International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 8 (7): 638-646, 2012
ISSN 1811-7775 / DOI: 10.3923/ijp.2012.638.646

Involvement of Transient Receptor Potential Melastatin 7 Channels in *Orostachys japonicus*-induced Apoptosis in Cancer Cells

[1]Min-Woo Hwang, [2]Hyung Woo Kim and [3]Byung Joo Kim
[1]Department of Sasang Constitutional Medicine, College of Korean Medicine,
Kyung-hee University, Seoul, 130-701, Republic of Korea
[2]Division of Pharmacology,
[3]Division of Longevity and Biofunctional Medicine, Pusan National University School of Korean Medicine,
Yangsan 626-870, Republic of Korea

Abstract: *Orostachys japonicus* (OJ) herbal preparations have a history of use in Korean folk medicine for the treatment of cancer. The aim of this study was to clarify the potential role of OJ extract on transient receptor potential melastatin 7 (TRPM7) channels in the growth and survival of AGS and MCF-7 cells, the most common human gastric and breast adenocarcinoma cell line. The AGS and MCF-7 cells were treated with varying concentrations of OJ extract. Caspase-3 activity, mitochondrial depolarization and sub-G1 analysis were conducted to determine if AGS and MCF-7 cell death occurs by apoptosis. Also, TRPM7 channels were overexpressed in Human Embryonic Kidney (HEK) 293 cells to identify the role of TRPM7 channels in AGS and MCF-7 cell growth and survival. Addition of OJ extract to the culture medium inhibited AGS and MCF-7 cells growth and survival. Experimental results showed caspase-3 activity, mitochondrial depolarization and sub-G1 were elevated. OJ extract inhibited the TRPM7-like current in AGS and MCF-7 cells and TRPM7 channel overexpressed HEK293 cells by whole cell voltage-clamp recordings. Furthermore, TRPM7 overexpression in HEK 293 cells exacerbated OJ extract-induced cell death. These findings indicate that OJ extract inhibits the growth and survival of gastric and breast cancer cell which is due to the blockade of TRPM7 channel activity. Therefore, OJ extract has an important role in regulation of the survival of gastric and breast cancer cells through TRPM7 channels.

Key words: *Orostachys japonicus*, transient receptor potential melastatin 7 (TRPM7) channel, gastric cancer, breast cancer

INTRODUCTION

Orostachys japonicas (OJ), a perennial herbaceous plant belongs to family Crassulaceae, is traditionally used as a folk medication with anti-inflammatory, anti-febrile, hemostatic, antidote and anti-cancer activities (Ryu *et al*., 2012). Friedelin, epi-friedlanol, grutinone, glutinol, triterpenoid, β-sitosterol, campesterol, fatty acid ester, kaempferol, quercetin, flavonoid and aromatic acids have been reported to be present in this plant (Jeong *et al*., 2011; Jung *et al*., 2007; Kim *et al*., 2011a; Ryu *et al*., 2012). However, the physiological activity and the signaling pathways involved in cancer cells, remain unknown.

Gastric and Breast cancer are leading causes of cancer-related mortality in Korea. In previous studies, we suggested that human gastric adenocarcinoma cells express transient receptor potential melastatin 7 (TRPM7) channel, which is essential for cell survival and is a potential target for pharmacological gastric cancer treatment (Kim *et al*., 2008). Also, Guilbert *et al*. (2009) suggested that TRPM7 is involved in breast cancer cell viability. TRPM7 belongs to the TRP family and is expressed in every cell type ubiquitously (Clapham, 2003; Nadler *et al*., 2001; Runnels *et al*., 2001). Also TRPM7 channels have a number of (patho)physiological and pharmacological functions (Jiang *et al*., 2007; Jin *et al*., 2008; Schmitz *et al*., 2003). However, the role of OJ extract on TRPM7 channel in the survival of gastric and breast cancer cells after incubation with OJ is unknown. In this study, we examined the potential role of OJ extract on TRPM7 channels in growth and survival of AGS and MCF-7 cells, the most common human gastric and breast adenocarcinoma cell line. Our data suggest that OJ extract plays an important role in the survival of these tumor cells through TRPM7 channels.

Corresponding Author: Byung Joo Kim, Division of Longevity and Biofunctional Medicine, Pusan National University School of Korean Medicine, Yangsan 626-870, Republic of Korea and Hyung Woo Kim, Division of Pharmacology, Pusan National University School of Korean Medicine, Beomeori, Mulgeum-eup, Yangsan, Gyeongsangnamdo 626-870, Republic of Korea Tel: (82) 51-510-8469

MATERIALS AND METHODS

Preparation of OJ extract: Whole plant of *O. japonicus* A. Berger (*O. japonicus* Herba, OJ) was purchased from Kwangmyungdang Medicinal Herbs (Ulsan, Korea). Fifty grams of OJ was immersed in 2,000 mL of methanol and sonicated for 30 min and then extracted for 24 h. The extract was filtered with Whatman filter paper (No. 20) and evaporated under reduced pressure using vacuum evaporator (Eyela, Japan). The condensed extract was then lyophilized using freeze dryer (Labconco, USA). Finally, 1.25 g of lyophilized powder was obtained (yield; 2.5%). The methanol extract of OJ (Voucher No. MH2011-0001) has been deposited at the Division of Pharmacology, School of Korean Medicine, Pusan National University.

Cells: The AGS and MCF-7 cell lines were used. AGS and MCF-7 cell lines were established at the Cancer Research Center, College of Medicine, Seoul National University, Korea. The cell lines were propagated in RPMI-1640 medium (Gibco-BRL) supplemented with 10% heat-inactivated fetal bovine serum and 20 μg mL^{-1} penicillin and streptomycin in an atmosphere of 5% CO_2 at 37°C.

