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Characteristics of the traditional Liu-Wei-Di-Huang prescription reassessed in modern pharmacology

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[ABSTRACT] Liu-Wei-Di-Huang (LW) is a *Yin* nourishing and *kidney* tonifying prescription in traditional Chinese medicine with promising pharmacological characteristics that can be further exploited and developed in modern medicine. We provide a comprehensive and detailed literature report on the clinical and experimental pharmacology of LW，including its quality control parameters, phytochemistry, pharmacokinetics, and toxicology. Our literature review indicates that the LW prescription possesses a unique combination of pharmacological characteristics that can be safely used for treating very different diseases. Quality control and pharmacokinetic parameters of LW are mostly based on its major bioactive phytochemical constituents. We postulate that modulating or rebalancing the neuroendocrine immunomodulation network in the body is the underlying mechanism of the multiple pharmacological activities displayed by LW.

[KEY WORDS] Liu-Wei-Di-Huang; Traditional Chinese medicine; Pharmacology; Phytochemistry; Pharmacokinetics; Toxicology

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Introduction

Effective treatment of multifactorial, complex, chronic illnesses, including neurodegenerative, metabolic, and circulatory system diseases, is limited by the availability, cost, and adverse effects of a therapy. Thus, the complementary and alternative medicine (CAM) including traditional Chinese medicine (TCM) is becoming well-regarded and frequently used among patients suffering from chronic diseases. In TCM, Liu-Wei-Di-Huang (LW) is the most common herbal formula prescribed by TCM doctors in the clinic. A study found that it has been used by 80.3% of Chinese practitioners and 64.2% of European practitioners [1]. It is also the most frequently prescribed herbal medicine in Korea [2].

LW, also called LiuweiDihuang, Liuweidihuang, Liuwei Dihuang, Rokumigan, Yukmijihwang-tang or Lokumijio-to, was developed by doctor *Qian Yi* (1035-1117) in 1114 during the *Beisong* dynasty and has been described in the ancient Chinese literature as "*Key to Therapeutics of Children's Diseases*" or "*Knack of Prescription in Pediatrics*" ('*xiao er yao zheng zhi jue*' in Chinese). LW is composed of six herbs, including *Rehmannia glutinosa* Libosch. var. *purpurea* Makino, *Cornus officinalis* Sieb. et Zucc., *Dioscorea japonica* Thunb., *Alisma orientale* Juzep., *Paeonia suffruticosa* Andrews, and *Poria cocos* Wolf at a ratio of 8 : 4 : 4 : 3 : 3 : 3 (Fig. 1). LW is orally administered as a decoction (soup) or in the form of pills, capsules or powder. LW has *Yin* nourishing and kidney tonifying properties that invigorates and strengthens or modulates and maintains the fundamental system to support reproduction, development, and performance over a lifetime.

In the 12th century, LW was originally developed and prescribed for treating dysplasia in children. Regardless of the original use, for nearly 1000 years, LW has been widely applied to nourish patients and prevent or treat a wide variety of diseases affecting the immune, endocrine, digestive, respiratory, urinary and nervous system [3-5]. There are at least 224 identified compounds in LW and 59 of those have an effect on diseases [6-8]. To date, the efforts continue to elucidate the medicinal properties and mechanisms of action of LW. In

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TCM, the medicinal properties of LW are described as *Yin* nourishing and *kidney* tonifying, whereas in modern pharmacology, the effect of LW is closely associated with maintaining and restoring the balance of the neuroendocrine immunomodulation (NIM) network. LW plays an integrative role in modulating the balance of the NIM network by regulating the bidirectional crosstalk between the neuroendocrine and immune system [9]. There are also local modulating networks within the NIM network at different levels of the neural, endocrine, and immune system, which maintain the homeostasis of those three systems via signaling molecules and pathways.

Fig. 1 Traditional Chinese medicine prescription Liu-Wei-Di-Huang. The LW prescription consists of six herbs, the Shu-Di-Huang (Radix Rehmanniae, *Rehmannia glutinosa* Libosch. var. *purpurea* Makino), Shan-Zhu-Yu (Corni Fructus, *Cornus officinalis* Sieb. et Zucc.), Shan-Yao (Dioscoreae Rhizoma, *Dioscorea japonica* Thunb.), Ze-Xie (Rhizoma Alismatis, *Alisma orientale* Juzep.), Mu-Dan-Pi (Moutan Cortex, *Paeonia suffruticosa* Andrews), and Fu-Ling (Hoelen, *Poria cocos* Wolf), mixed at a ratio of 8 : 4 : 4 : 3 : 3 : 3. LW is orally administered as a decoction (soup) or in the form of pills, capsules or powder

Clinical pharmacology

LW is a well-known prescription of nourishing *yin*, especially for invigorating kidney-*yin*. In the clinic, LW has been used to treat an array of conditions such as deficiency of liver-*yin* and kidney-*yin*, dizziness and tinnitus, soreness and weakness of waist and knees, osteopyrexia and tidal fever, night-sweat and spontaneous sweating, spermatorrhea, thirst, dripping discharge of urine, and emaciation (Fig. 2). Although LW has already been widely applied in the clinic to prevent and treat various diseases, there were and are ongoing research projects and clinical trials to evaluate the efficacy of LW.

Earlier studies also indicated that LW had a therapeutic effect on cancer and climacteric syndrome. Clinical studies showed that in patients with epithelial dysplasia of esophagus, a preneoplastic lesion, the recurrence rate within 1 year was 2.2% if the treatment included LW and 12.4% if the treatment

did not include LW, and the same rates within 5 years were 9% and 26%, respectively [10]. Data collected from 22 women with climacteric syndrome and 12 normal child-bearing-age women showed that LW treatment increased not only the plasma estradiol levels but also the leucocyte estrogen receptor levels [11].

Fig. 2 The Liu-Wei-Di-Huang has been used to treat various diseases in the clinic. LW is a classical prescription of nourishing yin, especially for invigorating kidney-yin. In clinical use of Traditional Chinese medicine, LW has been applied to treat deficiency of liver-yin and kidney-yin, dizziness and tinnitus, soreness and weakness of waist and knees, osteopyrexia and tidal fever, night-sweat and spontaneous sweating, spermatorrhea, thirst, dripping discharge of urine, and emaciation. Moreover, LW has already been used to prevent and treat cancer, hypertension, cognitive dysfunction, depression, diabetes, renal diseases, oral dryness, cardiovascular disease and climacteric syndrome

LW has beneficial effects on renal diseases, including pollakiuria, dysuria, and edema. The clinical long-term administration of LW improved serum protein concentration and edema in renal failure. Data from 6 healthy Japanese volunteers indicated that a single dose of LW increased serum levels of several amino acids, such as alanine, arginine, glutamate, glycine, and serine [12]. In addition, LW can markedly improve the therapeutic effectiveness and counteract the adverse effects of hormonotherapy for treatment of the nephrotic syndrome and reduce the recurrence rate of the disease [13].

A population-based study in Taiwan indicated that LW was prescribed by TCM doctors for the treatment of primary hypertension in 16 873 patients between 2006 and 2010 [14]. The potential effects included increasing the production of nitric oxide, blocking calcium channels, regulating the rennin-angiotensin system, increasing the diuretic effects, and acting on the central vasomotor center. In addition, LW combined with antihypertensive drugs appears to be effective in improving blood pressure and symptoms in patients with essential hypertension [15].

A double-blinded, placebo-controlled trial showed that LW prescription derivatives accelerated the speed of information processing and enhanced cognitive abilities in normal young adults [16]. Moreover, LW possesses therapeutic effects on fatigue or loss of energy in patients with prolonged major depressive disorder [17].

