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Full Length Research Paper

Study on the application of intestinal absorption *in vitro* coupled with bioactivity assessment in Yuanhu Zhitong preparation

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Generally, crude herbal extracts contain hundreds of components, and only the absorbed constituents among them may produce possible pharmacological effects. In previous studies, researchers directly utilized crude herbal extracts into cells or organs culture system for pharmacological test, which might easily result in false positive or false negative results. Therefore, reasonable pharmacological methods for investigation of crude herbal drugs *in vitro* were greatly needed. The objective of this research is to study the feasibility of combination of two simple biological methods, intestinal absorption test and vasorelaxation assay in the pharmacological research of Yuanhu Zhitong preparation (YZP). The intestinal absorption test was carried out to investigate the accounts of seventeen constituents in YZP and prepare the intestinal absorbed samples for vasorelaxation assay. The 17 absorbed constituents in three concentrations of YZP (0.16, 0.08, 0.04 g/ml) were detected by rapid resolution liquid chromatography coupled with a triple quadrupole electrospray tandem mass spectrometry. The vasorelaxation of absorbed YZP solutions were determined by isolated aorta test and a mixture containing 7 main detected constituents was chosen for bioassay. The accounts of 17 constituents in YZP significantly varied. The good vasorelaxation of YZP was first found on the endothelium-intact aorta precontracted by KCl (60 mmol/L), with the relaxation rate of 81.53 ± 3.98, 66.85 ± 10.01 and 43.64 ± 17.83% at three different concentrations (0.16, 0.08 and 0.04 g/ml). However, the bioassay results of the mixture showed that it had lower bioactivity than that of the preparation. Based on adequate biopharmaceutical properties, the combinative methods may be more reasonable than conventional method in pharmacological research, and the intestinal absorption test coupled with vasorelaxation evaluation can be used together to investigate vasoactive effects of Traditional Chinese Medicine (TCM) *in vitro*.

Key words: Intestinal absorption, vasorelaxation, Yuanhu Zhitong preparation, aortic rings, Traditional Chinese medicine.

INTRODUCTION

Traditional Chinese Medicine (TCM) has been gradually attracting broad interest around the world during recent years; however, the complexity of TCM provides a significant challenge for researchers to seek enough scientific evidences to support the efficacy of TCM (Graziose et al., 2011). In conventional TCM pharmacological research, crude herbal extracts are often directly added into cells or organs culture system *in vitro* (Kim et al., 2010; Li et al., 2010; Guerrero et al.,

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2010; Jin et al., 2010). Generally, TCM contains ten to hundreds of components and only the absorbed constituents can produce possible pharmacological effects. Thus, the effective compositions in crude drugs could probably not be the really effective compositions working *in vivo*. Therefore, it is difficult for us to achieve the same results *in vitro* and *in vivo* experimental studies. The false positive or the false negative results may occur easily. Although, the serum pharmacology which appeared in 1984 met the characteristics of TCM research to some extents (Iwama et al., 1987; Wang et al., 2005), many factors might cause inaccuracy of experimental data, such as the intricate system of serum and the low drug concentration in serum (Pan et al., 2002; Xue and Xie, 2003; Liu and Han, 2006; Ge et al., 2008; Li et al., 2009). By contrast, the everted intestinal sacs is a comparatively controllable absorption model, which is widely used in investigation of drug absorption (Xu et al., 2006; Arellano et al., 2007; Mei et al., 2010; Tactacan et al., 2011).

Based on these points, we propose that intestinal absorption test *in vitro* coupled with the bioactivity experiment should be a simple and feasible method of combinative bioassay for TCM pharmacological research. Furthermore, the operation is easy, and few factors are involved in the certainty of the experiments, and abundant intestinal absorption solutions can be collected for further studies.

Pain is an unpleasant sensation which has a serious effect on the quality of life and general functioning of people (Breivik et al., 2008). In the TCM theories, many factors can lead to migraine, such as disorders of blood vessel function, blood vessel convulsion, *etc* (Shi et al., 2005; Li et al., 2010). In those cases, migraine can be relieved by dredging vessels. Therefore, vasorelaxation is one of the effective ways to ease pain. Yuanhu Zhitong Preparation (YZP), a classical herb formula, is composed of Radix Angelicae dahuricae and Corydalis Rhizoma (processed with vinegar), which is widely used in the treatment of gastralgia, costalgia, headache and dysmenorrhea caused by qi stagnancy and blood stasis (China Pharmacopoeia Committee, 2010). It has been shown that two main components, dl-tetrahydropalmatine and imperatorin, in YZP have remarkable vasodilatation (Sun and Li, 1989; Jin et al., 2001; He et al., 2007; Zhang et al., 2010). Therefore, we determined the combinative bioassay method through investigation of vasorelaxation of YZP in this study.