Patch-clamp experiments: Whole-cell configuration of the patch-clamp technique experiments was performed at room temperature (22-25°C). The AGS or MCF-7 cells were transferred to a small chamber on an inverted microscope stage (IX70; Olympus, Japan) and were constantly perfused with a solution containing (mmol L^{-1}) KCl 2.8, NaCl 145, $CaCl_2$ 2, glucose 10, $MgCl_2$ 1.2 and HEPES 10, adjusted to pH 7.4 with NaOH. The pipette solution contained (mmol L^{-1}) Cs-glutamate 145, NaCl 8, Cs-2-bis(2-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid 10 and HEPES-CsOH 10, adjusted to pH 7.2 with CsOH. Axopatch I-D (Axon Instruments, Foster City, CA, USA) was used to amplify membrane currents and potentials. For data acquisition and the application of command pulses, pCLAMP software v.9.2 and Digidata 1322A (Axon Instruments) were used. Results were analyzed using pClamp and Origin software (Microcal Origin version 6.0).

TRPM7 expression in human embryonic kidney 293 cells: Human embryonic kidney (HEK)-293 cells were transfected with the Flag-murine LTRPC7/pCDNA4-TO construct and grown on glass coverslips in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, blasticidin (5 μg mL^{-1}) and zeocin (0.4 mg mL^{-1}). TRPM7 (LTRPC7) expression was induced by adding 1 μg mL^{-1} tetracycline to the culture medium. Whole-cell patch-clamp experiments were performed at 21-25°C with cells that were grown on glass coverslips.

MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay: Cell viability was assessed by MTT assay. The AGS or MCF-7 cells were seeded into each well of 12-well culture plates and then cultured in RPMI-1640 supplemented with other reagents for 72 h. After incubation, 100 μL of MTT solution (5 mg mL^{-1} in phosphate-buffered saline (PBS)) was added to each well and the plates were incubated at 37°C for 4 h. After removing the supernatant and shaking with 200 μL of dimethyl sulfoxide (Jersey Lab Supply, Livingston, NJ, USA) for 30 min, absorbance was measured at 570 nm. All experiments were repeated at least 3 times.

Caspase assay: Caspase-3 assay kits (Cellular Activity Assay Kit Plus) were purchased from BioMol (Plymouth, PA, USA). After experimental treatment, cells were centrifuged (10000 g, 4°C, 10 min) and washed with PBS. Cells were resuspended in ice-cold cell lysis buffer and incubated on ice for 10 min. Sample were centrifuged at 10 000 g (4°C, 10 min) and the supernatant was removed. Supernatant samples (10 μL) were incubated with 50 μL of substrate (400-μM Ac-DEVD-pNA) in 40 μL of assay buffer at 37°C. Absorbance at 405 nm was read at several time-points. pNA concentration in samples was extrapolated from a standard created with absorbances of sequential pNA concentrations. A pan-caspase inhibitor zVAD-fmk (Calbiochem) was used to validate the assay method.

Assessment of mitochondrial membrane depolarization: Mitochondrial membrane depolarization was evaluated using JC-1 fluorescence probe according to the manufacturer's instructions (Molecular Probes). AGS or MCF-7 cells were labeled with 2 μM JC-1 for 30 min at 37°C and then analyzed by flow cytometry using 488 nm excitation with 530/30 or 585/42 nm bypass emission filters. The cells without red fluorescence were regarded as the cells manifesting mitochondrial membrane depolarization.

Flow cytometric analysis: In order to investigate whether the cell cycle of AGS and MCF-7 cells was redistributed, flow cytometric analysis was used with Propidium Iodide (PI) stain (Nicoletti *et al*., 1991; Wang *et al*., 2005). The 1×10^6 cells were placed in an e-tube. The 700 μL of a ice-cold fixation buffer (ethyl alcohol) was slowly added with vortexing. Tubes were sealed with parafilm and incubated at 4°C overnight. Samples were spun for 3 min at 106 g at 4°C and the supernatant was aspirated and discarded. The cell pellet was resuspended by 200 μL of PI staining solution (PI 5 mg mL^{-1}) 2 μL and RNase 2 μL in PBS 196 μL) at 20817 g for 5 sec. After 30 min in the dark at room temperature, samples were analyzed in a fluorescence-activated cell sorter (FACScan; Becton-

Dickinson, Moutain View, CA, USA) at λ = 488 nm using Cell-Quest software (Becton-Dickinson). DNA content distribution of normal growing cells is characterized by two peaks, the G1/G0 and G2/M phases. The G1/G0 phase comprises the normal functioning and resting state of the cell cycle with the most diploid DNA content, while the DNA content in the G2/M phase is more than diploid. Cells in the sub-G1 phase have the least DNA content in cell cycle distribution; this is termed hypodiploid. The hypoploid DNA contents represent the DNA fragmentation (Wang *et al*., 2005).

Statistical analysis: Data are expressed as Mean±SEM. Differences between the data were evaluated by Student's t-test. A p-value of 0.05 was taken to indicate a statistically significant difference.

RESULTS

OJ extract induces cell death in AGS and MCF-7 cells: To ascertain whether OJ extract kills AGS and MCF-7 cells, we performed MTT assays. Viable cell population was gradually reduced in relation to concentrations of OJ extract with the IC_{50} value of 122.3 μg mL^{-1} in AGS cells (Fig. 1a) and 225.7 μg mL^{-1} in MCF-7 cells (Fig. 1c), which was verified by quantitating cell growth over time (Fig. 1b and d). Thus, our results demonstrate that OJ extract induces cell death in AGS and MCF-7 cells.

