL G3000 PWXL column, eluted with 0.1 mol/L Na_2SO_4 solution and detected by a RID-10A refractive index detector. Dextran standards with different molecular weights (T-2000, T-70, T-40, T-20 and T-10) were used to calibrate the column and establish a standard curve.

4.4. Monosaccharide composition analysis

ASPS was hydrolyzed and acetylated according to Lehrfeld (1985). Simply, the samples were hydrolyzed with TFA and then the hydrolyzed product was reduced with KBH_4 , followed by neutralization with acetic acid. After adding myo-inositol and Na_2CO_3 , the residue was concentrated. The reduced products were added with pyridine-propylamine and acetylated with pyridine-acetic anhydride. The acetylated products were analyzed by GC and identified and estimated with myo-inositol as the internal standard. GC was performed on an Agilent 6890 instrument equipped with HP-5 capillary column (30 m $\times$ 0.32 mm $\times$ 0.2 μm) and flame-ionization detector (FID) and temperatures programmed from 120 to 250 °C at a rate of 8 °C/min.

4.5. Detection of cell proliferation activity

Thawed HepG2 cells were routinely cultured in a culture flask containing RPMI-1640 culture medium with 10% newborn calf serum and antibiotics (100 U/mL of penicillin and 100 $\mu\text{g/mL}$ of streptomycin). The culture conditions were 5% CO_2 , saturated humidity, cultured in an incubator at 37 °C, regularly renewing the medium and routine passage. Logarithmic growth phase HepG2 cells digested with trypsin were prepared into a single cell suspension and the cell density was adjusted to $1 \times 10^5/\text{mL}$, and the cells in 100 μL of suspension per well were seeded in 96-well plates; 24 h later, ASPS were added to each well make the final concentrations become 0, 10, 20, 40 and 80 mg/L (0 mg/L for the control group and the rest concentrations for the experimental groups), the cells were routinely cultured for 24, 48, 72 and 96 h, and then incubated for 4 h after CCK-8 (10 μL) was added to each well. Values of the absorbance (A) of culture solutions at different concentrations were detected at 450 nm and the proliferation inhibition rate was calculated according to the following formula.

$$\text{Proliferation inhibition rate (\%)} = (1 - A_{\text{experimental group}} / A_{\text{control group}}) \times 100\%$$

4.6. Detection of cell apoptosis and cell cycle

Logarithmic growth phase HepG2 cells were seeded in 6-well plates at the density of 1×10^6 cells per well. After they were incubated with the different concentrations of ASPS for 48 h, based on instructions of the apoptosis detection kit, the dark staining was carried out, 5 μL of Annexin V-FITC and PI each were added to each well and the

apoptosis rate was measured by flow cytometry after staining for 15 min. Annexin V⁺/PI⁺ was taken as the early apoptotic cell, Annexin V⁺/PI⁻ was taken as the late apoptotic cell and the sum of the both was considered the total apoptotic cells. HepG2 cells cultured with the different concentrations of ASPS for 48 h were prepared into cell suspensions and the cell concentration was adjusted to 1.0×10^6 /mL, which were bathed in ice for 20 min. After the cells were washed twice, 50 µg/mL of PI were added to them for their staining. The cells were incubated at 4 °C in the dark for 30 min, and then they were counted by flow cytometer and the cell cycles were analyzed with Cell Quest software.

4.7. Western blotting analysis

After HepG2 cells cultured with the different concentrations of ASPS for 48 h were washed with pre-cooling PBS, the cell lysate was added to them for the lysis. The lysed solution was centrifuged at 12000 rpm and 4 °C for 5 min, and the precipitation was discarded. The protein concentration in the supernatant was detected with the BCA method, the protein concentration of each sample was adjusted and then an equal volume of loading buffer was added to each sample; 20 µg of boiled sample were added to each well, the electrophoresis on proteins in the samples was carried out using 10% SDS-PAGE gel and then they were transferred to PVDF membranes with semi-dry transfer method. According to the membrane area, an appropriate amount of primary antibodies of β -catenin, C-myc and Cyclin D1 (except 1:200 for β -catenin dilution, 1:100 for the rest) was in turn added to the membrane and it was kept at 4 °C overnight. An appropriate amount of secondary antibodies was added to it, which was incubated at room temperature for 2 h, colorated with ECL luminescence method and exposed on the film in a darkroom. Gel-pro Analyzer V4.0 image analysis software system was used to analyze the optical density of each developed band, and the semi-quantitative analysis was made based on the ratio of optical densities of each target protein and GAPDH protein.