The absorption test was firstly carried out, and then the bioassay samples and quantitative analysis of 17 constituents in YZP were obtained, followed by the thoracic aorta ring experiment. Based on the results of the aforementioned experiments, the combinative methods of intestinal absorption test *in vitro* and the formed vasorelaxation assessment, whose validity had been demonstrated by examination of pharmacological actions of YZP were used.

MATERIALS AND METHODS

Preparation of herbal extracts

Angelica Dahurica (Fisch. ex Hoffm.) Benth. et Hook. f. and *Corydalis yanhusuo* W. T. Wang were collected from Anguo medicinal material market (Hebei, China) in February 2011, and the drugs were identified by Pharmacist Xirong He, research assistant of China Academy of Chinese Medical Sciences. Voucher specimens were deposited in the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences. The dried and powered *C. yanhusuo* of 500 g and *A. Dahurica* of 250 g were mixed and soaked for 1 h, and then extracted under reflux with 3 L of 70% ethanol for 2 h. The extraction process was repeated twice. The extract was concentrated under reduced pressure using the rotary evaporator at 70, and followed by dilution with Tyrode buffer to three concentrations, 0.16, 0.08 and 0.04 g/ml.

Animals

Adult male Sprague-Dawley rats weighing 220 to 250 g (from the Experimental Animal Center of Peking University Health Science Center, Beijing, China) were used for the everted gut sac experiments and isolated vascular ring models. The animal welfare and experimental procedures comply with the Guide for the Care and Use of Laboratory Animals (National Research Council of the USA, 1996) and related ethical regulations of China Academy of Chinese Medical Sciences.

The intestinal absorbed solution preparation

Rats were fasted for 12 h before the experiment. Under anesthesia, the intestine of each rat was rapidly removed, washed with ice-cold Tyrode buffer solution (mmol/L, NaCl 136.75, KCl 3.76, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, $MgCl_2$ 1.05, $CaCl_2$ 1.80, glucose 5.56, pH 7.4), and divided into four 14 cm segments in length. Each segment was everted and ligated at both ends to form a sac, and then filled with the buffer. The filled sac was incubated in Magnus' bath with Tyrode buffer for 5 min to guarantee the equilibration, and then the buffer was exchanged with the YZP solution. During the incubation period, the solution was maintained at 37 and continuously aerated with O_2/CO_2 (95%/5%). After 2 h, the sacs were removed and blotted dry with gauze, and the serosal side solutions containing absorbed constituents were drained into small tubes. The intestinal absorbed solutions (three concentrations of YZP solutions) and blank Tyrode buffer were prepared. These samples were stored at -20 for further use.

Quantitative analysis of 17 constituents in YZP

The samples of intestinal absorbed solution were dried in nitrogen atmosphere, dissolved in the the same volume of 70% methanol, and then filtered through 0.22 μm nylon membrane filters. The filtrates were analyzed directly by rapid resolution liquid chromatography coupled with a triple quadrupole electrospray tandem mass spectrometry (RRLC-QQQ).

In our previous study, we established an analytical method that permitted to simultaneously quantify 17 constituents from Yuanhu Zhitong Tablet in 9 min, which was performed on an Agilent 1200 RRLC-QQQ (Zhang et al., 2011). The preliminary data suggested that the accounts of components in detected samples were all in the linear range of the aforementioned method, so the RRLC-QQQ was adopted in the analysis. The intestinal absorbed YZP solutions with three different concentrations and the YZP preparation were analyzed and 17 constituents were detected, namely scopoletin,

