Journal of Medicinal Plants Research Vol. 5(29), pp. 6564-6570, 9 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.1102

Full Length Research Paper

Evaluation of *in vitro* antioxidant and antitumour activities of *Astragalus membranaceus* aqueous extract

Xian-zhong Peng*, Shao-shui Chen, Fang-ling Ning, Mian-li Li

Department of Oncology, Affiliated Hospital of Binzhou Medical College, Binzhou 256603, China.

Accepted 22 September, 2011

Aqueous extracts of *Astragalus membranaceus* (AMEE) were prepared in this study. *In vitro* antioxidant and antitumour activities of aqueous extracts of *A. membranaceus* were evaluated. The aqueous extracts exhibited could effectively scavenge superoxide anion, Hydrogen peroxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and reduce 2,2'-azobis(2-amidinopropane) hydrochloride (AAPH)-induced human erythrocytes hemolysis. Moreover, aqueous extracts of *A. membranaceus* could still effectively dose-dependently inhibit against the human Gastric cancer cell line SGC-7901 and human hepatoma SMMC-7721 cells growth at the given dose range. The results indicate that the aqueous extracts of *A. membranaceus* have a very potent antioxidant and antitumour activity.

Key words: *Astragalus membranaceus* aqueous extracts, antioxidant, antitumour, SGC-7901, SMMC-7721.

INTRODUCTION

Astragalus membranaceus (Fisch.) Bunge (AM), Maxim of the Leguminosae family, is a traditional Chinese medicinal herb originated in Northern China. The dried root of AM, Huangqi, contains 2′4′-dihydroxy-5,6-dimethoxyisoflavone, kumatakenin, choline, betaine, polysaccharides, saponins, glucuronic acid, sucrose, amino acids, traces of folic acid and astraisoflavanin (Bensky and Gamble, 1993; Ma et al., 2002; Wu and Chen, 2004). *A. membranaceus* (AM) (root), a widely used Qi-tonifying and immunomodulating herb in traditional Chinese medicine, has gradually been recognized as a promising complementary medication in the management of diabetic renal disease (Zhang et al., 2009; Li et al., 2011). AM (root), also known as Huangqi in Chinese and Radix Astragali (RA) in Latin, is the dry root of *A. membranaceus* (Fisch.) Bge. or *A. membranaceus* var. Mongholicus (Bge.) Hsiao of the Fabaceae family. AM has been prescribed for centuries for cardiovascular disease and was believed to possess the effects of immune-stimulation, antiviral infection and antioxidative damage (Toda and Shirataki, 1999). The major bioactive constituents of AM (root) are flavonids, APS and saponins, and each has its own therapeutic properties. Evidences have indicated the importance of AM polysaccharide fractions in the modulation of immune functions both in human and experimental animals (Chen et al., 1981; Chu et al., 1988a, 1988b; McKenna et al., 2002; Wang et al., 2002; Block and Mead, 2003; Tan and Vanitha, 2004). Lee et al. (2002) also reported that flavonoid derivatives contained in *Rhus verniciflua* exhibit free radical-scavenging and anti-apoptotic properties. Previous studies have shown that the polysaccharides from *A. membranaceus* (Fisch.) Bunge (AM) are capable of restoring the immune functions in cancer subjects, as well as helping patients to improve the quality of life and therapeutic response (Shao et al., 2004; Yuan et al., 2008). Besides, AM injection supplemented with chemotherapy was found to inhibit the development of tumor, decrease the toxic-adverse effect of chemotherapy, elevate the immune function of organism and improve the quality of life in patients of malignant tumor (Duan and Wang, 2002).

Moreover, it was reported that AM injection could enhance the anti-tumor metastasis action of dendritic cells, effectively promote the immune response of tumor-bearing host and therefore had obviously inhibitory effect on cancer metastasis *in vivo*. Their immune protective function in normal animals was even more evident (Dong and Dong, 2005).