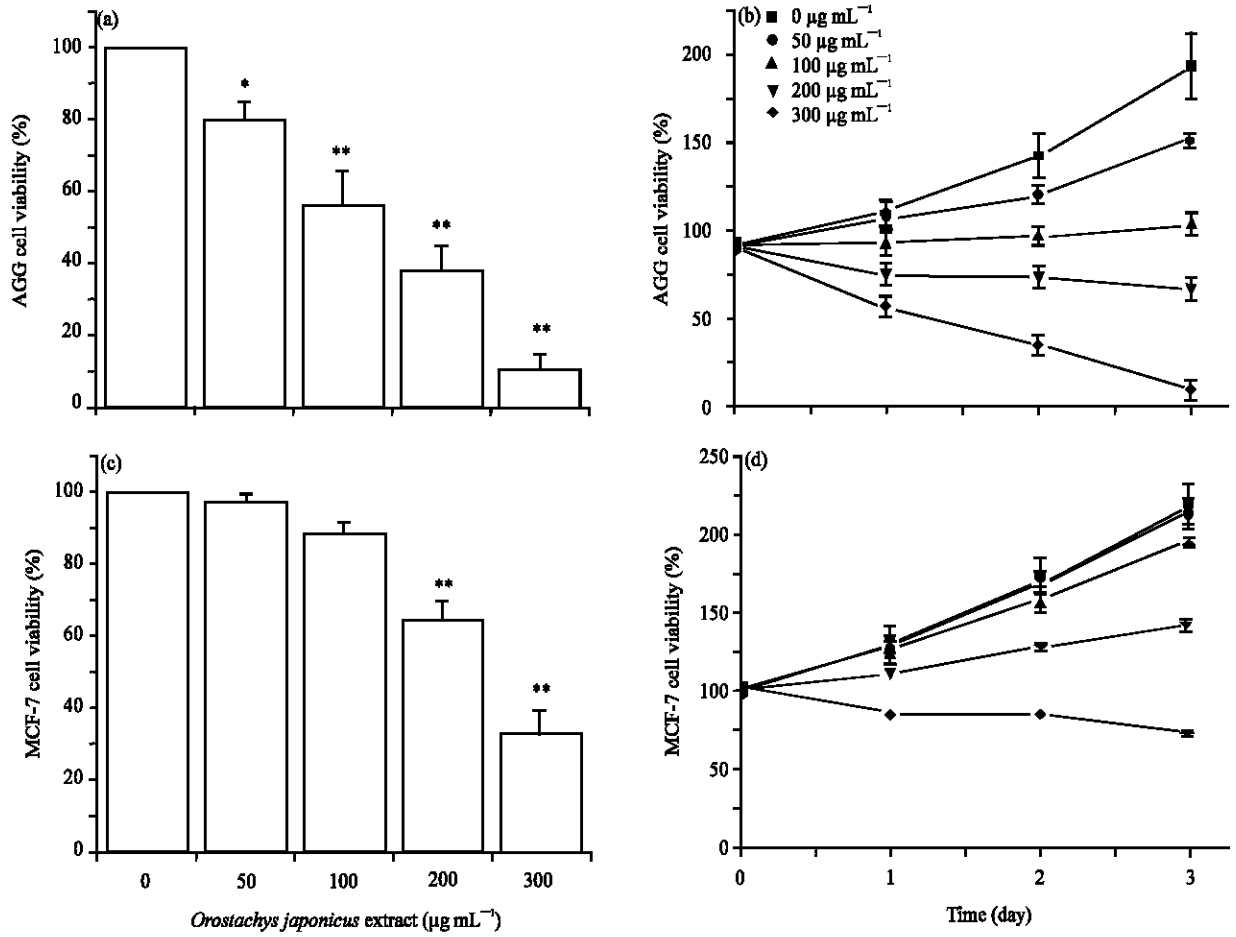

Fig. 1(a-d): *Orostachys japonicus* (OJ) induces cell death in AGS and MCF-7 cells incubated with OJ, (a) AGS cells viability, it is expressed as a relative value to that of the untreated cells which is set to 100%, (b) Time course response to OJ, AGS cell viability is expressed as a relative value to that of the cells treated with vehicle and harvested at zero time which is set to 100%, (c) MCF-7 cells viability, it is expressed as a relative value to that of the untreated cells which is set to 100% and (d) Time course response to OJ, MCF-7 cell viability is expressed as a relative value to that of the cells treated with vehicle and harvested at zero time which is set to 100%, Values are Mean±SEM, *p<0.05, **p<0.01