A population-based case control study showed that the integration of LW into diabetes care reduced the risk of developing kidney failure among type 2 diabetic patients and the treatment of LW may be beneficial in relieving diabetic nephropathy in type 2 diabetes patients [18]. In addition, adding LW to diabetes care might improve treatment outcomes of type 2 diabetes, including fasting blood glucose (FBG), post-

prandial blood glucose (2hPG), response and control rates [19]. Another population-based study indicated that LW and its derivatives were the most common herbal formulae prescribed by TCM doctors for the treatment of diabetes in Taiwan. The potential effects of LW in type 2 diabetes treatment include an increase in insulin secretion, enhancement of glucose uptake by adipose and muscle tissues, inhibition of glucose production by hepatocytes, and decrease in insulin resistance or enhancement of insulin sensitivity. But the therapy for symptom relief may have been negatively affected by the placebo effect [20]. Nonetheless, results from a randomized, double-blinded, placebo-controlled clinical trial showed after a 36-month treatment with LW, the urinary microalbumin to urinary creatinine ratio (Umalb/cr) level and diabetic nephropathy (DN) and diabetic retinopathy (DR) prevalence were significantly lower in the LW treatment group than that in the control group, indicating that LW was beneficial to diabetic microvascular complications [21]. In addition, adding LW to Western medicine might improve treatment outcomes in diabetic nephropathy, including hyperglycemia and renal functions [22].

The results from a randomized, placebo-controlled, double-blinded, two-center trial showed that LW improved oral moisture status and subjective oral dryness in elderly subjects with a lower BMI and greater tendency toward *Yin*-deficiency (YD) [23].

An integrated and personalized data analysis approach was used to evaluate the symptoms, clinical chemistry, and metabolomics profiles during LW treatment. Results showed that the treatment of LW relieved the symptoms of 'hectic fever' and 'spontaneous sweating', decreased low-density lipoprotein (LDL-C), total cholesterol, systolic blood pressure, and waist size, and reduced 10 of the 15 measured phosphatidylcholines [4]. This showed that LW improves the lipid profile indicating a reduction of cardiovascular risk.

A single-center, controlled, non-blinded, two-way crossover clinical trial showed that a 14-day administration of LW did not affect the activities of cytochrome P450 enzymes CYP2C19, CYP2D6, and CYP3A4 in healthy subjects, and is unlikely to cause pharmacokinetic interactions when it is combined with other medications predominantly metabolized by these enzymes [5]. A single-blind, randomized, placebo-controlled, two-phase crossover study demonstrated that LW can induced cytochrome P4501A2 (CYP1A2) and suppress cytochrome P4502A6 (CYP2A6) and N-acetyltransferase 2 (NAT2) activities, and affect caffeine metabolism [24]. These two clinical trials provided data on the pharmacokinetic properties of LW.

Based on the recent clinical trial outcomes and monitoring data, LW has not only therapeutic effects on cancer, hypertension, cognitive dysfunction, depression, diabetes, renal diseases, oral dryness, cardiovascular disease, and climacteric syndrome (Fig. 2), but also promising pharmacokinetic properties. However, further research studies and clinical trials are needed to evaluate the efficacy of LW in treating disease.

Experimental pharmacology

A large body of pharmacological study has revealed that LW exerts therapeutic effects on various experimental disease models of the central nervous, peripheral nervous, immune, metabolic, reproductive, skeletal, endocrine, oral, renal, and circulatory system (Table 1). The underlying mechanism for the broad spectrum of the pharmacological effects exerted by LW may be that it can modulate and restore the balance of the NIM network disturbed by a diversity of pathological factors [9].

Table 1 Liu-Wei-Di-Huang has therapeutic effects on various experimental models

Disease or condition	Model	Period of treatment	Pharmacological effects	Reference
Cognitive impairment in AD	SAMP8 mice	5 months	Enhanced the memory and retention ability in passive avoidance performance. Promoted the spatial memory ability in water maze test. Partially improved the leaning behavior in conditioned avoidance performance.	[26]
	Hippocampal slice; Primary cultured hippocampal neurons.	30 minutes	Facilitated the induction of LTP in hippocampal slices. Increased intracellular $[Ca^{2+}]_i$, suppressed the I_{Ca} and promoted NMDA-evoked currents in primary cultured hippocampal neurons.	[27]
	Primary cultural hippocampal neuron	48 hours	Decreased the amplitude of delay rectifying I_K and voltage-gated I_{Ca}. Increased the frequency of sEPSC and mEPSC.	[28]
	SAMP8 mice	20 days	Improved the shortened estrus, increased the ovary weigh and significantly reduced the pituitary content of luteinizing hormone (LH).	[29]
		30 days	Regulated expressions of DUSP12, NSF, STUB1, CaMKII*α*, AMFR, UQCRFS1 in the brain of SAMP8.	[30-33]
Non-AD type cognitive impairment	*D*-gal-induced brain injury in rats	6 weeks	Alleviated spatial learning and memory deficit induced by *D*-gal. Increased ACh content and ChAT activity, and decreased AChE activity in visual cortex. Attenuated loss of neurons in hippocampus and visual cortex. Increases of ChAT and AChE expressions.	[34]

Continued

Disease or condition	Model	Period of treatment	Pharmacological effects	Reference
Non-AD type cognitive impairment	SCOP- and PCA-induced amnesia rats	1 or 2 weeks	Prolonged the shortened step-through latency induced by SCOP and PCA.	[35]
	CXM-induced amnesia rats	1 or 2 weeks	Prolonged the shortened step-through latency induced by CXM. Muscarinic antagonist scopolamine, peripheral cholinergic antagonist scopolamine methylbromide, serotonin precursor 5-hydroxytryptamine and serotonin releaser *p*-chloroamphetamine, $GABA_A$ receptor antagonist bicuculline, and $GABA_B$ receptor agonist baclofen could block the ameliorating effects of LW.	[36]
	STZ-induced DE rats.	one month	Reduced the escape latency time and path length, enhanced the spent time in the target quadrant and platform crossings in Morris-water maze test. Decreased the level of fasting blood glucose, increased Na^+-K^+-ATP enzyme and ChAT activities, enhanced GSH level while decreased AChE and iNOS activities in hippocampus. Improved the expressions of IGF-1 and BDNF, attenuated the neural apoptosis, over-expression of caspase-3 and Aβ deposition in the hippocampus and cerebral cortex of STZ-induced DE rats.	[37]
	Rats with ibotenic acid lesion of the medial septum	21 days	Decreased the latencies to find the platform on acquisition trials, spent a greater proportion of the probe trial searching in the training quadrant in Morris-water maze test. Attenuated ibotenic acid-induced ChAT neuron damage in the medial septum. Reduced the loss of ChAT immunoreactivity in the medial septum.	[38]
	MPP^+-treated primary mesencephalic neurons and MPTP-treated C57BL/6 mice.	14 days	Decreased MPP+-induced loss of tyrosine hydroxylase (TH)-positive neurons and increase of annexin V-positive neurons. Reduced MPP^+-induced oxidative damage via increasing antioxidant defense (SOD, GSH), decreasing ROS production, and down-regulating NADPH oxidases (Nox2 and Nox4). Inhibited neuronal apoptosis by improving mitochondrial membrane potential, increasing antiapoptotic protein Bcl-2 expression, and down-regulating apoptotic signaling (Bax, cytochrome c, cleaved-caspase-3) in MPP+-treated neurons. Attenuated TH-positive neuronal loss in substantianigra pars compacta, and improved locomotor activity of mice.	[39]
	Transgenic *C. elegans* (CL4176) expressesing human Aβ 1-42	3 days	Delayed Aβ induced paralysis. Reduced ROS. Increased expression of hsp16.2-GFP after thermal stress whereas a minute induction was observed for sod3-GFP. Repressed the expression of *amy1* in CL4176 while up-regulating hsp16.2 induced by elevating temperature.	[41]
Normal cognition	Male Sprague-Dawley rats	10 days	Increased memory latency in passive avoidance test. Regulated mRNA expression of 17 genes, including neuronal pentraxin precursor (pentraxin), transthyretin, and neuronal specific protein PEP-19 (PEP-19)	[42]
		18 days	Lowered the frequency of error in radial-arm maze test. Increased the number of BRDU-positive cells in the dentate gyrus.	[43]
Ddiabete	STZ-induced diabetic rats; BbeseZucker rats; Wistar rats with insulin resistance	3 days	Enhanced the plasma glucose-lowering action induced by tolbutamide in obese Zucker rats. Delayed the formation of insulin resistance in rats. Increased the plasma glucose lowering action of exogenous insulin in STZ induced diabetic rats.	[45]
	OLETF rats	40 weeks	Prevented the pancreatic islets developing fibrosis and islet atrophy. Lowered the increment of the blood glucose. Postponed the onset of hyperglycemia. Increased the level of adiponectin and improved the status of insulin resistance. Decrease the incidence of T2DM.	[46-48]
	Rats induced by STZ	4 weeks	Decreased blood glucose, increased plasma insulin level. Decreased malondialdehyde and increased activity of glutathione peroxidase and superoxide dismutase in the renal cortex. Declined inducible nitric oxide synthetase, total nitric oxide synthase and constitutive nitric oxide synthase and enhanced nitric oxide. Decreased extracellular matrix indicated by MMP-2 and MMP-9 activities and hydroxyproline. Down-regulated ET-1 level and decreased mRNA expression of endothelin-converting enzyme, preproET-1 and ET (A) receptor.	[50]