4.8. Statistical analysis

The data in this study were analyzed with SPSS 17.0 software. The data were expressed as "mean $\pm$ SD". $P < 0.05$ or $P < 0.01$ was considered statistically significant.

Acknowledgement: This work was funded by project "YYZX 201124-1" supported by Sci-tech Department of Jilin Province.

References

- Ando M, Yonemori K, Katsumata N (2012) Phase- I and pharmacokinetic study of nab-paclitaxel, nanoparticle albumin-bound paclitaxel, administered weekly to Japanese patients with solid tumors and metastatic breast cancer. *Cancer Chemother Pharmacol* 69: 457-465.
- Auyeung KK, Cho CH, Ko JK (2009) A novel anticancer effect of Astragalus saponins: Transcriptional activation of NSAID-activated gene. *Int J Cancer* 125: 1082-1091.
- Bae JS, Park HS, Park JW (2012) Red ginseng and 20(S)-Rg3 control testosterone-induced prostate hyperplasia by deregulating androgen receptor signaling. *J Nat Med* 66: 476-485.
- Bai Y, Tohda C, Zhu S (2011) Active components from Siberian ginseng (*Eleutherococcus senticosus*) for protection of amyloid β (25-35)-induced neuritic atrophy in cultured rat cortical neurons. *J Nat Med* 65: 417-423.
- Chen R, Liu Z, Zhao J (2011) Antioxidant and immunobiological activity of water-soluble polysaccharide fractions purified from *Acanthopanax senticosus*. *Food Chemistry* 127: 434-440.
- Dizon DS, Kemeny NE (2002) Intrahepatic arterial infusion of chemotherapy: Clinical results. *Semin Oncol* 29: 126-135.
- El-Serag HB, Rudolph KL (2007) Hepatocellular carcinoma: Epidemiology and molecular carcinogenesis. *Gastroenterology* 132: 2557-2576.
- Fang JN, Proksch A, Wagner H (1985) Immunologically active polysaccharides of *Acanthopanax senticosus*. *Phytochemistry* 24: 2619-2622.
- Fu J, Fu J, Yuan J (2012) Anti-diabetic activities of *Acanthopanax senticosus* polysaccharide (ASP) in combination with metformin. *Int J Biol Macromol* 50: 619-623.
- Fujikawa T, Miguchi S, Kanada N (2005) *Acanthopanax senticosus* Harms as a prophylactic for MPTP-induced Parkinson's disease in rats. *J Ethnopharmacol* 97: 375-381.
- Gao K, Liu M, Cao J (2014) Protective effects of *Lycium barbarum* polysaccharide on 6-OHDA-induced apoptosis in PC12 cells through the ROS-NO pathway. *Molecules* 20: 293-308.
- Gao LB, Tian H, Wang XG (2015) Early enteral and parenteral nutritional support after hepatectomy in patients with hepatic carcinoma: A systematic review and meta-analysis. *Onco Targets Ther* 8: 623-631.
- Jiang HT, Cao JY (2015) Impact of laparoscopic versus open hepatectomy on perioperative clinical outcomes of patients with primary hepatic carcinoma. *Chin Med Sci J* 30: 80-83.
- Lee S, Son D, Ryu J (2004) Anti-oxidant activities of *Acanthopanax senticosus* stems and their lignan components. *Arch Pharm Res* 27: 106-110.
- Li XL, Zhou AG (2007) Preparation of polysaccharides from *Acanthopanax senticosus* and its inhibition against irradiation-induced injury of rats. *Carbohydrate Polymers* 67: 219-226.
- Lin QY, Jin LJ, Cao ZH (2008) Inhibition of inducible nitric oxide synthase by *Acanthopanax senticosus* extract in RAW264.7 macrophages. *J Ethnopharmacol* 118: 231-236.
- Kim KJ, Hong HD, Lee OH (2010) The effects of *Acanthopanax senticosus* on global hepatic gene expression in rats subjected to heat environmental stress. *Toxicology* 278: 217-223.
- Kuo HS, Hsu FN, Chiang MC (2009) The role of Cdk5 in retinoic acid-induced apoptosis of cervical cancer cell line. *Chin J Physiol* 52: 23-30.
- Lachenmayer A, Alsinet C, Savic R (2012) Wnt-pathway activation in two molecular classes of hepatocellular carcinoma and experimental modulation by sorafenib. *Clin Cancer Res* 18: 4997-5007.
- Lehrfeld J (1985) Simultaneous gas-liquid chromatographic determination of aldonic acids and aldoses. *Anal Chem* 57: 346-348.