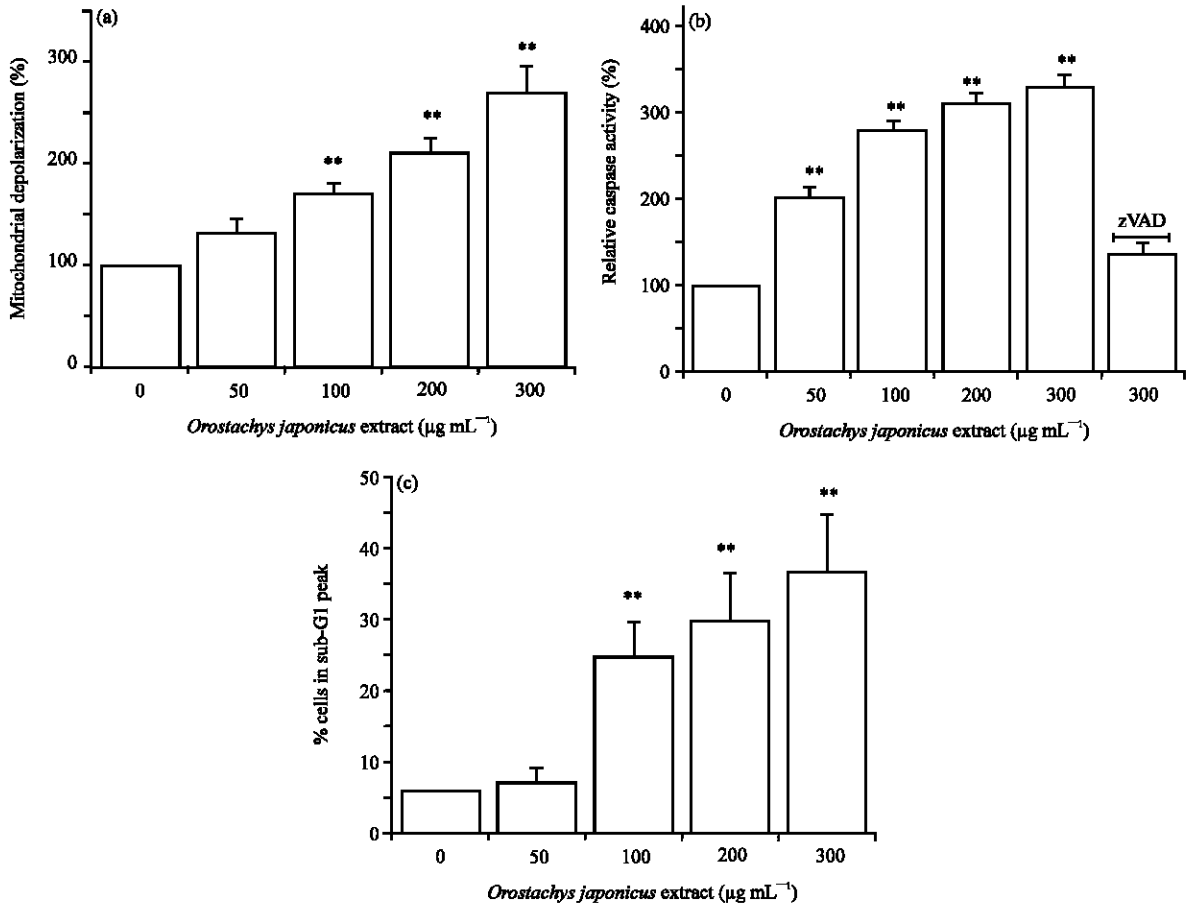

Fig. 2(a-c): OJ triggers apoptosis in AGS cells, (a) Mitochondria membrane depolarization is expressed as a relative value to that of untreated cells which is set to 100%, (b) Caspase activity from untreated cells, it is expressed as 100%, Pan-caspase inhibitor zVAD-fmk (zVAD) at 20 μM was used to validate the analytical method employed and (c) Sub-G1 peak measured by FACScan, Values are Mean±SEM of three independent experiments, *p<0.05, **p<0.01

OJ extract triggers apoptosis in AGS and MCF-7 cells: To determine whether AGS and MCF-7 cell death occurs by apoptosis, we conducted the mitochondrial membrane depolarization assay. OJ extract elevated mitochondrial membrane depolarization, an early event of an intrinsic apoptosis signaling (Fig. 2a, 3a). Thus, our findings suggest that OJ extract induces apoptosis via intrinsic apoptotic mechanism(s). Caspase-3 activation is one of the hallmarks of apoptotic cell death. We also measured the enzyme activity in AGS and MCF-7 cells after OJ extract incubation. Using a synthetic substrate, we detected the caspase-3 activity in AGS and MCF-7 cells. OJ extract increased the activity of caspase-3, which was restrained by zVAD-fmk, a pan-caspase inhibitor (Fig. 2b, 3b). Also, as a method to analyze the mode of cell death in AGS and MCF-7 cells after OJ extract incubation, we used sub-G1 analysis (Hotz *et al*., 1994; Vermes *et al*., 2000). In this protocol, incubated cells are stained with a fluorescent DNA stain (such as PI). Due to the action of endogenous endonucleases in apoptotic cells, the DNA is cleaved into endonucleosomal fragments of typical sizes. These DNA fragments are extracted from the cells. This loss of DNA is detectable by FACS analysis, as the reduced nuclear staining of apoptotic cells results in a novel (sub-G1) fluorescence peak to the left of the regular fluorescence peak. The sub-G1 after 300 μg mL^{-1} OJ extract incubation was markedly increased by 37.1±5.2% in AGS and 33.2±6.3% in MCF-7 cells (Fig. 2c, 3c).

Involvement of TRPM7 in cell death: It has been proposed that TRPM7 is required for cell survival, on the basis of experiments in genetically engineered DT-40 B-cells (Nadler *et al*., 2001). Furthermore, Guilbert *et al*.