Continued

Disease or condition	Model	Period of treatment	Pharmacological effects	Reference
Ddiabete	Sprague-Dawley rats induced by high-sugar and high-fat diet with small dose STZ injection	12 weeks	Decrease the level of FBG and FINS in serum, improve the cellular morphology of liver, kidney, pancreas tissue, and the expression of IRS2, PI3K, Akt mRNA and phosphorylated IRS2, PI3K, Akt protein involved in the canonical PI3K/Akt signaling pathway of T2DM rats in liver were significantly up-regulated, while the total IRS2, PI3K, and Akt protein had no obvious changes.	[51]
Obesity	Obese rats	9 weeks	Lowered serum CRP and TNF-alpha levels. Increased liver SOD activity, serum adiponectin levels.	[54]
	OP-CD rats	10 weeks	Decreased body weight after 3 weeks of the treatment. Reduced visceral and epididymal fat and improved metabolic phenotypes by lowering the serum total cholesterol (TC), non-high-density lipoprotein cholesterol, triacylglycerol, free fatty acids (FFA), and leptin levels. Dose-dependently increased fat and carbohydrate oxidations, energy expenditure, and the relative efficiency of fat oxidation for energy expenditure. Reduced food intake only in week 5 and did not affect the accumulative food intake in every week and the entire treatment period.	[53]
		10 weeks	Reduced body weight, food intake. Lowered serum triglyceride (tg) and nonesterified fatty acid (nefa) levels and body fat. Lowered serum leptin and insulin levels. Liver function testing revealed no adverse side effects under the current experimental conditions.	[52]
Bone diseases	RAW264.7 cells	4 days	Inhibited receptor activator for nuclear factor- kappa B ligand (RANKL)-induced tartrate-resistant acid phosphatase (TRAP) activity and the formation of multinucleated osteoclasts in RAW264.7 cells. Decreased RANKL-induced expression of osteoclast differentiation-specific genes (TRAP, MMPs-9, cathepsin K, and the d2 isoform of vacuolar ATPase V0 domain). Inhibited RANKL-induced phosphorylation of mitogen-activated protein kinases (extracellular signal-regulated kinase, c-Jun N-terminal kinase, and p38), phosphorylation of I-κBα, phosphorylation of NF-κB p65, and the expression of transcription factors Fra-2 and nuclear factor of activated T-cells, cytoplasmic 1. Inhibited the bone-resorptive activity of differentiated osteoclasts.	[59]
	Mouse being intraperitoneal injected with cyclophosphamide	13 days	Accelerated bone marrow HSPCs in marrow-suppressed mice and enhancedcell proliferation by promoting cell cycles from G_0/G_1 phase to access into S, G_2/M phase. Increased the mRNA expression level of TPO and c-Mpl in spleen.	[58]
	Kunming mice induced by horse serum and steroid	10 weeks	Reduced the percentage of empty osteocyte lacunae. Increased the osteoprotegerin expression and decreased the osteoprotegerin ligand expression. Reduced the apoptosis index.	[55]
	OVX rats	12 weeks	Decreased the level of ALP and BGP in serum, increase the BMD of femurs, and improved the biomechanical capabililty of vertebral body in maximum loading and elastic modulus. Ordered arrangement of trabeculae, slightly thinning of trabeculae and none obvious slight fractures in femurs. Increase in cell viability, alkaline phosphatase activity and amount of calcified nodules. The expression of Lrp-5, β-catenin, Runx2 and Osx involved in the canonical Wnt/β-catenin signaling pathway were significantly up-regulated.	[57]
Perimenopausal period syndrome	OVX rats	14 days	Enhanced *Lactobacillus* and *Bifidobacterium* contents in the intestine and intestinal β-glucosidase activity. Elevated steady-state concentrations of genistein, daidzein, and equol in serum.	[60]
	ApoE$^{-/-}$ OVX mice administrated with high-fat diet.	14 weeks	Prevented plaque formation and reduced plasma lipid and Hcy levels. Inhibited CHOP and cleaved caspase-3 expression *in vivo* and *in vitro* while maintaining GPR30 expression. Hcy-induced HUVECs apoptosis was weakened by LW-medicated serum pretreatment *in vitro*. Up regulated NO release and eNOS activity in HUVECs. Optimized the balance between Bax and Bcl-2, and attenuated intracellular ROS production.	[61]
	OVX rats	8 weeks	Reduced retroperitoneal and peri-renal fat accumulation, serum lipids, the atherogenic index, cardiac risk factors, intima-media thickness, and NASH.	[62]

Continued

Disease or condition	Model	Period of treatment	Pharmacological effects	Reference
Immune disease	EAE model induced by MOG (35−55) and CFA supplemented with PTX	40 days	Reduced inflammatory cells, demyelination and axonal loss. Decreased the protein and mRNA expression of TNF-alpha and the ratio of TNF-alpha/TGF-beta. Enhanced the levels of cAMP.	[63]
	Sprague-Dawley rats with injection of carrageenan	3 days	Discriminated phenotypes of metabolites from carrageenan-stimulated inflammation model. Restore the metabolite network that disturbed by inflammation.	[64]
Reproductive problems	CP-treated Wistar rats	56 days	Increased the weight of the testes, epididymal sperm count and sperm motility. Reduced lipid peroxidation by CP. Increased the expression of cAMP-CREM in male germ cells.	[66]
DMD	Dystrophin-deficient mice	3 months	Facilitate locomotor activity with the parameters of horizontal activity, total distance, number of movements, movement time, vertical activity, number of vertical movements, vertical movement time, stereotypy, number of stereotyped movements, and stereotyped movement time.	[68]
Hypertension	High-salt and high-fat diet-fed rats	4 weeks	Decreased the levels of MAP, FG, INS, high-density lipoprotein cholesterol (HDL-c), homeostasis model assessment of basal insulin resistance (HOMA-IR) and angiotensin II (Ang II) from plasma and Ang II and renin from kidney. Promoted the excretion of urinary Na^+, reducing the loss of urinary K^+ and MAU, and improved the glomerular afferent arteriole, arterioles and each kidney unit.	[69]
ARF	Rats with ischemia/reperfusion-induced ARF	4 days	Polyuria was markedly restored with restoring expression of AQP 2 in the kidney. The expressions of Na, K-ATPase α1 and β1 subunits in the renal medulla and cortex were restored. Lowered the expression of HO-1. The renal functional parameters including creatinine clearance, urinary sodium excretion, urinary osmolality, and solute-free reabsorption were markedly restored. The renal damages were abrogated.	[70]
BPH	TP-induced BPH rat	4 weeks	Decreased absolute and relative prostate weights, dihydrotestosterone levels in the serum or prostate and PCNA expression in the prostate.	[71]
Spontaneous breast carcinoma	Mouse model of spontaneous breast carcinoma	to the agonal stage of control group	Compared with the control group, cancer tissue volume and weight were lower in the LW-treated groups, and survival time was longer. The expression of VEGF, ERK and Cyclin D1 were inhibited in the LW-treated groups, and cell differentiation was increased. Tumor weights and volumes and VEGF, ERK and Cyclin D1 expression in LW-treated groups.	[72]
Periodontal diseases	Cell line OBA-9 and HGF-1	3 days	Prevented biofilm formation by *Fusobacteriumnucleatum*. Inhibited IL-6 secretion in both epithelial cells and fibroblasts stimulated with lipopolysaccharide. Increased proliferation and migration of gingival fibroblasts in a wound healing assay.	[73]