*Corresponding author. E-mail: byfypxz6137@126.com. Tel: Tel/Fax +86-13854380030

In this study, *in vitro* antioxidant activities and antitumour activities of the *A. membranaceus* aqueous extract were also evaluated.

MATERIALS AND METHODS

Plant material

A. membranaceus sample was purchased from a local herb shop in Shanghai.

Sample preparation

A. membranaceus (50 g) was grind into fine powder and steeped in 2000 ml of boiling water using reflux for 3 h. The water phase was filtered and freeze-dried. All extracts were stored at −20℃ until use.

Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of *A. membranaceus* aqueous extract was based on the method described by Liu et al. (1997) with slight modification of Oktay et al. (2003). Superoxide radicals are generated non-enzymatically in phenazine metho- sulfate (PMS)–NADH systems by the oxidation of NADH and assayed by the reduction of nitro blue tetrazolium (NBT). In this experiment, the superoxide radicals were generated in 1 ml of Tris–HCl buffer (16 mM, pH 8.0) containing NBT (50 µM) solution and NADH (78 µM) solution. The reaction was started by adding PMS solution (10 µM) to the mixture. The reaction mixture was incubated at 25℃ for 5 min, and the absorbance at 560 nm in a spectrophotometer was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

Percentage Inhibition = $[(A_0-A_1)/A_0\times100]$, where A_0 was the absorbance of the control and A_1 was the absorbance of extract and the standard compound.

Scavenging of hydrogen peroxide

The ability of the *A. membranaceus* aqueous extract to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (2 mmol/L) (Fine Chem Industries, Mumbai) was prepared in phosphate buffer (pH 7.4). Extracts (0.2 to 1.6 mg/ml) were added to hydrogen peroxide solution.

Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage scavenging activity of hydrogen peroxide by extract was calculated using the following formula,

Percentage scavenging activity $[H_2O_2]$ = [Abs (control) – Abs (standard) / Abs (control)] × 100.

Where, Abs (control): Absorbance of the control and Abs (standard): Absorbance of the extract/standard.

Quantitative 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

A methanolic solution of DPPH (0.15%) was mixed with serial dilutions of *A. membranaceus* aqueous extract and after 30 min; the absorbance was recorded at 515 nm using a spectrophotometer (HACH 4000 DU UV – visible spectrophotometer). Ascorbic acid was used as a positive control. The inhibition curve was plotted and the IC50 values were determined.

Erythrocyte hemolysis assay

EDTA-treated tubes (3 ml) were used to collect human blood from healthy volunteers at the 9th hospital in Shanghai. Erythrocytes were isolated as described by Souza et al. (2008). The protocol proposed by Souza et al. (2008) was used to evaluate the improved resistance conferred by the polyphenolic preparations to erythrocytes submitted to free radical attacks induced by AAPH. Hemolysis was followed at 534 nm every 90 s for 3 h at 37℃. The protection of erythrocytes was deduced from the time required (T0.5) for half-hemolysis (50% reduction at Abs540 nm) compared to control values (phosphate buffer). The inhibition of erythrocyte hemolysis was calculated as follows:

Percentage Inhibition $_{hemolysis}$ = $[1- (T_{0.5\ (No\ antioxidant)}/T_{0.5\ (with\ antioxidant)})] \times 100$

Cytotoxicity assays

The cytotoxicity of *A. membranaceus* aqueous extracts was evaluated against four human tumour cell lines: SGC-7901 (human Gastric Cancer) and SMMC-7721 cells (human hepatoma). The general viability of cultured cells was determined by the reduction of the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product, as previously described by Mosmann (1983).

The tumour cells were maintained in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin at 37℃ with 5% CO_2. For all experiments cells were seeded at 0.3×10^6 cells/ml and incubated during 24, 48 or 72 h with *A. membranaceus* aqueous extracts (25 to 200 mg/L), under the conditions described above. After centrifugation and solution removing, MTT solution was added and the plates were incubated, centrifuged, and the solids dissolved in pure and sterile DMSO. The absorbance was measured in a plate spectrophotometer DTX-800 (Beckman Coulter) at 595 nm.