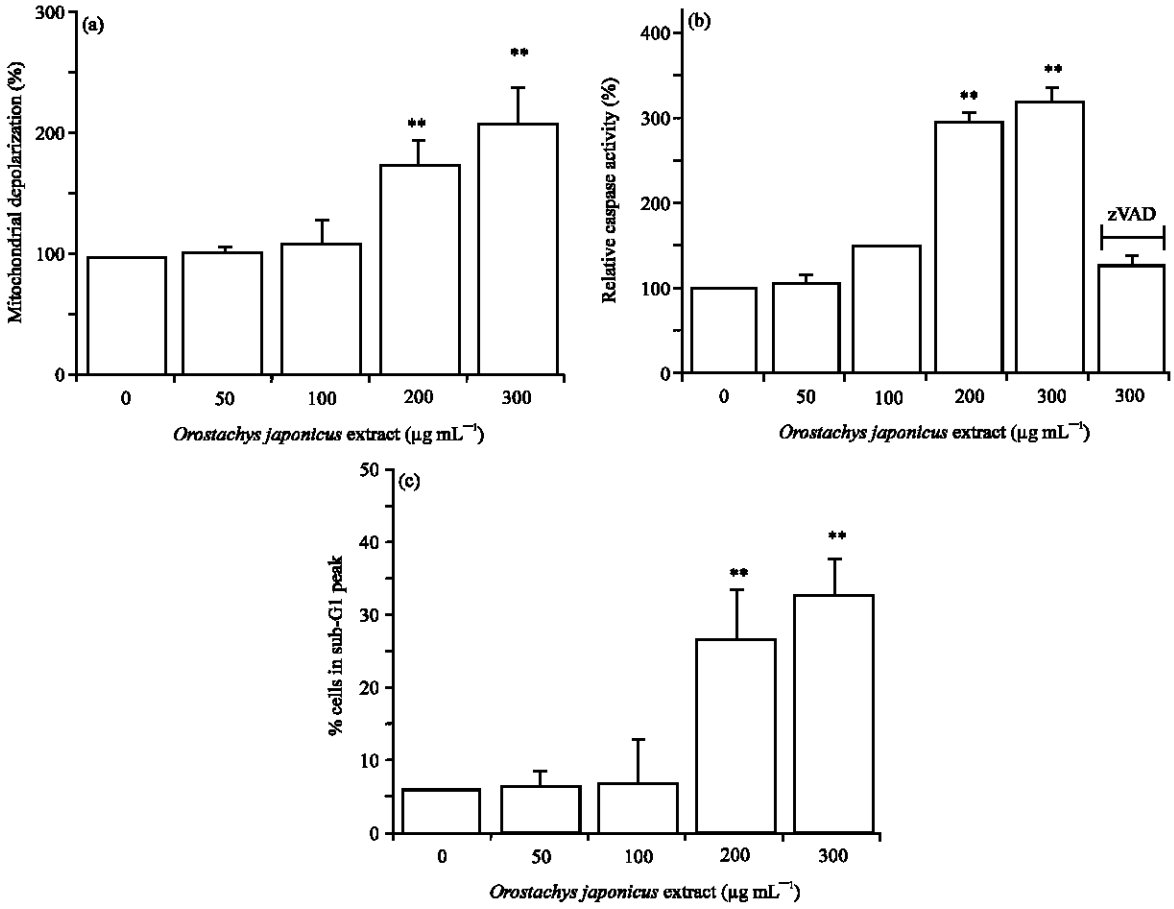

Fig. 3(a-c): OJ triggers apoptosis in MCF-7 cells, (a) Mitochondria membrane depolarization is expressed as a relative value to that of untreated cells which is set to 100%, (b) Caspase activity from untreated cells is expressed as 100%, Pan-caspase inhibitor zVAD-fmk (zVAD) at 20 μM was used to validate the analytical method employed and (c) Sub-G1 peak measured by FACScan, Values are Mean±SEM of three independent experiments, *p<0.05, **p<0.01

(2009) suggested that TRPM7 is required for breast MCF-7 cancer cell proliferation. Also, we recently suggested, as in previous reports, that AGS cells express the TRPM7 channel and suppression of the TRPM7 channel induces cell death (Kim *et al*., 2008). Therefore, we investigated the effects of OJ extract on TRPM7 channels. We performed whole cell voltage-clamp recordings to investigate the effects of OJ extract in TRPM7-like current in AGS and MCF-7 cells. A voltage ramp with voltage ranging from +100 mV to -100 mV evoked small inward currents at negative potentials, whereas larger outward currents were evoked at positive potentials, showing outward-rectifying cation currents (n = 6; Fig. 4a, c). In the presence of OJ extract, the amplitude of the currents was inhibited outwardly by 81.3±4.2% and inwardly by 57.2±3.4% in AGS cells and 71.3±4.6% and inwardly by 58.3±2.1% in MCF-7 cells (n = 6; Fig. 4b, d). In addition, similar results were obtained in HEK293 cells overexpressing TRPM7. A voltage ramp with voltage ranging from +100 mV to -100 mV evoked small inward currents at negative potentials, whereas larger outward currents were evoked at positive potentials, showing outward-rectifying cation currents (n = 5; Fig. 5a). In the presence of OJ extract, the amplitude of the currents was inhibited outwardly by 80.7±5.1% and inwardly by 95.3±2.5% in TRPM7 overexpressed HEK293 cells (n = 5; Fig. 5b). To provide additional evidence that supports the contribution of the TRPM7 channel to OJ extract toxicity, we investigated changing expression levels of TRPM7 channel and its influences on OJ extract-mediated cell death. We used HEK293 cells with inducible TRPM7 channel expression

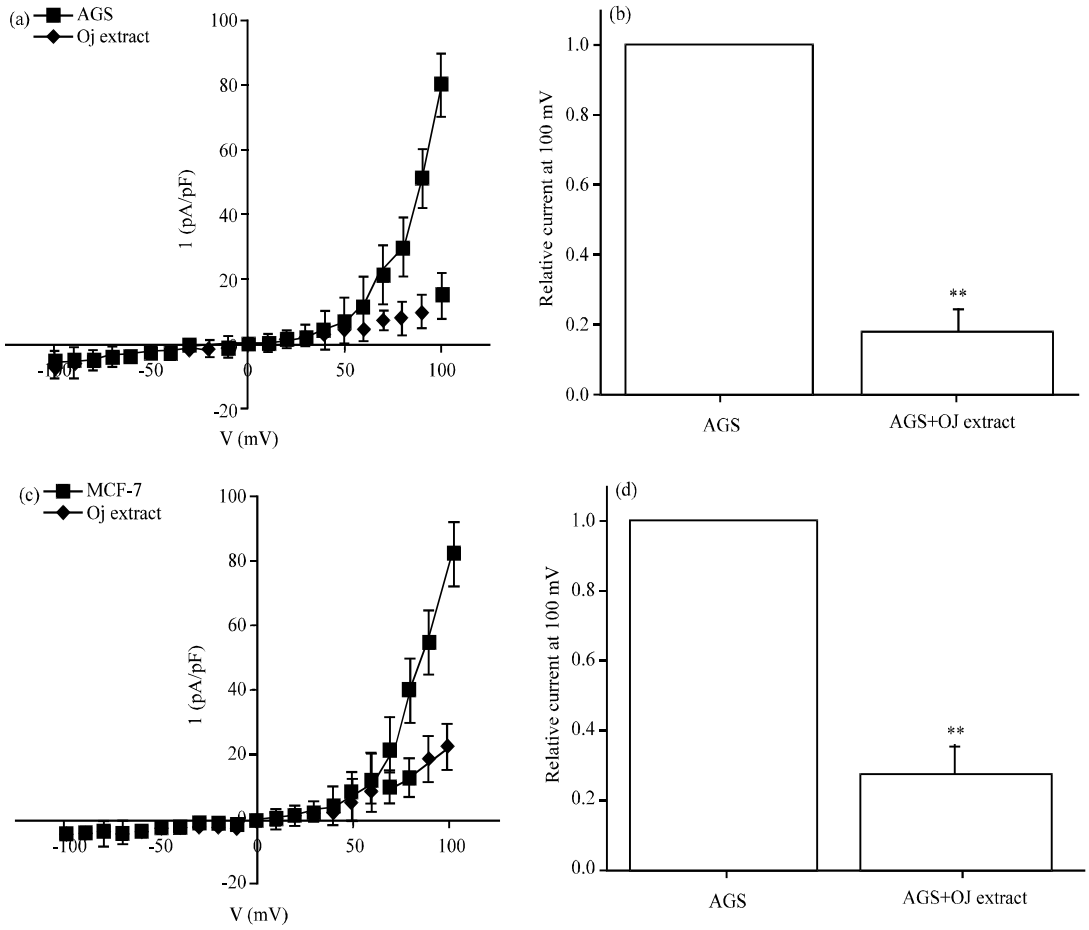

Fig. 4(a-d): Inhibition of transient receptor potential melastatin (TRPM7)-like currents by OJ in AGS cells (a) I/V curves and (b) Summary bar graph in the absence or presence of OJ and in MCF-7 cells (c) I/V curves and (d) Summary bar graph in the absence or presence of OJ, **p<0.01