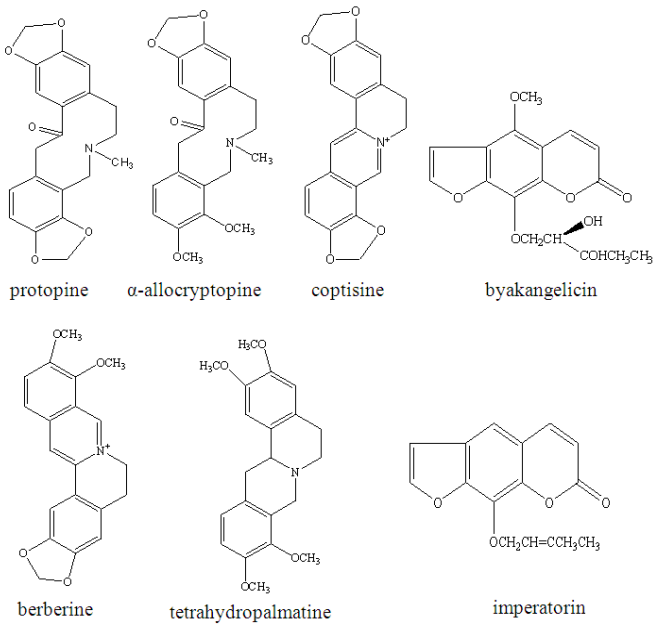

Figure 1. The chemical structures of the seven constituents.

protopine, α-allocryptopine, dl-tetrahydropalmatine, coptisine, tetrahydroberberine, corydaline, berberine, byakangelicin, byakangelicol, xanthotoxin, bergapten, pimpinellin, oxypeucedanin, imperatorin, osthole and isoimperatorin. Three parallel samples were performed for each concentration.

Preparation of thoracic aorta rings

After rat was killed by cervical dislocation, the thoracic aorta was carefully removed and immediately placed into ice-cold Krebs' solution (mmol/L, NaCl 118.96, KCl 4.73, KH_2PO_4 1.17, $MgSO_4$ 1.17, $NaHCO_3$ 25.0, $CaCl_2$ 2.54, glucose 11.1, pH 7.4) (Zhang et al., 2011). Arterial vessel was carefully handled by removing connective fat and tissues, and then cut into four 3 to 4 mm segments in length. The segments were mounted in an organ bath containing Krebs' solution by two L-shaped stainless-steel wire hooks inserted into the lumen, and bubbled with O_2/CO_2 (95%/5%) at 37°C. After incubated under no tension for 30 min, the ring segments were allowed to equilibrate for 1 h at a resting tension of 1 g and washed every 20 min. The changes in tension were recorded by isometric transducers connected to a data acquisition system (Shanghai Alcott Biotech Co., LTD. Shanghai, China).

Bioactivity evaluation of intestinal absorbed YZP solutions on rat aortic rings with endothelium

After the equilibration, the presence of endothelium was confirmed by induced relaxation with acetylcholine (ACh, 10^{-5} mol/L) in aortic rings precontracted with phenylephrine (PE, 10^{-6} mol/L). A relaxation value higher than 60% indicated a satisfactory endothelium activity and only these tissues were used in the experiments.

All rings were exposed repeatedly to KCl (60 mmol/L) until the tensions stabilized. After that, six cumulative volumes of the intestinal absorbed solution were added directly to the organ bath from 50 to 1600 µl.. The intervals were 10 min for the first four volumes namely 50, 100, 200 and 400 µl, and 15 and 30 min for 800 and 1600 µl, respectively (Since the larger volume would take the longer time for response, the intervals for 800 and 1600 µL were longer than that of other volumes). Three intestinal absorbed samples of YZP and blank intestinal absorbed solutions were evaluated and each experiment performed on rings from a single rat was repeated eight times.

Bioassay of the mixture of 7 constituents from detected 17 components

Seven constituents from the detected 17 components including protopine, α-allocryptopine, dl-tetrahydropalmatine, coptisine, corydaline, byakangelicin and imperatorin (Figure 1) were chosen for bioactivity assay, because the amounts of those were more than 90% of the detected components. The standards were diluted with blank intestinal solution to the same concentrations as those in YZP. Eight parallel experiments were performed for each bioassay.

Statistical analysis

All data of bioassay experiments are represented as mean ± S.D. Student's *t*-test was used when the analysis involved the comparison of two means, and a *P*-value of less than 0.05 ($P < 0.05$) was considered significance, which were all computed using SPSS 16.0 software.