RESULTS AND DISCUSSION

Superoxide anion scavenging activity

Superoxide anions were generated *in vitro* enzymatically by hypoxanthine/xanthine oxidase system that reduces NBT and forms a blue coloured chromophore, formazone that can be measured at 560 nm. Superoxide radicals generated *in vitro* by the system was determined by NBT photo reduction method. The decrease in absorbance at

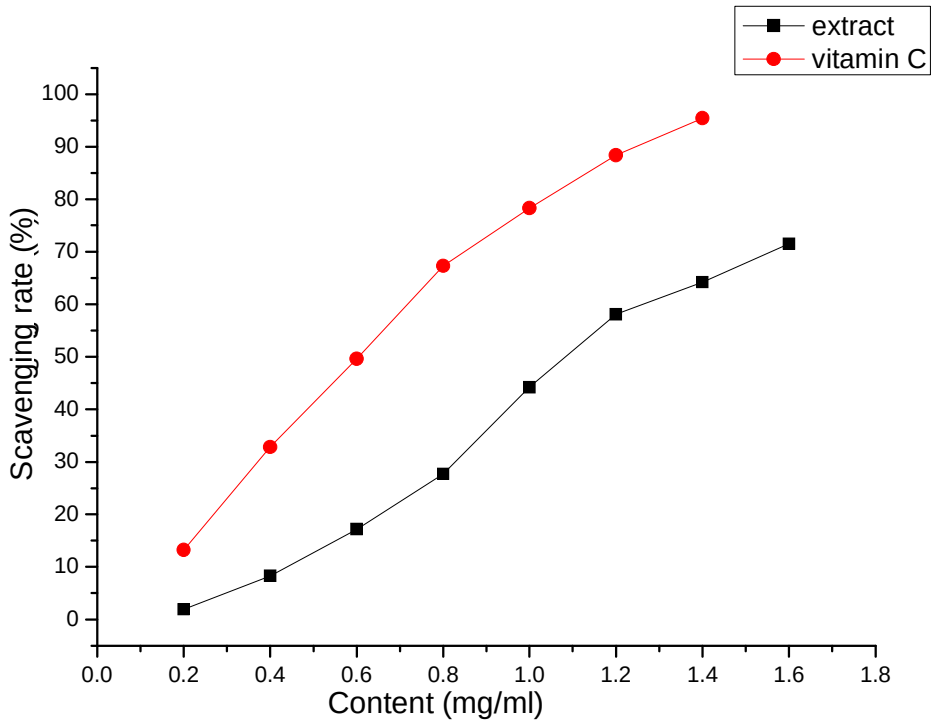

Figure 1. Scavenging activity of *Astragalus membranaceus* aqueous extract against the superoxide radical.

560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. Superoxide radical was converted by superoxide dismutase (SOD) to hydrogen peroxide, which subsequently can produce extremely reactive hydroxyl radicals in the presence of transition metal ions such as iron and copper or by UV photolysis. Determination of the mean rate of increase in absorbance over a 1 min period provided a measure of the extent to which the EEVZ capable of inhibiting NBT reduction by the superoxide anion radical and thus superoxide scavenging activity (Joubert et al., 2004; Das et al., 2009).

From the investigations on the superoxide radical scavenging capacities, it was found that the AME inhibits superoxide radicals in a dose dependent manner. The aqueous extract of *A. membranaceus* exhibited superoxide scavenging activity (Figure 1) at all the concentrations (0.2 to 1.6 mg ml^{-1}). The IC50 value for scavenging of O_2^- for AME was 1.13 mg/ml while IC50 value for ascorbic acid was 0.62 mg/ml. Ascorbic acid is a potent reducing agent and acts as a free radical scavenger. However, it may act as a pro-oxidant in the presence of metals (Bendich et al., 1986).