(Nadler *et al*., 2001; Jiang *et al*., 2007). In the absence of induced TRPM7 channel expression (TRPM7 (-) cells, Tet(-), HEK293 cells incubation with OJ extract induced cell death in the MTT assay (n = 5; Fig. 5c). However, when TRPM7 channel overexpression was induced by adding tetracycline (TRPM7(+) cells, Tet(+), HEK293 cells incubation with OJ extract induced cell death at an increased rate in the MTT assay, which suggests that increased expression of TRPM7 channels leads to increased rate of OJ extract-induced cell death. Taken together, our data suggest that OJ has an important role in regulation of TRPM7 channels and TRPM7 channels play important roles in the survival of AGS and MCF-7 cells.

DISCUSSION

Transient receptor potential (TRP) channels were first cloned in Drosophila species and had a functions of Ca^{2+}-permeable nonselective cation channels (NSCCs) (Clapham, 2003). The TRP family is divided into 3 subfamilies. i.e., the classic (TRPC), vanilloid (TRPV) and melastatin (TRPM) types. TRPC channels mediate cation entry in response to phospholipase C activation, whereas TRPV proteins respond to physical and chemical stimuli such as changes in temperature, pH and mechanical stress. The eight TRPM family members differ markedly from the other TRP channels in terms of domain structure, cation selectivity and activation mechanism (Clapham, 2003).

TRPM7 is a ubiquitously distributed ion channel that belongs to the TRP ion channel family (Clapham, 2003; Fleig and Penner, 2004; Harteneck *et al*., 2000; Montell, 2001). It shows pronounced outward rectifying currents when overexpressed in mammalian cells (Monteilh-Zoller *et al*., 2003; Nadler *et al*., 2001; Runnels *et al*., 2001; Schmitz *et al*., 2003). Expression levels of TRPV1 are known to be increased in

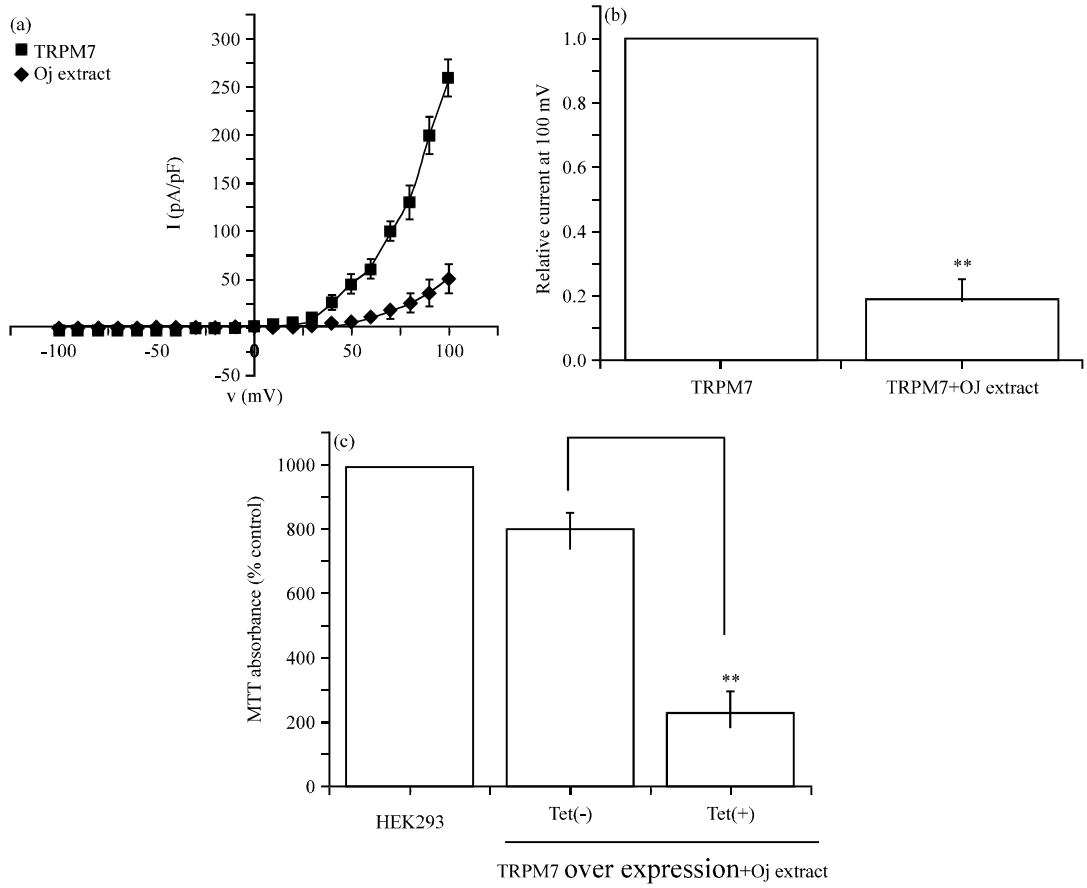

Fig. 5(a-c): Effects of OJ on TRPM7 channel overexpression in human embryonic kidney (HEK) cells (a) I/V curves and (b) Summary bar graph in the absence or presence of OJ and in (c) TRPM7 cells treated or not treated with tetracycline for 1 day, Cells were incubated with OJ, followed by MTT assay, **p<0.01