Anti-amnestic and cognitive-enhancing activities

Several of our studies have described the beneficial effects and possible mechanisms of LW in cognitive deterioration of Alzheimer's disease (AD). The senescence-accelerated mouse prone 8 (SAMP8) strain is a robust model for exploring the etiopathogenesis of sporadic AD and a reliable experimental model for developing preventive and therapeutic treatments for late-onset/age-related AD [25]. The treatment with LW significantly ameliorated cognitive deterioration of SAMP8 mice [26], modulated the synaptic plasticity by facilitating the induction of long-term potentiation (LTP) in hippocampal slices of SAMP8 mice [27], improved neuronal function by increasing intracellular $[Ca^{2+}]_i$, the frequency of spontaneous excitatory post-synaptic current (sEPSC) and miniature excitatory post-synaptic current (mEPSC), suppressing the Ca^{2+} current (I_{Ca}) and promoting *N*-methyl-D-aspartate (NMDA)-evoked currents, decreasing the amplitude of delay rectifying K^+ current (I_K) and voltage-gated I_{Ca} in primary cultured hippocampal neurons [27-28]. Moreover, LW modulated the imbalance of hypothalamus-pituitary- ovary (HPO) axis in SAMP8 mice during the aging process by improving the shortened estrus, increasing the ovary weight and significantly reducing the pituitary content of the luteinizing hormone (LH) [29]. In addition, LW regulated expression of autocrine motility factor receptor (AMFR) [30], calcium/calmodulin-dependent protein kinase type II alpha chain (CaMKIIα) [31], STIP1 homologous and U box containing protein 1(STUB1) [32], dual specificity protein phosphatase containing protein (DUSP12), *N*-ethylmaleimide sensitive fusion protein (NSF), and ubiquinol-cytochrome c reductase subunit

(UQCRFS1) [33] in the brain of SAMP8 mice.

Some recent studies have demonstrated that LW has beneficial effects on amnesia. LW can reverse the *D*-galactose (*D*-gal)-induced spatial learning and memory impairments and neuronal damage by restoring acetylcholine (ACh) levels and cholineacetyltransferase (ChAT) activity, inhibiting acetylcholinesterase (AChE) activity in the central nervous system (CNS) [34]. LW has ameliorative effects on scopolamine (SCOP)-, p-chloroamphetamine (PCA)- [35] or cycloheximide (CXM)- induced impairment of avoidance performance [36] by increasing the central cholinergic and GABAergic system, and decreasing serotonergic neuronal activity. LW had a potential benefit for the treatment of spatial learning and memory deficits of streptozotocin (STZ)-induced diabetic encephalopathy (DE) *via* regulating blood glucose and reducing hyperglycemia-induced damages to nerve cells, promoting the function of antioxidant defense systems, inhibiting neuronal apoptosis, increasing neurotrophic factor expression, and reducing β-amyloid (Aβ) deposition [37]. Moreover, LW prescription derivatives had beneficial effects on spatial learning and memory impairment in amnesia rats with ibotenic acid-induced lesions in the medial septum by reducing the loss of ChAT immunoreactivity and cell losses in the medial septum [38]. In addition, LW has beneficial effects on Parkinson's disease (PD). LW protected dopaminergic neurons and improved locomotor activity in the MPTP-treated mouse model of PD by enhancing antioxidant defense and decreasing apoptotic death [39]. LW protected motor neurons against survival motor neuron (SMN) deficiency-induced neurodegeneration [40]. The treatment of LW alleviated Aβ-induced paralysis in *Caenorhabditis elegans* by increasing heat shock proteins (HSP16.2), lowering *amy1*, improving antioxidant activity, and reducing ROS [41].

Besides exerting protective effects on cognitive deficits, LW also showed significant memory enhancement on normal cognition. In normal male Sprague-Dawley rats, LW significantly increased memory retention abilities in the passive avoidance test by inducing several genes that are involved in protecting neuronal cells and enhancing cell proliferation and neurite growth [42]. Treatment by LW enhanced the spatial learning ability and increased the neurogenesis in the dentate gyrus in normal male Sprague-Dawley rats [43]. Therefore, these studies have characterized LW as a cognitive enhancer.

Beneficial effects on diabetes

Many studies indicate that LW is potentially useful in managing diabetic disorders. One interesting study showed that LW had the ability to stimulate the secretion of insulin, which appeared to be helpful in improving the diabetic condition, specifically for hyperglycemia in type-II diabetes mellitus (T2DM) [44]. The oral administration of LW increased insulin sensitivity and delayed the development of insulin resistance in rats. The effect of LW on insulin sensitivity appears to develop more rapidly than that of metformin [45]. Additionally, treatment with LW prevented the pancreatic islets developing fibrosis and islet atrophy, significantly lowered the increment of the blood glucose and postponed the onset of hyperglycemia, increased the level of adiponectin and improved the status of insulin resistance, and decreased the incidence of type 2 diabetes in Otsuka Long-Evans Tokushima Fatty (OLETF) rats [46-48].

In a study on the mechanism of activity, LW stimulated the secretion of insulin, decreasing the plasma glucose levels. Further analysis showed that LW may enhance the release of Ach from nerve terminals to stimulate the muscarinic M3 receptors for augmenting insulin release, which produces the plasma glucose lowering action [49]. The benefits of LW in relieving the abnormalities in early diabetic nephropathy are likely to be mediated by the suppression of the renal endothelin-1-reactive oxidative species (ET-ROS) system and escalating the activity of matrix metalloproteinases (MMPs) [50]. LW intervened in the insulin resistance of the T2DM model, induced by high sugar and high-fat diet in combination with a STZ injection in male Sprague-Dawley rats by regulation of the canonical PI3K/Akt signaling pathway in the liver [51].

Preventive effects on obesity

Some studies indicate that LW is a potential therapeutic agent for the prevention of obesity. The study in obesity-prone (OP-CD) rats showed that the LW treatment lowered body weight and improved insulin and leptin sensitivity [52]. Interestingly, LW ethanol extract decreased body weight, significantly reduced visceral and epididymal fat and improved metabolic phenotypes in male OP-CD rats possibly by increasing energy metabolism and expenditure, along with a possible effect on decreasing energy intake [53]. These studies indicate, that LW might be a potential natural weight-lowering product. A further study indicated that LW had anti- inflammatory, anti-oxidative, and adiponectin-ameliorating effects in obese rats by lowering serum C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-alpha) levels, but increasing liver superoxide dismutase (SOD) activity and serum adiponectin levels [54].

Alleviative effects on bone diseases

The treatment of LW prevented steroid-induced osteonecrosis of the femoral head (ONFH) by improving lipid metabolism, relieving bone lesion, and protecting against cell death in mice [55]. LW could alleviate osteoporosis induced by ovariectomy [56], in part, through up-regulation of canonical Wnt/β-catenin signaling pathway in osteoblasts [57]. In addition, LW accelerated the bone marrow hematopoietic stem progenitor cells (HSPCs) in marrow-suppressed mice and enhanced cell proliferation by promoting cell cycles from the G_0/G_1 phase to S and, eventually, G_2/M phase [58]. Therefore, LW may have a therapeutic potential in bone diseases by preventing osteoclast differentiation and inhibiting receptor activator for nuclear factor-kappa ligand-induced osteoclast differentiation [59].

Beneficial effects on the perimenopausal period syndrome

LW may play a role in the treatment and prevention of the perimenopausal period syndrome by enhancing the *Lactobacillus* and *Bifidobacterium* content and the intestinal

β-glucosidase activity in the intestine of rat [60]. LW treatment can significantly reduce plaque formation in ovariectomized (OVX) ApoE-deficient mice, an animal model of menopausal atherosclerosis. It may act by inhibiting homocysteine (Hcy)-induced endothelial cell apoptosis via modulating G protein coupled estrogen receptor 30 (GPR30) [61]. In OVX rats, the treatment with LW helped in the arrangement of trabeculae and the slightly thinning of trabeculae and prevented obvious slight fractures in femurs. It decreased the level of alkaline phosphatase (ALP) and osteocalcin (BGP) in serum, increased the bone mineral density (BMD) of femurs and improved the biomechanical capability of the vertebral body in maximum loading and elastic modulus [58]. LW had cholesterol-lowering effects both *in vivo* and *in vitro*, suggesting that LW may have a potential as a therapeutic agent for the treatment of hyperlipidemia in postmenopausal females [62].