RESULTS AND DISCUSSION

Quantitative analysis of 17 constituents in YZP by RRLC-QQQ

Based on the retention behavior and mass spectra of the related standards, 17 constituents in YZP were identified and quantitatively analyzed by RRLC-QQQ. As the data shown in Table 1, the higher dosage levels yielded comparatively larger absorption accounts. The absorption accounts of protopine at three doses were 5.5964, 10.7184 and 14.7537 µg, respectively. We obtained the similar results of other 16 components as well. Therefore, the accounts of 17 absorbed constituents were presented in a dose-dependent manner.

The seven components with the high content in extract solutions of YZP were corydaline, coptisine, imperatorin, protopine, isoimperatorin, α-allocryptopine and dl-tetrahydroberberine; whereas components that had the high content in intestinal absorbed solutions were protopine, corydaline, α-allocryptopine, dl-tetrahydropalmatine, coptisine, byakangelicin and imperatorin. It suggested that the accounts of these constituents in extract solutions differed from those in intestinal absorbed YZP solutions. Furthermore, the absorption rate of each component had great difference, which might result from varying absorption mechanisms. Byakangelicin and dl-tetrahydropalmatine had a high absorption rate since the mechanism might be considered as active transport. The accounts of these constituents significantly varied during the process of intestinal absorption. Considering adequate

Table 1. The absorbed doses of 17 constituents in the YZP intestinal absorbed solutions.

Compound	High concentration		Middle concentration		Low concentration	
	Dose (μg)	Total absorption (μg, $\bar{X}$±SD)	Dose (μg)	Total absorption (μg, $\bar{X}$±SD)	Dose (μg)	Total absorption (μg, $\bar{X}$±SD)
scopoletin	0.1910	0.0070 ±0.0005	0.0955	0.0063±0.0007	0.0478	0.0045±0.0003
protopine	1101.8288	14.7537±0.7345	550.9144	10.7184 ±1.3507	275.4572	5.5964±1.2246
α-allocryptopine	508.4640	6.2933±0.4822	254.2320	5.4267±2.9694	127.1160	1.1224±0.7273
tetrahydropalmatine	67.7160	6.1807±0.0461	33.8580	4.1373±0.6966	16.9290	2.7587±0.3678
coptisine	1609.2560	4.5720±0.1342	804.6280	2.9513±0.8415	402.3140	2.0020±1.1814
tetrahydroberberine	234.9600	0.7121±0.0409	117.4800	0.4499±0.0977	58.7400	0.2638±0.0303
corydaline	1843.3800	6.7553±0.2318	921.6900	4.0227±0.6325	460.8450	2.6620±0.2494
berberine	225.0600	0.7549±0.0338	112.5300	0.5299±0.1835	56.2650	0.3105±0.1961
byakangelicol	0.5988	0.4035±0.0057	0.2994	0.3457±0.0666	0.1497	0.1225±0.0862
byakangelicin	3.0254	1.8543±0.0590	1.5127	1.5901±0.2307	0.7564	0.6936±0.5327
xanthotoxin	7.5372	0.1430±0.0013	3.7686	0.0956±0.0075	1.8843	0.0572±0.0136
bergapten	78.5840	0.6039±0.0096	39.2920	0.4947±0.0628	19.6460	0.3873±0.0277
pimpinellin	24.4112	0.3182±0.0021	12.2056	0.2314±0.0353	6.1028	0.1666±0.0177
oxypeucedanin	2.7544	0.0257±0.0016	1.3772	0.0197±0.0031	0.6886	0.0188±0.0013
imperatorin	1273.8000	1.2031±0.1028	636.9000	1.2610±0.1512	318.4500	0.9745±0.0234
Osthole	1.0617	0.0007±0.0001	0.5309	0.0007±0.0001	0.2654	0.0006±0.0000
Isoimperatorin	1005.4000	0.2522±0.0417	502.7000	0.3337±0.0337	251.3500	0.2309±0.0299

biopharmaceutical properties involved in the test, the intestinal absorbed YZP solutions might be more reasonable as samples of pharmacological research *in vitro* compared with the extract solutions. The absorbed doses and absorption rates of 17 markers in YZP were displayed in Tables 1 and 2.

Vascular effect of the intestinal absorbed YZP solutions on rat aortic rings

The cumulative addition of the intestinal absorbed YZP solutions on rat aortic rings precontracted by KCl (60 mmol/L) produced significant bioactivities. As shown in Figure 2, the blank intestinal solution had an average effect of -4.31 ± 1.91% on the vasorelaxation, thus it had little effect with the experiment and as control group. It also showed that the intestinal absorbed YZP solutions could induce vasorelaxation. In addition, the high concentration of YZP had the strongest effect in vasodilatation (81.53 ± 3.98%), compared with the middle and low concentrations (66.85 ± 10.01 and 43.64 ± 17.83%, respectively). However, different volumes of absorbed YZP solutions caused different vasodilative effects.