prostate, colon, bladder and pancreas cancers (Domotor *et al*., 2005; Hartel *et al*., 2006; Kim *et al*., 2011b; Shapovalov *et al*., 2011). TRPM1 has a functions of marker for melanoma metastasis (Duncan *et al*., 1998; Kim *et al*., 2011a). In addition, the TRPM8 channel has a functions of marker for prostate cancer (Heshall *et al*., 2003; Kim *et al*., 2011b; Tsavaler *et al*., 2001). TRPM7 is expressed ubiquitously, for example brain, hematopoietic tissues (Minke and Cook, 2002), kidney and heart (Aarts *et al*., 2003; Hanano *et al*., 2004). The TRPM7 cation channel supports multiple cellular and physiological functions, including cellular Mg^{2+} homeostasis (He *et al*., 2005; Schmitz *et al*., 2003), cell viability and growth (Hanano *et al*., 2004; He *et al*., 2005; Nadler *et al*., 2001), anoxic neuronal cell death (Aarts *et al*., 2003), synaptic transmission (Krapivinsky *et al*., 2006), cell adhesion (Su *et al*., 2006) and intestinal pacemaking (Kim *et al*., 2005; Kim *et al*., 2011a). Wykes *et al*. (2007) suggested that TRPM7 channels are critical to human mast cell survival. Jiang *et al*., 2007 showed that TRPM7 has an important role in the growth and proliferation of human head and neck carcinoma cells. Abed and Moreau, 2007 proposed the importance of TRPM7 in human osteoblast-like cell proliferation. As in previous studies, Guilbert *et al*. (2009) suggested that TRPM7 is required for breast cancer cell proliferation. Also, we suggested that TRPM7 channels play an important role in the growth and survival of gastric cancer cells (Kim *et al*., 2008). In line with these studies, our studies show that OJ induces apoptosis in human gastric and breast adenocarcinoma cells and may due to the blockade of TRPM7 channel activity.

OJ herbal preparations have a history of use in Korean folk medicine for the treatment of cancer (Jung and Shin, 1990). Apoptosis is essential for normal cell development and cancer treatment. In GT1-1 mouse hypothalamic neuronal cell line, chloroform fraction among fractions of OJ had the highest protective effect of OJ on H_2O_2-induced apoptosis (Yoon *et al*., 2000). OJ extract had a functions of anti-ulcerogenic effects in mice

(Jung *et al*., 2007). OJ extract inhibited the growth of HT-29 human colon cancer cells by various apoptosis-aiding activities as well as apoptosis itself (Ryu *et al*., 2012). OJ extract has been used as a constituent of oriental herb medicine prescriptions for the variety of physiologic or pharmacologic effects in various regions. However, only a few reports have described the effects of OJ extract on gastric and breast cancer. In a previous report, evidence indicated that TRPM7 channel activation influences the growth and survival of human gastric and breast adenocarcinoma cells (Guilbert *et al*., 2009; Kim *et al*., 2008).

In summary, OJ extract inhibited the growth and survival of AGS and MCF-7 cells. Caspase-3 activity, mitochondrial depolarization and sub-G1 were elevated. OJ extract inhibited the TRPM7-like current in AGS and MCF-7 cells and TRPM7 channel overexpressed HEK293 cells by whole cell voltage-clamp recordings. Furthermore, overexpression of TRPM7 channels in HEK 293 cells increases the rate of OJ-induced cell death.

In conclusion, OJ extract inhibits the growth and survival of gastric and breast cancer and that the OJ extract induced apoptosis is due to the blockade of TRPM7 channel activity. Therefore, OJ extract may play an important role in survival in cases of gastric and breast cancer through TRPM7 channels.

ACKNOWLEDGMENT

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0021347).

REFERENCES

Aarts, M., K. Iihara, W.L. Wei, Z.G. Xiong and M. Arundine *et al*., 2003. A key role for TRPM7 channels in anoxic neuronal death. Cell, 115: 863-877.

Abed, E. and R. Moreau, 2007. Importance of melastatin-like transient receptor potential 7 and cations (magnesium, calcium) in human osteoblast-like cell proliferation. Cell Prolif., 40: 849-865.

Clapham, D., 2003. TRP channels as cellular sensors. Nature, 426: 517-524.

Domotor, A., Z. Peidl, A. Vincze, B. Hunyady and J. Szolcsanyi *et al*., 2005. Immunohistochemical distribution of vanilloid receptor, calcitonin-gene related peptide and substance P in gastrointestinal mucosa of patients with different gastrointestinal disorders. Inflammopharmacology, 13: 161-177.

Duncan, L.M., J. Deeds, J. Hunter, J. Shao and L.M. Holmgren *et al*., 1998. Down-regulation of the novel gene melastatin correlates with potential for melanoma metastasis. Cancer Res., 58: 1515-1520.

Fleig, A. and R. Penner, 2004. Emerging roles of TRPM channels. Novartis Found. Symp., 258: 248-258.

Guilbert, A., M. Gautier, I. Dhennin-Duthille, N. Haren, H. Sevestre and H. Ouadid-Ahidouch, 2009. Evidence that TRPM7 is required for breast cancer cell proliferation. Am. J. Physiol. Cell. Physiol., 297: C493-C502.

Hanano, T., Y. Hara, J. Shi, H. Morita and C. Umebayashi *et al*., 2004. Involvement of TRPM7 in cell growth as a spontaneously activated Ca^{2+} entry pathway in human retinoblastoma cells. J. Pharmacol. Sci., 95: 403-419.

Hartel, M., F.F. di Mola, F. Selvaggi, G. Mascetta and M.N. Wente *et al*., 2006. Vanilloids in pancreatic cancer: Potential for chemotherapy and pain management. Gut, 55: 519-528.

Harteneck, C., T.D. Plant and G. Schultz, 2000. From worm to man: Three subfamilies of TRP channels. Trends. Neurosci., 23: 159-166.

He, Y., G. Yao, C. Savoia and R.M. Touyz, 2005. Transient receptor potential melastatin 7 ion channels regulate magnesium homeostasis in vascular smooth muscle cells: Role of angiotensin II. Circ. Res., 96: 207-215.

Heshall, S.M., D.E. Afar, J. Hiller, L.G. Horvath and D.I. Quinn *et al*., 2003. Survival analysis of genome-wide gene expression profiles of prostate cancers identifies new prognostic targets of disease relapse. Cancer Res., 63: 4196-4203.

Hotz, M.A., J. Gong, F. Traganos and Z. Darzynkiewicz, 1994. Flow cytometric detection of apoptosis: Comparison of the assays of in situ DNA degradation and chromatin changes. Cytometry, 15: 237-244.