Regulatory effects on immune disease

LW possesses potential therapeutic activities against immune dysfunction. A study showed that LW had a regulatory role in the experimental autoimmune encephalomyelitis (EAE), a model for human multiple sclerosis (MS) by regulating the cytokine balance in favor of T helper 1 (Th1)/regulatory T (Treg) cells, which depend on increased cAMP levels [63]. LW could restore the metabolite network that was disturbed by the inflammation in a carrageenan-stimulated inflammation rat model [64]. In addition, the administration of LW inhibited the RNA and protein expression of Th2-type cytokines: IL-4, IL-5, IL-10, or IL-13 and Th1-type: IL-2 and IFN-gamma in human peripheral blood mononuclear cells (PBMC) [65].

Improved effects on reproduction

A few studies indicated that LW may have a potential effect on the reproductive system. LW protects against cyclophosphamide (CP)-induced reproductive toxicity. The treatment of LW increased the weight of the testes, epididymal sperm count, and sperm motility by inhibiting the increases of lipid peroxidation and enhancing the cAMP-responsive element modulator (CREM) expression in rats [66]. During an evaluation of the effects of LW on diabetic impotence in diabetic rats, LW significantly improved mounting performance by preserving the intromission and ejaculation, but not by lowering the blood sugar [67].

Ameliorative effects on other diseases

Multiple studies showed that treatment with LW ameliorated muscular dystrophy, hypertension, renal disease, benign prostatic hyperplasia, breast carcinoma, and periodontal diseases (Table 1).

LW had effects on Duchenne muscular dystrophy (DMD). Consuption of LW can facilitate locomotor activity according to the parameters of horizontal activity, total distance, number of movements, movement time, vertical activity, number of vertical movements, vertical movement time, stereotypy, number of stereotyped movements, and stereotyped movement time. This suggested that LW can act as a potent herbal remedy for the pharmacological treatment of DMD patients [68].

LW possesses anti-hypertension properties. It is capable of moderately reducing the mean arterial pressure (MAP) in salt-sensitive hypertension rats. The LW treatment lowered the blood pressure by significantly inhibiting the renal rennin-angiotensin system (RAS) activation, improving renal function by decreasing urinary K^+ and microalbuminuria (MAU) loss and increasing urinary Na^+ excretion, lowering plasma fasting blood glucose (FG) and insulin (INS), as well as significantly improving insulin sensitivity index (ISI) [69].

LW was also used for the treatment of renal diseases. Administration of LW ameliorates renal defects in rats with ischemia/reperfusion-induced acute renal failure (ARF) by improving the renal functional parameters including creatinine clearance, urinary sodium excretion, urinary osmolality, and solute-free reabsorption, in association with the expression of aquaporin 2 (AQP 2), Na, K-ATPase, and heme-oxygenase-1 (HO-1) [70].

LW effectively inhibits the development of benign prostatic hyperplasia (BPH) by decreasing absolute and relative prostate weights, lowering dihydrotestosterone levels in the serum or prostate and reducing proliferating cell nuclear antigen (PCNA) expression in the prostate [71].

In mice with spontaneous breast carcinoma, the LW treatment not only decreased tissue volume and weight but also extended survival time. LW suppressed the expression of the vascular endothelial growth factor (VEGF), extracellular signal-regulated kinase (ERK) and Cyclin D1, which increased cell differentiation [72].

LW has a potential application in periodontal diseases by suppressing the formation of *Fusobacterium nucleatum* biofilms, inhibiting IL-6 secretion in gingival epithelial cells and fibroblasts, and promotion wound healing [73].

Quality control and phytochemistry

LW is a well-balanced mixture of different herbs that have several biological and physiological effects. In the past, the high-performance liquid chromatography-diode array detection (HPLC-DAD) method has been employed for the analysis and the quality control of LW [28, 74]. Recently, some quality control methods for LW have been further developed based on chemical standards and biomarkers. For instance, the HPLC fingerprinting with chemical standards or biomarker components including 5-hydroxymethyl-2-furaldehyde, morroniside, loganin, paeoniflorin, verbascoside, and paeonol was typically used as quality control for LW preparations [39, 75-76]. CHENG *et al.* developed a method, M-TCM, for the biological assessment of the quality of TCM preparations based on high-throughput sequencing and metagenomic analysis that should be used to determine both prescribed and contaminated species simultaneously and indiscriminately [77-78]. An ultra-high performance liquid chromatography dual-wavelength method was developed to simultaneously determine 11 constituents in LW preparations [79]. These 11 constituents were gallic acid, protocatechuic acid, 1, 2, 3, 4, 6-*o*-penta-

galloylglucose, paeonol, 5-hydroxylmethyl furfural, morroniside, loganin, sweroside, paeoniflorin, benzoic acid, and benzoylpaeoniflorin. A combination method using five markers for HPLC fingerprinting coupled with diode array detection was developed to monitor the quality consistency of LW [80]. The five marker compounds were 5-hydroxylmethyl furfural, gallic acid, loganin, paeoniflorin, and paeonol. The integration of ultraviolet spectroscopic fingerprints and multi-wavelength fusion fingerprints provided a rapid and effective approach to monitor the quality consistency of LW [81].

The chemical constituents of LW have been extensively studied since the early 1900s. Nonetheless, identification of the bioactive ingredient from LW remains a challenging task using the traditional approach that focuses on chemical isolation coupled with biological activity screening. Although many analytical methods have been established for the constituent analysis of LW, such as Q-TOF-MS-IDA-MS/MS [82-83], DEAE-cellulose and gel-filtration chromatography [84], gel-column chromatography and HPLC-TLC-IR-^{13}CNMR [85], super-filtration [86], RP-HPLC [87], HPLC-ESI-TOF-MS [88], the sulfuric acid-phenol method [89], and HPLC/DAD/ ESI-MS [90], the main components of LW that are responsible for the therapeutic effects still remain unknown. To identify and study the active substances of LW that have the effect on the NIM network, a close collaboration between pharmacological and phytochemical studies was established for our research. We isolated and identified several active fractions and bioactive ingredients from LW. We found that LW was mainly composed of oligosaccharides (levidulinose, TMAN, stachyose), polysaccharides (polygalacturonic acid, rhamnogalacturonic acid polysaccharide, arabinogalactan, dextran), and glycosides. Glycosides included iridoid glycosides (loganin, morroniside, 7-O-isidecosidesdmorroniside, 6harideeractions amorroniside, loganic acid, sweroside, PAE, glucose loganin), peoniflorin (peoniflorin, hydroxypeoniflorin, 4-chlorine- hydroxy-peoniflorin), phenethanol-glycosides (phenylpropionic acid, acteoside, isoacteoside, jionoside A_1, jionoside A_2, jionoside B_1, jionoside B_2), 5-hydroxymethyl-furaldehyde and derivatives (5-methoxyl-methyl-furaldehyde, 5-ortho-butoxy-methyl-furaldehyde, 5-hydroxymethyl-pyromucic acid), and others (ursolic acid, *β*-sitosterol, daucosterol, alismoxide, gallic acid, para hydroxybenzoic acid, benzoic acid, succinic acid, and stearic acid) [9, 91-94]. It was reported, there were at least 224 different compounds in LW [6-8]. HPLC fingerprinting of LW was used to identify 22 components, including succinate, gallic acid, 5-hydroxy methyl furfural, 2, 5-furan-2-formaldehyde, 7-alpha-O-D-glucose morroniside, 7-beta-O-D-glucose morroniside, loganic acid, morroniside, hydroxyl group, loganin, *Swertia japonica* glucoside, 7-O-apple acid loganin, Coke *Rehmannia* benzene ethanol glycoside A1/A2, paeoniflorin, galloyl paeoniflorin, a new glycoside I from *Cornus officinalis*, mudanpioside C, benzoylpaeoniflorin, and paeonol [82]. Sangha *et al*. also determined some main bioactive components in LW extracts [41]. 5-Hydroxymethyl furfural, phenolic compound gallic acid, iridoidsloganin and sweroside, and paeoniforin were identified in LW ethanol extract and LW water extract, but phenolic compound paeonol was only found in the LW ethanol extract using ^{1}H NMR and HPLC-MS analysis [41]. The response surface methodology (RSM) is applied for the determination of optimal conditions with maximum yields of bioactive compounds, *i.e.*, gallic acid, 5-hydroxymethylfurfural, morroniside, loganin, paeoniflorin, benzoic acid, and paeonol, in LW [95]. According to literature reports, the total polysaccharide content in LW should be approximately 25%. The oral bioavailability of other effective components (including gallic acid, morroniside, peoniorin, loganin and paeonol) was very low, accounting for a content of only 3% in the LW preparation [96]. However, it is well known that paeoniflorin, morroniside, loganin, gallic acid, paeonol, and 5-hydroxymethyl furfural are the main bioactive compounds of LW and employed by the quality control of LW [37, 97].