Scavenging of hydrogen peroxide

Hydrogen peroxide is a weak oxidizing agent which inactivates enzymes by oxidation of the essential thiol (SH^-) groups. It rapidly transverses cell membranes and once inside the cell interior, interacts with Fe^{2+} and Cu^{2+} to form hydroxyl radicals, which is harmful to the cell (Contreras-Guzmán and Strong, 1982). It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. Figure 2 shows that AME shows less scavenging activity (H_2O_2) than that of Ascorbic acid. The IC50 value for scavenging of H_2O_2 for AME was 1.23 mg/ml while IC50 value for ascorbic acid was 1.07 mg/ml.

Scavenging of DPPH radical

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The model of scavenging the stable DPPH radical is widely used for relatively rapid evaluation of antioxidant activities compared to other methods (Yokozawa et al., 1998). In the quantitative assay the extract exhibited a notable dose dependent inhibition of the DPPH activity, with a 50% inhibition (IC50) at a concentration of 0.91 mg/ml while the IC50 value of the positive control, ascorbic acid, was found 0.74 mg/ml (Figure 3).

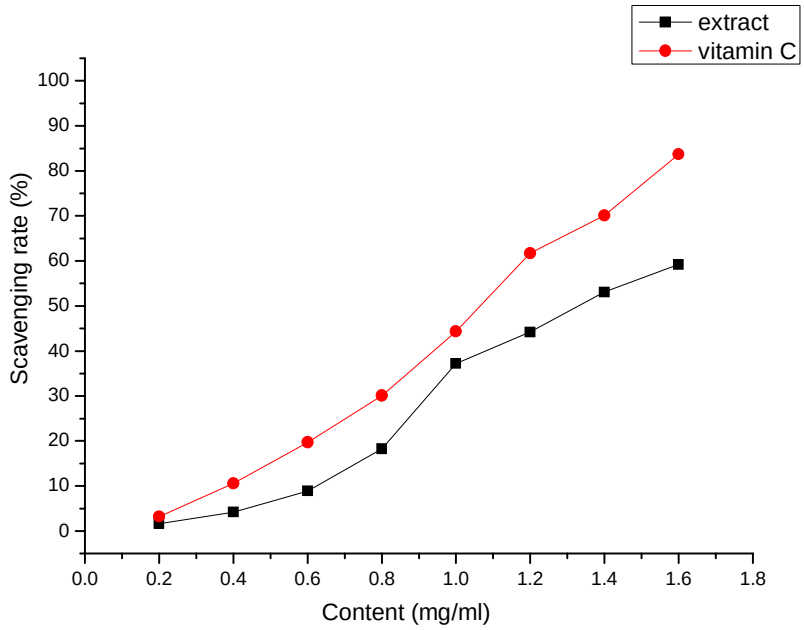

Figure 2. Scavenging activity of *Astragalus membranaceus* aqueous extract against the hydrogen peroxide radical.