Jeong, J.H., D.S. Ryu, D.H. Suk and D.S. Lee, 2011. Anti-inflammatory effects of ethanol extract from Orostachys japonicus on modulation of signal pathways in LPS-stimulated RAW 264.7 cells. BMB Rep., 44: 399-404.

Jiang, J., M.H. Li, K. Inoue, X.P. Chu, J. Seeds and Z.G. Xiong, 2007. Transient receptor potential melastatin 7-like current in human head and neck carcinoma cells: Role in cell proliferation. Cancer Res., 67: 10929-10938.

Jin, J., B.N. Desai, B. Navarro, A. Donovan, N.C. Andrews and D.E. Clapham, 2008. Deletion of Trpm7 disrupts embryonic development and thymopoiesis without altering Mg^{2+} homeostasis. Science, 322: 756-760.

Jung, B.S. and M.K. Shin, 1990. Dictionary of the Medical Plants. Younglim-Sa, Seoul, Korea, pp: 600-601.

Jung, H.J., J. Choi, J.H. Nam and H.J. Park, 2007. Anti-ulcerogenic effects of the flavonoid-rich fraction from the extract of *Orostachys japonicus* in mice. J. Med. Food, 10: 702-706.

Kim, B.J., E.J. Park, J.H. Lee, J.H. Jeon, S.J. Kim and I. So, 2008. Suppression of transient receptor potential melastatin 7 channel induces cell death in gastric cancer. Cancer Sci., 99: 2502-2509.

Kim, B.J., H.H. Lim, D.K. Yang, J.Y. Jun and I.Y. Chang *et al*., 2005. Melastatin-type transient receptor potential channel 7 is required for intestinal pacemaking activity. Gastroenterology, 129: 1504-1517.

Kim, B.J., S.Y. Nah, J.H. Jeon, I. So and S.J. Kim, 2011a. Transient receptor potential melastatin 7 channels are involved in ginsenoside Rg3-induced apoptosis in gastric cancer cells. Basic Clin. Pharmacol. Toxicol., 109: 233-239.

Kim, Y.I., S.W. Park, I.H. Choi, J.H. Lee, H.J. Woo and Y. Kim, 2011b. Effect of OROSTACHYS japonicus on cell growth and apoptosis in human hepatic stellate cell line LX2. Am. J. Chin. Med., 39: 601-613.

Krapivinsky, G., S. Mochida, L. Krapivinsky, S.M. Cibulsky and D.E. Clapham, 2006. The TRPM7 ion channel functions in cholinergic synaptic vesicles and affects transmitter release. Neuron, 52: 485-496.

Minke, B. and B. Cook, 2002. TRP channel proteins and signal transduction. Physiol. Rev., 8: 429-472.

Monteilh-Zoller, M.K., M.C. Hermosura, M.J. Nadler, A.M. Scharenberg, R. Penner and A. Fleig, 2003. TRPM7 provides an ion channel mechanism for cellular entry of trace metal ions. J. Gen. Physiol., 121: 49-60.

Montell, C., 2001. Physiology, phylogeny and functions of the TRP superfamily of cation channels. Sci. STKE.,

Nadler, M.J., M.C. Hermosura, K. Inabe, A.L. Perraud and Q. Zhu *et al*., 2001. LTRPC7 is a Mg.ATP-regulated divalent cation channel required for cell viability. Nature, 411: 590-595.

Nicoletti, I., G. Migliorati, M.C. Pagliacci, F. Grignani and C. Riccardi, 1991. A rapid and simple method for measuring thymocyte apoptosis by *Propidium iodide* staining and flow cytometry. J. Immunol. Meth., 139: 271-279.

Runnels, L.W., L. Yue and D.E. Clapham, 2001. TRP-PLIK, a bifunctional protein with kinase and ion channel activities, Science, 291: 1043-1047.

Ryu, D.S., H.S. Lee, G.S. Lee and D.S. Lee, 2012. Effects of the ethylacetate extract of *Orostachys japonicus* on induction of apoptosis through the p53-mediated signaling pathway in human gastric cancer cells. Biol. Pharm. Bull., 35: 660-665.

Schmitz, C., A.L. Perraud, C.O. Johnson, K. Inabe and M.K. Smith *et al*., 2003. Regulation of vertebrate cellular Mg^{2+} homeostasis by TRPM7. Cell, 114: 191-200.

Shapovalov, G., V. Lehenkyi, R. Skryma and N. Prevarskaya, 2011. TRP channels in cell survival and cell death in normal and transformed cells. Cell Calcium, 50: 295-302.

Su, D., J.M. May, M.J. Koury and H. Asard, 2006. Human erythrocyte membranes contain a cytochrome b561 that may be involved in extracellular ascorbate recycling. J. Biol. Chem., 281: 39852-39859.

Tsavaler, L., M.H. Shapero, S. Morkowski and R. Laus, 2001. Trpp8, a novel prostatespecific gene, is up-regulated in prostate cancer and other malignancies and shares high homology with transient receptor potential calcium channel proteins. Cancer Res., 61: 3760-3769.

Vermes, I., C. Haanen and C. Reutelingsperger, 2000. Flow cytometry of apoptotic cell death. J. Immunol. Methods, 243: 167-190.

Wang, B.J., S.J. Won, Z.R. Yu and C.L. Su, 2005. Free radical scavenging and apoptotic effects of cordycepin sinensis ractionated by supercritical carbon dioxide. Food Chem. Toxicol., 43: 543-552.

Wykes, R.C., M. Lee, S.M. Duffy, W. Yang, E.P. Seward and P. Bradding, 2007. Functional transient receptor potential melastatin 7 channels are critical for human mast cell survival. J. Immunol., 179: 4045-4052.

Yoon, Y., K.S. Kim, S.G. Hong, B.J. Kang, M.Y. Lee and D.W. Cho, 2000. Protective effects of *Orostachys japonicus* A. Berger (Crassulaceae) on H_2O_2-induced apoptosis in GT1-1 mouse hypothalamic neuronal cell line. J. Ethnopharmacol., 69: 73-78.