Pharmacokinetics

LW is prepared from six different herbs, and each herb has a great variety of compounds. However, to our knowledge, there is not yet a well-established profile of the major chemical constituents of LW. Therefore, although LW has a long history with successful applications in the clinic, the scientific and systematic pharmacokinetics assessment is far from comprehensive. There are currently only a few studies on the pharmacokinetic parameters and characteristics of LW with a focus on its bioactive chemical constituents.

In the clinic, Y. Chen assessed the effect of LW on the activities of cytochrome P4502C19 (*CYP2C19*), cytochrome P4502D6 (*CYP2D6*), and P4503A4 (*CYP3A4*) in 12 healthy Chinese subjects in a single-center, controlled, non-blinded, two-way crossover clinical trial [5]. As the results showed, there was no difference in the activities of the three tested enzymes before and after a 14-day administration of LW, which had no effect on the pharmacokinetic parameters of the substrates and their metabolites and did not affect the activities of *CYP2C19*, *CYP2D6* and *CYP3A4* [5]. However, LW induced cytochrome P4501A2 (*CYP1A2*), suppressed cytochrome P4502A6 (*CYP2A6*) and NAT2 activities, and affected the caffeine metabolism in humans [24].

After the oral administration of LW, 9 compounds were identified in blood as constituents derived from LW. Out of the 9 compounds, 5-hydroxymethyl-2-furoic acid (HMFA), morroniside, sweroside, loganin, paeonol, and paeoniflorin were the main constituents absorbed into the blood, whereas the others were the metabolites of paeonol [98-99]. The pharmacokinetic analysis of LW indicated the $t_{1/2}\alpha$ and $t_{1/2}\beta$ were 2.62/32.66, 0.46/4.71, and 1.30/23.51 h, and the climax times and concentrations were 0.56/683.75, 0.70/2826.11, and 0.62 h/ 4030.48 ng·mL^{-1}for HMFA, loganin, and paeonol, respectively [100]. Specifically, the absorption and distribution of HMFA was fast ($t_{1/2}\kappa\alpha$ 0.1 h, $t_{1/2}\alpha$ 2.62 h), but the elimination was slow ($t_{1/2}\beta$ 32.66 h). Loganin was absorbed promptly ($t_{1/2}\alpha$

0.46 h) and eliminated moderately ($t_{1/2}\beta$ 4.71 h) as compared to that of HMFA [100]. A study on the pharmacokinetic profiles of morroniside and loganin in rat plasma indicated that morroniside and loganin could be simultaneously determined within 7.4 min. Linear calibration curves were obtained over the concentration ranges of 45.45−4800 ng·mL^{-1} for all the analytes [101].

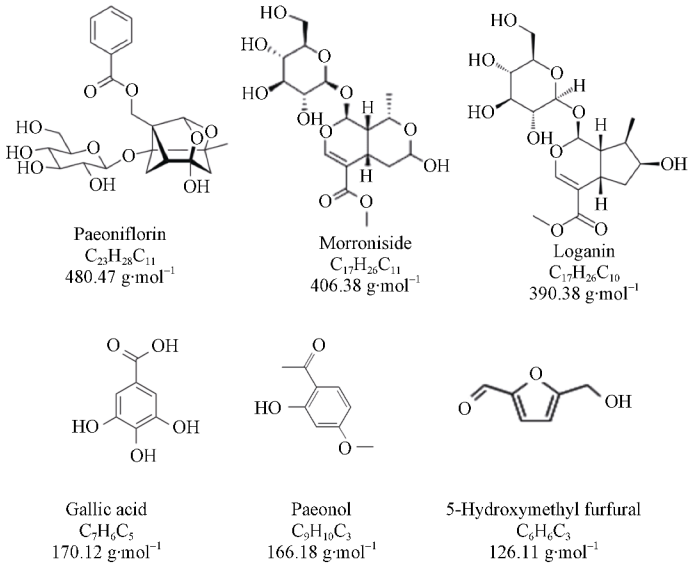

Fig. 3 Chemical markers as quality control of Liu-Wei-Di-Huang

Absorbed Paeoniflorin has a low bioavailability and is mainly excreted in the urine [102-103]. Paeoniflorin is not metabolized by the gut wall, liver or lung. The unabsorbed fraction of paeoniflorin is degraded by the intestinal flora [103]. After i.v. administration, paeoniflorin could quickly penetrate the blood-brain barrier to reach brain tissues such as the hippocampus [104].

After administrating morroniside in rats, the highest level was observed in the small intestine, then in the kidney and stomach. No morroniside was detected in the brain, and there was no long-term accumulation of morroniside in rat tissues [105]. The determination of morroniside concentration in rat plasma indicated that area under the plasma concentration-time curve ($AUC_{0\text{-inifinity}}$) was (587.6 +/–290. 7) μg·L^{-1}·min^{-1}, C_{max} was (334.2+/–148.0) μg·L^{-1}, T_{max} was (0.6 +/–0.3) h, and $t_{1/2}$ was (0.7+/–0.3) h [106]. The determination of morroniside in the plasma of beagles indicated that the relationship between the dose and the $AUC_{0\text{-inifinity}}$ showed a good linearity. Linear pharmacokinetic properties were proposed for morroniside [107]. In addition, the metabolites of morroniside in rat urine, bile and feces included mor-1 and mor-2 identified as nitrogen-containing compounds along with the known aglycones [108].

A study on the tissue distribution of loganin in rats after a single administration of loganin showed that the highest level was observed in the kidney, then in the stomach, lungs and small intestine. The lowest level was found in the brain. The peak levels were reached after 90 min in most tissues [109]. The absolute bioavailability of loganin was calculated to be 13.2%. Only 5% of administered prototype medicine was detected in the urine, very little was detected in the bile, and it was undetectable in the feces [110-111]. Loganin is probably metabolized by the liver microsomes or by intestinal bacteria [112].

Pharmacokinetic parameters of gallic acid in rat serum were C_{max} at 2.5 μg·mL^{-1}, T_{max} at 1.08 h, area under concentration-time curve ($AUC_{0\text{-tn}}$) 2.82 μg·mL^{-1}·h, area under concentration-time curve ($AUC_{0\text{-t}\alpha}$) 3.09 μg·mL^{-1}·h, elimination half-life ($t_{1/2\ el}$) 0.98 h, elimination rate constant (K_{el}) 0.71 h, clearance (cl) 32.39 L·h^{-1}, volume of distribution (V_d) 45.55 L [113]. Gallic acid showed a relatively targeted distribution in the kidney tissue. The concentration of gallic acid in the kidney tissue reached 1218.62 ng·g^{-1}, at one hour after oral administration. Gallic acid followed a concentrated elimination over a 4 h period. The amount of unchanged gallic acid that survived the metabolism was about 14.60% of the total intake [114]. In general, the bioavailability of gallic acid is very low. In one experiment conducted in healthy human volunteers, the mean maximum concentration of gallic acid in plasma was 1.83 μmol·L^{-1} after intake of 50 mg gallic acid [115].