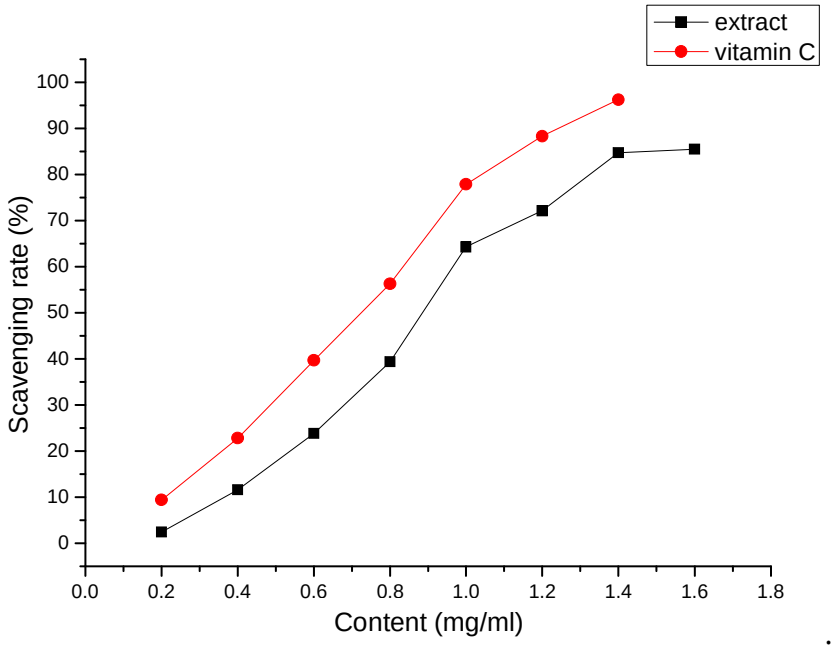

Figure 3. Scavenging activity of *Astragalus membranaceus* aqueous extract against the DPPH radical.

Effects of *A. membranaceus* aqueous extracts on human erythrocytes hemolysis

Figure 4 shows the effects of *A. membranaceus* aqueous extracts (40, 80, 120 mg/l) on human erythrocytes exposed to the water-soluble radical initiator AAPH. Erythrocytes incubated at 37℃ as a 2% suspension in PBS were stable with little hemolysis observed within 5 h

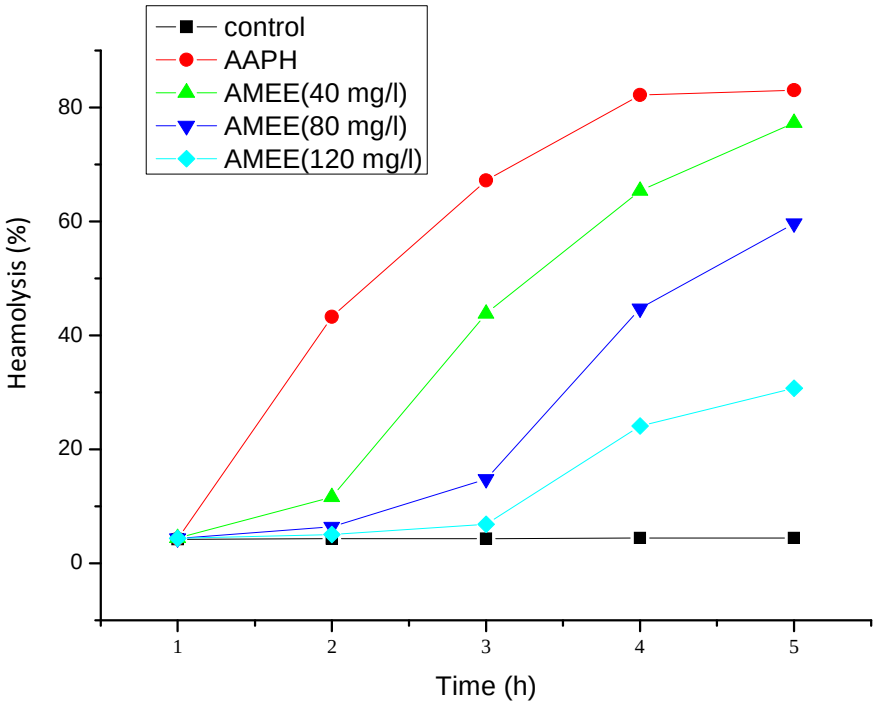

Figure 4. Effects of Astragalus membranaceus aqueous extracts on AAPH-induced hemolysis in erythrocytes.