The high and middle concentrations of YZP solutions produced remarkable difference at 400 μl, while the low concentration was at 800 μl. All solutions of three concentrations had extremely significant bioactivities at 1600 μl compared to the control group. Thus a dose-dependent vasorelaxant effect was produced.

Bioassay of the mixture of 7 constituents

As shown in Figure 3, the mixture of 7 standards with the high and middle concentrations produced vasorelaxation of 28.74 ± 4.25 and 12.09 ± 11.73%, respectively. However, there was no bioactivity in vasorelaxation for the low concentration. The high concentration of the mixture produced significant vasodilative effect at 800 μl, compared with the control group, while the middle concentration was at 1600 μl. The mixture showed a lower bioactivity of vasorelaxation compared with the intestinal absorbed YZP solutions with the same concentration. Additionally, the results implied that some micro-constituents and unknown constituents might contribute to the vasorelaxation of YZP. Therefore, one or several constituents may not stand for the whole preparation, and the comprehensive effects of the multicomponent in YZP still need to be explored.

Conclusions

The vasoactive effects of YZP were investigated in our study. The results indicated that the accounts of main constituents in YZP significantly varied in intestinal absorption experiments, and the bioassay of seven-constituent mixture indirectly showed a comprehensive effect of the multicomponent in YZP. The combinative method of intestinal absorption test *in vitro* and bioactivity assessment in vasorelaxation was successfully performed for YZP study, which implied that the

Table 2. The absorption rate of 17 markers of YZP intestinal absorbed solutions.

Compound	Absorption rate (%)		
	High concentration (n=3)	Middle concentration (n=3)	Low concentration (n=3)
scopoletin	3.6517±0.2671	6.5494±0.7577	9.3981±0.6289
protopine	1.339±0.0667	2.5895±1.1287	2.0317±0.4446
α-allocryptopine	1.2377±0.0948	2.1345±1.168	0.883±0.5721
tetrahydropalmatine	9.1273±0.0681	12.2197±2.0574	16.2955±2.1728
coptisine	0.2841±0.0083	0.3668±0.1046	0.4976±0.2936
tetrahydroberberine	0.3031±0.0174	0.3829±0.0831	0.4491±0.0516
corydaline	0.3665±0.0126	0.4364±0.0686	0.5776±0.0541
berberine	0.3354±0.015	0.4709±0.1631	0.5519±0.3485
byakangelicol	67.3858±0.9577	115.4454±22.2299	81.8471±57.5661
byakangelicin	61.2914±1.9496	105.1175±15.2521	91.705±70.4331
xanthotoxin	1.8978±0.0173	2.5366±0.1988	3.0353±0.7207
bergapten	0.7685±0.0123	1.259±0.1599	1.9712±0.1411
pimpinellin	1.3035±0.0087	1.8959±0.2895	2.7305±0.2896
oxypeucedanin	0.9335±0.0581	1.4271±0.2227	2.7282±0.1909
imperatorin	0.0944±0.0081	0.198±0.0237	0.306±0.0074
osthole	0.062±0.0048	0.135±0.0122	0.2268±0.0094
Isoimperatorin	0.0251±0.0041	0.0664±0.0067	0.0919±0.0783