- Luo Z, Zeng H, Ye Y (2015) Afflower polysaccharide inhibits the proliferation and metastasis of MCF-7 breast cancer cell. *Mol Med Rep* 11: 4611-4616.
- Minelli R, Cavalli R, Ellis L (2012) Nanosponge-encapsulated camptothecin exerts anti-tumor activity in human prostate cancer cells. *Eur J Pharm Sci* 47: 686-694.
- Mochizuki M, Yoo YC, Matsuzawa K (1995) Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside-Rb2, 20(R)- and 20(S)-ginsenoside-Rg3, of red ginseng. *Biol Pharm Bull* 18: 1197-1202.
- Nishibe S, Kinoshita H, Takeda H (1990) Phenolic compounds from stem bark of *Acanthopanax senticosus* and their pharmacological effect in chronic swimming stressed rats. *Chem Pharm Bull* 38: 1763-1765.
- Park EH, Kim YJ, Yamabe N (2014) Stereospecific anticancer effects of ginsenoside Rg3 epimers isolated from heat-processed American ginseng on human gastric cancer cell. *J Ginseng Res* 38: 22-27.
- Poon RT, Fan ST, Tsang FH (2002) Locoregional therapies hepatocellular carcinoma: Critical review from the surgeon's perspective. *Ann Surg* 235: 466-486.
- Ramesh H (2014) Resection for hepatocellular carcinoma. *J Clin Exp Hepatol* 4(Suppl 3): S90-96.
- Shen L, Du, G (2012) *Lycium barbarum* polysaccharide stimulates of MCF-7 cells by the ERK pathway. *Life Sci* 91: 353-357.
- Smalinskiene A, Savickiene N, Zitkevicius V (2014) Effect of *Acanthopanax senticosus* on the accumulation of cadmium and on the immune response of spleen cells. *J Toxicol Environ Health A* 77: 1311-1318.
- Stewart DJ, Chang DW, Ye Y (2014) Wnt signaling pathway pharmacogenetics in non-small cell lung cancer. *Pharmacogenomics J* 14: 509-522.
- Sun YX, Wang SS, Li TB (2008) Purification, structure and immunobiological activity of a new water-soluble polysaccharide from the mycelium of *Polyporus albicans* (Imaz.) teng. *Bioresour Technol* 99: 900-904.
- Tohda C, Ichimura M, Bai Y (2008) Inhibitory effects of *Eleutherococcus senticosus* extracts on amyloid beta(25-35)-induced neuritic atrophy and synaptic loss. *Pharmacol Sci* 107: 329-339.
- Tsim NC, Frampton AE, Habib NA (2010) Surgical treatment for liver cancer. *World J Gastroenterol* 16: 927-933.
- Vivarelli M, Montalti R, Risaliti A (2013) Multimodal treatment of hepatocellular carcinoma on cirrhosis: An update. *World J Gastroenterol* 19: 7316-7326.
- Wang N, Yang J, Lu J (2014) A polysaccharide from *Salvia miltiorrhiza* Bunge improves immune function in gastric cancer rats. *Carbohydr Polym* 111: 47-55.
- Xin T, Zhang F, Jiang Q (2012) The inhibitory effect of a polysaccharide from *Codonopsis pilosula* on tumor growth and metastasis in vitro. *Int J Bio Macromol* 51: 788-793.
- Xu C, Liu, Y, Yuan G (2012) The contribution of side chains to antitumor activity of a polysaccharide from *Codonopsis pilosula*. *Int J Bio Macromol* 50: 891-894.
- Xu Y, Zhang J, Jiang W (2015) Astaxanthin induces angiogenesis through Wnt/ β -catenin signaling pathway. *Phytomedicine* 22: 744-751.
- Yoon TJ, Yoo YC, Lee SW (2004) Anti-metastatic activity of *Acanthopanax senticosus* extract and its possible immunological mechanism of action. *J Ethnopharmacol* 93: 247-253.
- Zhang X, Yang XR, Huang XW (2012) Sorafenib in treatment of patients with advance hepatocellular carcinoma: A systematic review. *Hepatobiliary Pancreat Dis Int* 11: 458-466.
- Zhang Y, Wei W, Cheng N (2012) Hepatitis C virus-induced up-regulation of micro RNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. *Hepatology* 56: 1631-1640.
- Zhang RJ, Qian JK, Yang GH (1990) Medicinal protection with Chinese herb-compound against radiation damage. *Aviat Space Environ Med* 61: 729-731.
- Zhao B, Li L, Liu J (2014) Exposure to perfluorooctane sulfonate in utero reduces testosterone production in rat fetal Leydig cells. *PLoS One* 9: e78888.