5-Hydroxymethyl furfural had a high bioavailability and was rapidly absorbed into the bloodstream from the gastrointestinal tract without being metabolized. After a single oral administration of 50 mg·kg^{-1} body weight in transgenic sickle mice, the pharmacokinetic parameters were *AUC* 133 μg·mL^{-1}·min^{-1}, $T_{1/2}$ 0.83 h, C_{max} 68 μg·mL^{-1}, C_{max} 0.54 mmol·L^{-1}, $V_{d(ss)}$ 0.45 L·kg^{-1}, MRT 1.8 h, C_l 5.2 mL·kg^{-1}·min^{-1} [116].

Toxicology

The increase in the global use of LW gives cause for concerns over its safety or possible toxicity. However, there is not much known about the toxicology of LW based on the

literature. In fact, in the clinical practice of traditional Chinese medicine, LW is used based on a holistic diagnosis and treatment according to the theory of "*Bian Zheng Lun Zhi*", which believes that LW has no side effects at all. Our study may add some information. LW-AFC was prepared from LW, containing a polysaccharide fraction, a glycoside fraction, and an oligosaccharide fraction [117-122]. Our study showed that the CNS, respiratory system, and cardiovascular system were normal in mice and rats receiving orally administrated LW-AFC 15 g·kg^{-1} (equal to the standard dose of LW). The study on acute toxicity of LW-AFC indicated that there was no death after 14 days treatment with 15 g·kg^{-1} LW-AFC in the mice or rats. Results of a study on long-term toxicity of LW-AFC showed that the nontoxic dosage of oral and repeat administration was 0.42 g·kg^{-1} in rats for 180 days and 0.5 g·kg^{-1} in beagle dogs for 270 days, which is the equivalent of 25 and 30 times of the clinical dosage for humans, respectively.

Concluding remarks

LW is an ancient and classical TCM prescription of *Yin* nourishing and *kidney* tonifying. There are adequate data on the clinical safety and efficacy of LW. The LW prescription consisting of six herbs is the integral administration mode. The synergy of the combinatorial intervention reinforces the overall effects and eliminates the adverse effects of certain drugs associated with single drug prescriptions. To our knowledge, the total number of compounds and the identities of the bioactive or therapeutic constituents in LW are still unknown. Indeed, the various compounds may interact with one another in complex ways resulting in synergistic activities that can produce enhanced or even novel effects as compared to those of the characterized compounds, such as paeoniflorin, morroniside, loganin, gallic acid or paeonol. Nonetheless, LW with still unknown pharmacologic mechanisms is used worldwide as nourishing prescription and for treating many different diseases. This phenomenon reflects the synergetic effect caused by the intervention using LW with its constituents.

This paper has systematically reviewed clinical and experimental pharmacology of LW. The same prescription has beneficial effects on various disease. The pharmacologic effects of LW might be due to a synergistic effect by multi-layered, multi-dimensional networks of the potential combination of the LW components and might follow the concept of a system-based intervention strategy which use multiple inputs (i.e., more than one drug) to treat a complex disease. Based on the similarity of the information on the symptoms and holistic physiological role, we postulate that the role of "*kidney*" in TCM might be consistent with the NIM network in physiology (Fig. 4). The "*kidney*" in TCM is the foundation of innateness and the regulative hub of zang-fu viscera. The function of "*kidney*" in TCM lies in modulating and maintaining a balanced body function and the homeostasis of the internal environment. The NIM network shares functional similarities with this concept. The deficiency of "*kidney*" in TCM is similar to the physiopathology of the imbalance of the NIM (Fig. 4). The deficiency of "*kidney*" results in aging, dementia,

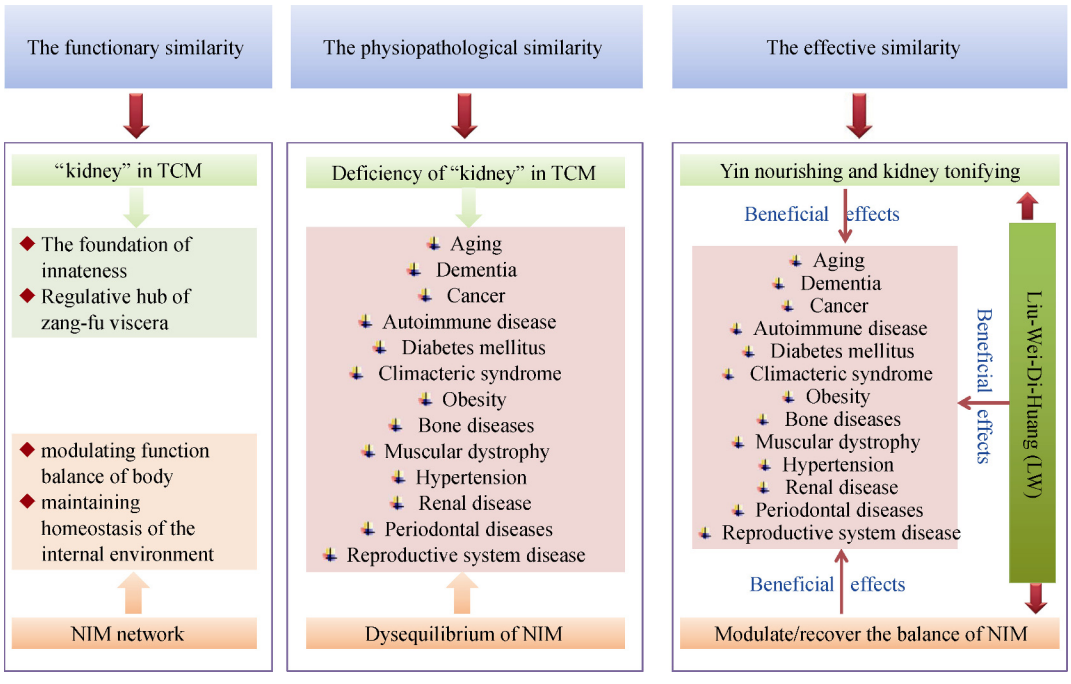

Fig.4 The schematic diagram of the pharmacological theory on Liu-Wei-Di-Huang. Based on the similarity of symptoms information and the holistic physiological role, we postulate that the role of "*kidney*" in Traditional Chinese medicine (TCM) might be consistent with the neuroendocrine immunomodulation (NIM) network in physiology. The deficiency of "*kidney*" in TCM shares the physiopathological feature of imbalance with NIM. Therefore, the mechanism of *Yin* nourishing and *kidney* tonifying by LW in TCM might be to modulate or recover the balance of NIM in the body

cancer, autoimmune disease, diabetes mellitus, climacteric syndrome, obesity, bone disease, muscular dystrophy, hypertension, renal disease, periodontal diseases, and reproductive system disease. The imbalance of NIM in the body is considered to be closely associated with those diseases [9], which and has high phenotypical similarity with the deficiency of "*kidney*". Therefore, the mechanism of *Yin* nourishing and *kidney* tonifying by LW in TCM might be to modulate or recover the balance of NIM in the body (Fig. 4). LW may regulate the NIM network [117, 120-121] by affecting the gene network [118], protein-protein interaction network [123], protein modification network[119], and intestinal microbiome [122].

The pharmacological effects of LW could be explained as maintaining the homeostasis of the NIM network. In the nervous system, the administration of LW modulated the release of neurotransmitters and neuropeptides, the expressions of genes and proteins related to learning and memory and facilitating the induction of LTP [26-28, 30-33, 118-119]. In the endocrine system, LW modulated the levels of hormones and cognate receptors, hypothalamic peptides and monoamine neurotransmitters, and also the pituitary gonadotropic hormone function [29, 124-128]. In the immune system, the administration of LW modulated the functions of immunocompetent cells, the interactions between the immunocytes and the productions of cytokines, metabolites, and the intestinal microbiome [129-134]. Using bioactive molecules and pathways, LW not only maintains the local homeostasis of nervous, endocrine, and immune systems, but also regulats the balance among these three functional systems [117, 120, 135]. LW plays an integrative role in modulating the balance of the NIM network by regulating communications and interactions among the neuro-endocrine-immune system in a bi-directional manner [9, 135]. Based on the network pharmacology, LIANG *et al*. showed that the key pharmacological effects and therapeutic indications of LW may be in the maintaining homeostasis of the endocrine system, the immune system and the metabolism [136]. Based on the genetic and phenotype information associated with both LW herbs and LW-treated diseases, LI *et al.* found that LW-treated diseases have a high phenotypic similarity and identified by certain "co-modules" enriched in cancer pathways and neuro-endocrine-immune pathways, which may be responsible for the action of treating different diseases by the same LW formula [137].