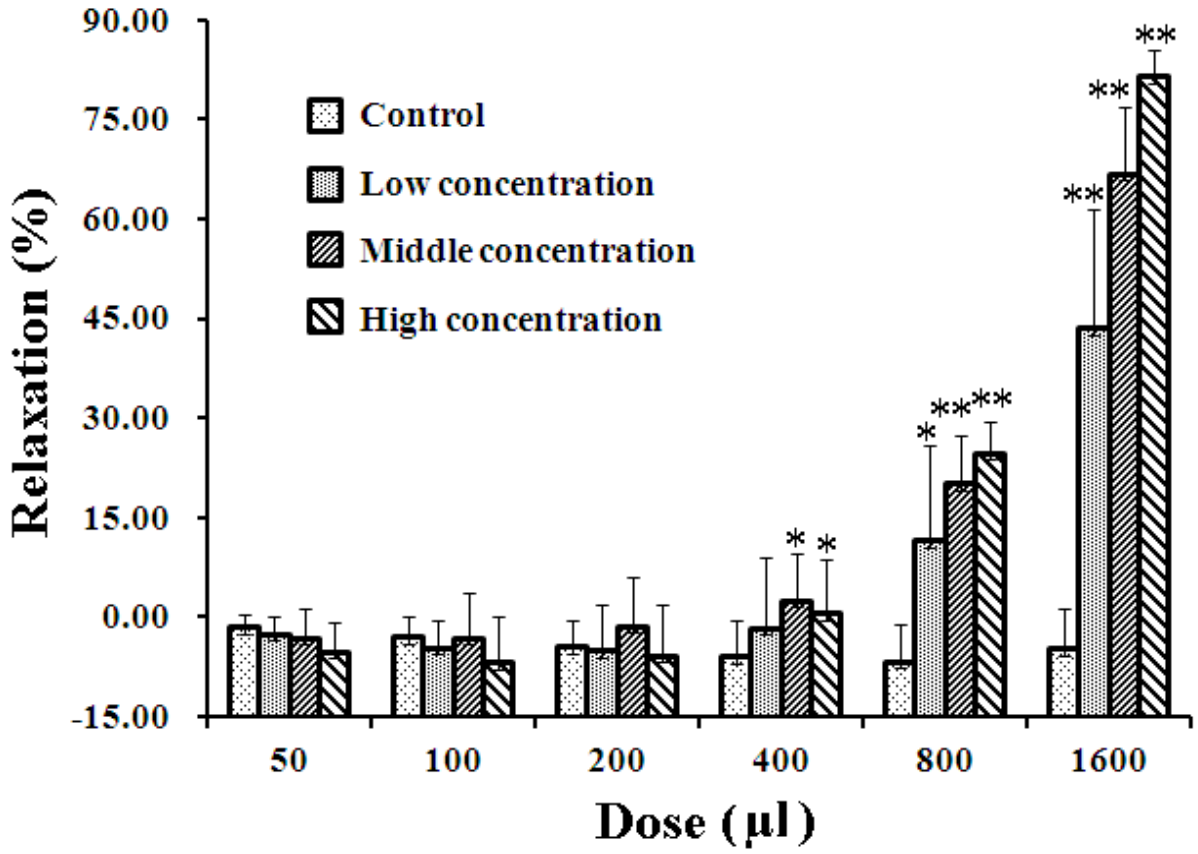

Figure 2. The effects of intestinal absorbed YZP solutions on rat aortic rings pre-contracted with KCl (60 mmol/L). Contractions are expressed as percentage of the initial contraction induced by KCl (60 mmol/L), n=8, *****, $P<0.05$, ******, $P<0.001$, significantly different compared to the control.