(4.4 ± 0.5%). When the water-soluble radical generator, AAPH (25 mM), was added to the aqueous suspension of erythrocytes, the hemolysis induction was time dependent. The hemolysis lagged, indicating that endogenous antioxidants in the erythrocytes can trap radicals to protect them against free radical-induced hemolysis, as described previously (Zou et al., 2001). When production of peroxyl radicals (and other reactive species formed throughout the free radical chain propagation) overcomes the numerous antioxidant barriers of defence, an “oxidative stress” condition will develop, inducing oxidative damage on erythrocyte constituents, namely on membrane and haemoglobin, which may lead ultimately to hemolysis (Cimen, 2008). It is clearly evident from Figure 4 that *A. membranaceus* aqueous extracts significantly protected the erythrocyte membrane from hemolysis induced by AAPH in a concentration- and time-dependent manner. Moreover, a correlation can be demonstrated between lag time and extract concentration.

Evaluation of cytotoxic activity against human tumor cell lines

In our preliminary experiment, the anticancer activity of *A. membranaceus* aqueous extracts was evaluated against human Gastric cancer cell line SGC-7901 and human hepatoma SMMC-7721 cells. As illustrated in Figure 5A and B, incubation with *A. membranaceus* aqueous extracts significantly inhibited the cells’ proliferation in a dose-dependent and time-dependent manner, over the dose range of 20 to 200 mg/ml. At the dose of 200 mg/ml, inhibition rate of *A. membranaceus* aqueous extracts against SGC-7901 cells were 32.4% for 24 h, 60.3% for 48 h, and 75.5% for 72h, respectively. At the dose of 200 mg/ml, inhibition rate of *A. membranaceus* aqueous extracts against SMMC-7721 cells were 27.83% for 24 h, 59.13% for 48 h, and 66.3% for 72 h, respectively. Altogether, the results revealed that the *A. membranaceus* aqueous extracts was active against SGC-7901 cells and SMMC-7721 cells. All these data provided a strong evidence for the high anticancer effect of *A. membranaceus* aqueous extracts. In our preliminary study, we also examine effect of *A. membranaceus* aqueous extracts on normal gastic cells growth. We found that *A. membranaceus* aqueous extracts (20 to 200 mg/ml) did not significantly affect the growth of normal gastic cells. These results indicated that pharmacological activities of *A. membranaceus* aqueous extracts would be more significant in clinical medicine.

Conclusion

Exposure to O_2^- causes cell injuries or death to the aerobic organisms via cytotoxicity, carcinogenicity or mutagenicity.