To decipher the molecular mechanisms of LW employed for maintaining the homeostasis of the NIM network, we combined chemical and therapeutic properties with the network pharmacology (Fig. 5). We found that there were 42, 63, 37, 29, 36, and 25 compounds in *Rehmannia glutinosa* Libosch.

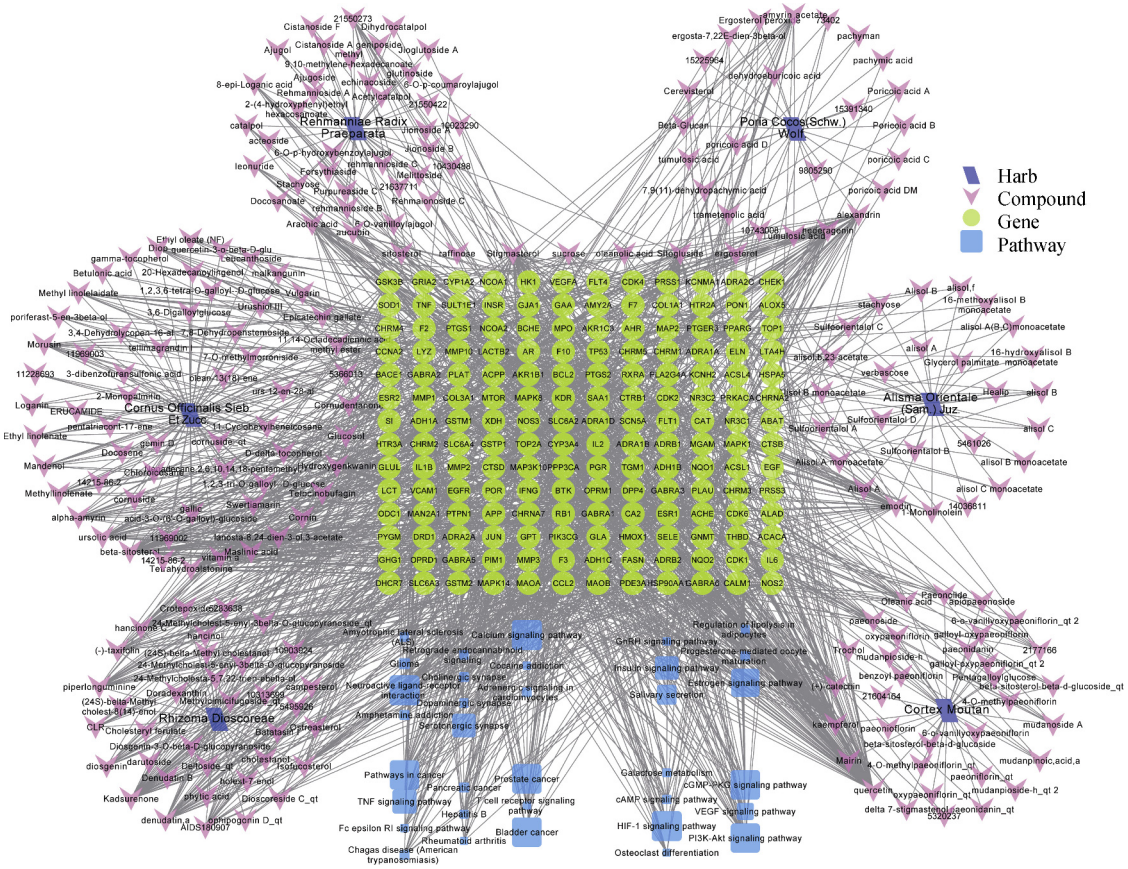

Fig.5 The molecular network mechanism of the pharmacological effects of Liu-Wei-Di-Huang

var. *purpurea* Makino, *Cornus officinalis* Sieb. et Zucc., *Dioscorea japonica* Thunb., *Alisma orientale* Juzep., *Paeonia suffruticosa* Andrews, and *Poria cocos* Wolf respectively. The compounds of the six herbs targeted 181, 388, 287, 110, 249, and 124 bio-molecules, respectively. There were 168 non-redundant genes among the targets. These 168 genes were enriched in 34 pathways ($P < 0.001$ and FDR < 0.25) (Fig. 5). The pathways relating to the nervous system include neuroactive ligand-receptor interaction, calcium signaling pathway, serotonergic synapse, glioma, amphetamine addiction, cholinergic synapse, retrograde endocannabinoid signaling, amyotrophic lateral sclerosis (ALS), dopaminergic synapse, cocaine addiction, *etc*. The pathways correlating with the endocrine system included estrogen signaling pathway, insulin signaling pathway, salivary secretion, progesterone-mediated oocyte maturation, GnRH signaling pathway, *etc*. The pathways relating to the immune system included TNF signaling pathway, bladder cancer, prostate cancer, pathways in cancer, pancreatic cancer, T cell receptor signaling pathway, rheumatoid arthritis, Fc epsilon RI signaling pathway, hepatitis B, etc. There were other pathways, such as HIF-1 signaling pathway, cGMP-PKG signaling pathway, PI3K-Akt signaling pathway, VEGF signaling pathway, cAMP signaling pathway, regulation of lipolysis in adipocytes, galactose metabolism, osteoclast differentiation, *etc*. (Fig. 5).

We collected all compounds and targets of LW from TCMSP database (http: //lsp.nwu.edu.cn/tcmsp.php) [138]. Drug-likeness (DL) is a qualitative concept used in drug design to estimate a "drug-like" profile of a prospective compound, which helps optimize pharmacokinetics and drug properties such as solubility and chemical stability [139]. In this work, Tanimoto Similarity (TS) was used to screen compounds which are promising to be chemically suitable for drugs [140] between herbal ingredients and the average molecular properties of all drugs in FDA [141]. The TS index is defined as $T(A,B)=\frac{A \cdot B}{\|A\|^2 + \|B\|^2 - A \cdot B}$. Where A represents the molecular descriptors of herbal compounds, B represents the average drug-likeness index of all drugs in FDA. In this study, compounds with DL $\geqslant$ 0.15 were selected as the candidate bioactive compounds. The potential targets of the compounds in this dataset were predicted by an in silico CSDT model [142]. In order to probe the meaningful functional annotation of our achieved targets, in this work, KEGG pathway enrichment analysis was performed by linking the targets to DAVID v 6.8 (https: //david.ncifcrf.gov/) [143-144]. Only KEGG pathway with $P < 0.001$ and the FDR (the false discovery rate) <0.25 were selected. Here, the FDR was introduced to perform a multiple-hypothesis testing error measure of P values. This figure shows relationships among herbs, compounds, targets, and pathways. Pathway nodes are represented with different node size and font size, which is inversely proportional to the P value. If the P value is smaller, the size of node and font are bigger. And the P value indicates the significance of the genes in this pathway.

Due to the complexity of the composition and the complexity of the interactions among components in LW, we can imagine the LW prescription as a complex system. Therefore, further investigation needs to be conducted to achieve a complete understanding of the pharmacology of LW. It will be necessary to integrate system biology, bioinformatics, computational biology, and pharmacogenomics for exploring the potential interactions occurring during a holistic treatment. In addition, the LW prescription is suitable for the different symptomatology classifications and present typological groups of patients based on the discriminating theory of "*Bian Zheng Lun Zhi*" in TCM. It is worth noting that there is a need for well-conducted, randomized, double blinded, placebo-controlled, multi-center clinical trials to further evaluate efficacy of LW in a modern clinical setting.

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