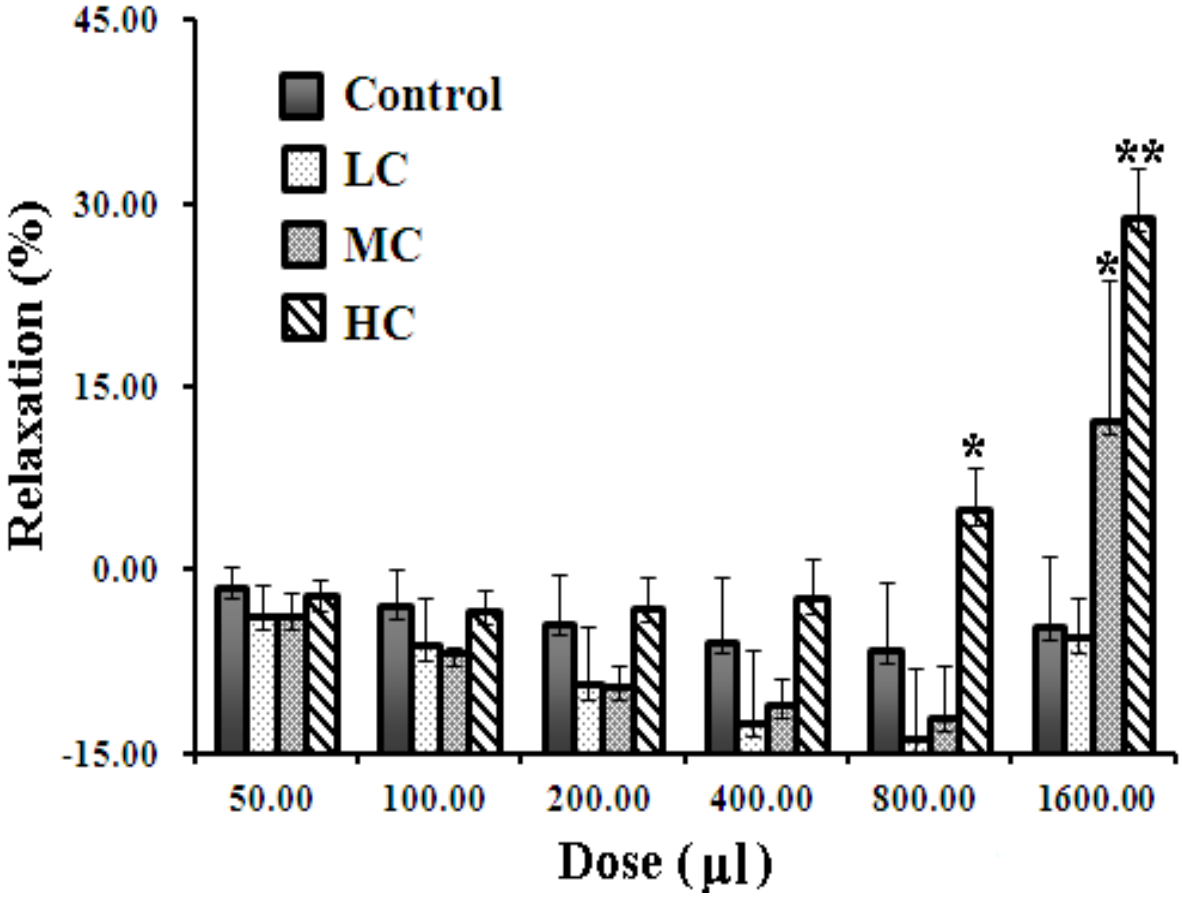

Figure 3. Effects of high concentration (LC), middle concentration (MC), low concentration (LC) of 7 mixed standards and the control group on rat aortic rings pre-contracted with KCl (60 mmol/L), n=8. *****, *P*<0.05, ******, *P*<0.001, significantly different compared to control.

combinative method might be more reasonable to reflect the bioactivity of multicomponent preparation *in vitro,* compared with the conventional method.

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REFERENCES

Arellano C, Philibert C, Vachoux C, Woodley J, Houin G (2007).The metabolism of midazolam and comparison with other CYP enzyme substrates during intestinal absorption: in vitro studies with rat everted gut sacs. J. Pharm. Pharm. Sci., 10: 26-36.

Breivik H, Borchgrevink PC, Allen SM, Rosseland LA, Romundstad L, Hals EK, Kvarstein G, Stubhaug A (2008). Assessment of pain. Br. J. Anaesth., 1: 17-24.

Ge JW, Zhu HB, Wang YH, He R, He SL (2008). Rethinking the validity of Chinese medicine serum pharmacology. World Sci. Technol. Modernization Tradit. Chin. Med. Mater. Med., 10: 16-22.

Graziose R, Lila MA, Raskin I (2011). Merging traditional Chinese medicine with modern drug discovery technologies to find novel drugs and functional foods. Curr. Drug Discov. Technol., **7**: 2-12.

Guerrero E, Morán-Pinzón JA, Ortíz LG, Olmedo D, Olmo E, López-Pérez JL, Feliciano AS, Gupta MP (2010). Vasoactive effects of different fractions from two Panamanians plants used in Amerindian traditional medicine. J. Ethonpharmacol., 131: 497-504.

He JY, Zhang W, He LC, Cao YX (2007). Imperatorin induces vasodilatation possibly via inhibiting voltage dependent calcium channel and receptor-mediated Ca^{2+} influx and release. Eur. J. Pharmacol., 573: 170-175.

Iwama H, Amagaya S, Ogihara Y (1987). Effect of shosaikoto, a Japanese and Chinese traditional herbal medical mixture, on the mitogentic activity of lipopolysaccharid: a new pharmacological testing method. J. Ethonpharmacol., 21: 45-53.