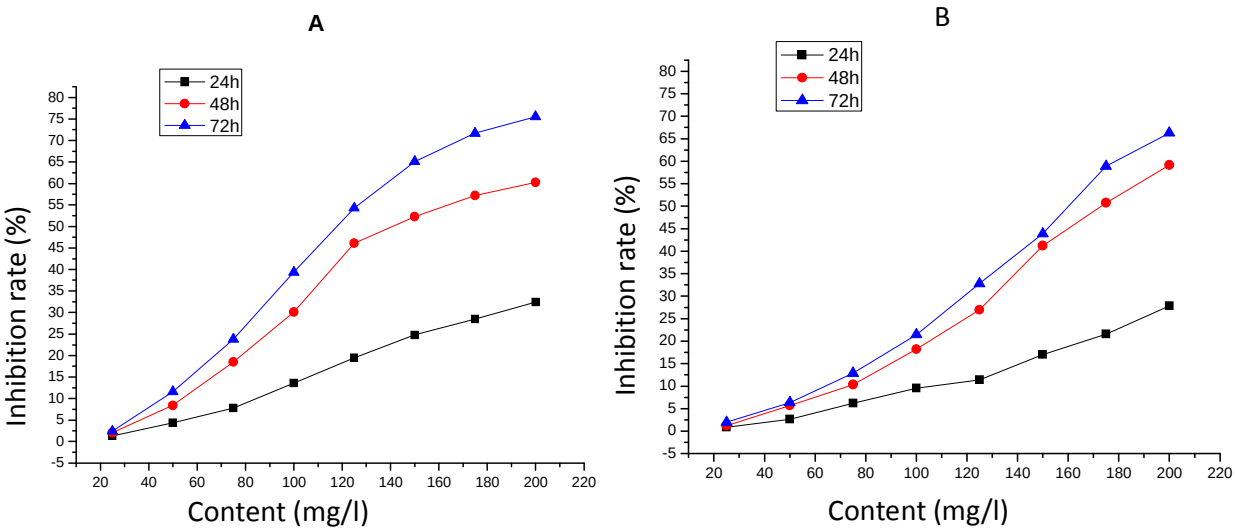

Figure 5. Cytotoxic effect of Astragalus membranaceus aqueous extracts against human Gastric Cancer cell line SGC-7901 (A), and human hepatoma SMMC-7721 cells (B). Cell survival was determined as the percentage of the control from three independent experiments.

Removing O_2^- can successfully protect cells against this damage. Therefore, small molecules, with antioxidant properties, are the new tools for the prevention of such diseases. In the present study, the *A. membranaceus* aqueous extracts displayed strong antioxidant activities, including scavenging O_2^-, $H_2O_2^-$, $DPPH^-$ radicals and decreasing AAPH-induced human erythrocytes hemolysis. In addition, the *A. membranaceus* aqueous extracts displayed strong antitumour activities against the human Gastric cancer cell line SGC-7901 and human hepatoma SMMC-7721 cells. The results of this study suggest that the *A. membranaceus* aqueous extracts possess both strong free radicals scavenging and antitumour activities, and further studies are needed to determine the efficacy *in vivo* or clinical usefulness.

REFERENCES

Bendich A, Machlin LJ, Scandurra O, Burton GW, Wayner DDM (1986). The antioxidant role of vitamin C. Adv. Free Radic. Biol. Med., 2: 419-444.

Bensky D, Gamble A (1993). Chinese Herbal Medicine: Materia Medica (revised ed.), Eastland Press, Seattle.

Block KI, Mead MN (2003). Immune system effects of echinacea, ginseng, and astragalus: a review, Integr Cancer Ther., 2: 247-267.

Chen LJ, Shen ML, Wang MY, Zhai SK, Liu MZ (1981). Effect of Astragalus polysaccharides on phagocytic function in mice, Zhongguo Yao Li Xue Bao, 2: 200-204.

Chu DT, Wong WL, Mavligit GM (1988a). Immunotherapy with Chinese medicinal herbs I. Immune restoration of local xenogeneic graft-versus-host reaction in cancer patients by fractionated Astragalus membranaceus *in vitro*, J. Clin. Lab. Immunol., 25: 119-123.

Chu DT, Wong WL, Mavligit GM (1988b). Immunotherapy with Chinese medicinal herbs II. Reversal of cyclophosphamide-induced immune suppression by administration of fractionated Astragalus membranaceus *in vitro*, J. Clin. Lab. Immunol., 25: 125-129.

Contreras-Guzmán ES, Strong FC (1982). Determination of tocopherols (vitamin E) by reduction of cupric ion. J. Assoc. Anal. Chem., 65: 1215-1221.

Das S, Neogy S, Gautam N, Roy S (2009). *In vitro* nicotine induced superoxide mediated DNA fragmentation in lymphocytes: Protective role of Andrographis paniculata Nees. Toxicol. *in vitro*, 23: 90-98.

Duan P, Wang ZM (2002). Clinical study on effect of Astragalus in efficacy enhancing and toxicity reducing of chemotherapy in patients of malignant tumor, Zhongguo Zhong Xi Yi Jie He Za Zhi, 22: 515-517.

Dong JC, Dong XH (2005). Comparative study on effect of Astragalus injection and interleukin-2 in enhancing anti-tumor metastasis action of dendrite cells. Zhongguo Zhong Xi Yi Jie He Za Zhi, 25: 236-239.