Jin GZ (2001). New discoveries from a traditional Chinese medicine: Corydalis Rhizoma, Shanghai Scientific and Technical Publishers, Shanghai.
Jin SN, Wen JF, Kim HY, Kang DG, Lee HS, Cho KW (2010). Vascular relaxation by ethanol extract of Xanthoceras sorbifolia via Akt-and SOCE-eNOS-cGMP pathways. J. Ethonpharmacol., 132: 240-245.
Kim EY, Kim JH, Rhyu MR (2010). Endothelium-independent vasorelaxation by *Ligusticum wallichii* in isolated rat aorta: comparison of a butanolic fraction and tetramethylpyrazine, the main active component of *Ligusticum wallichii*. Biol. Pharm. Bull., 33: 1360-1363.
Li J, Yin F, Yao SK. (2009). Advance in studies of serum pharmacology. Chin. J. Based Med. Tradit. Chin. Med., 15: 234-236.
Li JC, Meng XL, Zhang Y (2010). Research thinking on the effect of preventive treatment on migraine by traditional Chinese medicine. Harm. Clin. Chin. Mater. Med., 5: 165-167.
Li X, Kim HY, Cui HZ, Cho KW, Kang DG, Lee HS (2010). Water extract of *zanthoxylum piperitum* induces vascular relaxation via endothelium-dependent NO-cGMP signaling. J. Ethonpharmacol., 129: 197-202.
Liu JX, Han X (2006). Study on time and dose effect of serum containing Chinese herbal medicine. China J. Chin. Mater. Med., 31: 829-831.
Mei YX, Kim CH, Lee CK, Shin JS, Cho IH, Sohn UD (2010). Intestinal absorption of fibrinolytic and proteolytic lumbrokinase extracted from Earthworm, Eisenia andrei. Korean J. Physiol. Pharmacol., 14: 71-75.
Pan WS, Liu MF, Shi Y, Xing DM, Du LJ, He XH, Zhang HY (2002). Plasma pharmacology and plasma chemistry and pharmacokinetics of traditional Chinese Medicines. World Sci. Technol., Modernization Trad. Chin. Med. Mater. Med., 3: 53-56.
Shi DH, Chen LY, Zeng SJ, Xie ZJ (2005). Vasorelaxation of headache pill on Rabbit carotid artery. Chin. J. Exp. Tradit. Med. Formul., 6: 53-55.
Sun F, Li DX (1989). Effects of l-dl-dl-tetrahydropalmatine on isolated rabbit arterial strips. Acta Pharmacol. Sin., 10: 30-33.
Tactacan GB, Rodriguez-Lecompte JC, Karmin O, House JD (2011). Functional characterization of folic acid transport in the intestine of the laying hen using the everted intestinal sac model. Poult. Sci., 90: 83-90.
Wang BC, Zhu LC, Chen Q (2005). Primary study on the application of serum pharmacology in Chinese traditional medicine. Colloids Surf. B: Biointerfaces, 43: 194-197.
Xu F, Xiao SY, Lu XW, Yang DH, Yang XD, Li CL, Shang MY, Tu PF, Cai SQ (2006). Absorption and metabolism of astragali radix decoction: in silico, in vitro, and a case study in vivo. Drug metob. dispos., 34: 913-924.
Xue J, Xie ML (2003). Advances in studies on methodology in serum pharmacology of Chinese materia medica. Chin. Tradit. Herb Drugs, 34: 9-11.
Zhang Y, Wang QL, Zhan YZ, Duan HJ, Cao YJ, He LC (2010). Role of store-operated calcium entry in imperatorin-induced vasodilatation of rat small mesenteric artery. Eur. J. Pharmacol., 647: 126-131.
Zhang YC, Chen C, Li SJ, Xu HY, Li DF, Wu HW, Yang HJ (2011). Chemical analysis and vascular activity of essential oil from Ligusticum sinensis Oliv., Conioselinum tataricum Hoffm. and Ligusticum jeholense Nakai et Kitag. Chin. J. Exp. Tradit. Med. Form., 14: 205-210.
Zhang YC, Xu HY, Chen XM, Chen C, Wang HJ, Meng FY, Yang HJ, Huang LQ (2011). Simultaneous quantification of 17 constituents from Yuanhu Zhitong Tablet using rapid resolution liquid chromatography coupled with a triple quadrupole electrospray tandem mass spectrometry. J. Pharm. Biomed. Anal., 56: 497-504.