Joubert E, Winterton P, Britz TJ, Ferreira D (2004). Superoxide anion and α,α-diphenyl-β-picrylhydrazyl radical scavenging capacity of rooibos (Aspalathus linearis) aqueous extracts, crude phenolic fractions, tannin and flavonoids. Food Res. Int., 37: 133-138.

Lee JC, Lim KT, Jang YS (2002). Identification of Rhus verniciflua Stokes compounds that exhibit free radical scavenging and anti-apoptotic properties. Biochim. Biophys. Acta, 1570: 181-191.

Li MX, Wang WX, Xue J, Gu Y, Lin SY (2011). Meta-analysis of the clinical value of Astragalus membranaceus in diabetic nephropathy. J. Ethnopharmacol., 133: 412-419.

Liu F, Ooi VEC, Chang ST (1997). Free radical scavenging activity of mushroom polysaccharide extracts. Life Sci., 60: 763-771.

Ma XQ, Shi Q, Duan JA, Dong TT, Tsim KW (2002). Chemical analysis of Radix Astragali (Huangqi) in China: a comparison with its adulterants and seasonal variations. J. Agric. Food Chem., 50: 4861-4866.

McKenna DJ, Hughes K, Jones K (2002). Astragalus. Int. J. Integr. Med., 4: 40-46.

Mosmann T (1983). Rapid colourimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65: 55-63.

Oktay M, Gülcin I, Küfrevioglu Öİ (2003). Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Food Technol. Wissenchaft, 36: 263-271.

Ruch RJ, Cheng SJ, Klaunig JE (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from chinese green tea. Carcinogenesis, 10: 1003-1008.

Shao BM, Xu W, Dai H, Tu PF, Li ZJ, Gao XM (2004). A study on the immune receptors for polysaccharides from the roots of Astragalus membranaceus, a Chinese medicinal herb. Biochem. Biophys. Res. Commun., 320: 1103-1111.

Souza JNS, Silva EM, Loir A, Rees J-F, Rogez H, Larondelle Y (2008). Antioxidant capacity of four polyphenol-rich Amazonian plant extracts: A correlation study using chemical and biological *in vitro* assays. Food Chem., 106: 331-339.

Tan BK, Vanitha J (2004). Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: a review. Curr. Med. Chem., 11: 1423-1430.

Toda S, Shirataki Y (1999). Inhibitory effects of Astragali Radix, a crude drug in Oriental medicines, on lipid peroxidation and protein oxidative modification by copper. J. Ethnopharmacol., 68: 331-333.

Wang RT, Shan BE, Li QX (2002). Extracorporeal experimental study on immuno-modulatory activity of Astragalus membranaceus extract. Zhongguo Zhong Xi Yi Jie He Za Zhi, 22: 453-456.

Wu F, Chen X. (2004). A review of pharmacological study on Astragalus membranaceus (Fisch.) Bge, Zhong Yao Cai, 27: 232-234.

Yuan CT, Pan XP, Gong Y, Xia AJ, Wu GH, Tang JQ, Han XD (2008). Effects of Astragalus polysaccharides (APS) on the expression of immune response genes in head kidney, gill and spleen of the common carp, *Cyprinus carpio* L. Int. Immunopharmacol., 8: 51-58.

Yokozawa T, Chen CP, Dong E, Tanaka T, Nonaka GI, Nishioka I (1998). Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2 picrylhydrazyl radical. Biochem. Pharmacol., 56: 213-222.

Zhang JQ, Xie XS, Li C, Fu P (2009). Systematic review of the renal protective effect of Astragalus membranaceus (root) on diabetic nephropathy in animal models. J. Ethnopharmacol., 126: 189-196.

Zou C, Liebert M, Zou C, Grossman HB, Lotan R (2001). Identification of effective retinoids for inhibiting growth and inducing apoptosis in bladder cancer cells. J. Urol., 165: 986-992.

Cimen MY (2008). Free radical metabolism in human erythrocytes. Clin. Chim. Acta, 390: 1-11.