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Review Article

Carthami flos: a review of its ethnopharmacology, pharmacology and clinical applications

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ABSTRACT

Carthami flos, the dried floret of *Carthamus tinctorius* L., Asteraceae (safflower), has been widely used in traditional Chinese medicine to treat a broad range of ailments, such as coronary heart disease, angina pectoris, gynecologic disease, stroke, and hypertension. However, although several studies on *Carthami flos* have been done consecutively, the results are usually scattered across various documents. This review aims to provide up-to-date information on the traditional uses, pharmacology, clinical applications, and toxicology of *Carthami flos* in China and thereby to provide a basis for further investigation of its use to treat dissimilar diseases. Various ethnomedical uses of *Carthami flos* have been documented in many ancient Chinese books. Crude extracts and isolated compounds from *Carthami flos* show a broad range of pharmacological properties, such as protective effects on brain tissue, on osteoblasts, and in myocardial ischemia, as well as anti-inflammatory, antithrombotic, antitumor, and antidiabetic activities. To date, safflower and safflor yellow injections have been used to treat coronary heart disease, chronic pulmonary heart disease, cerebrovascular diseases, orthopedic diseases, and diabetes mellitus. Regarding the toxicology of *Carthami flos*, among the side effects that have been observed are allergic reaction, spermatogenetic failure, fatty liver, and nephrotoxicity.

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Introduction

Carthami flos, the dried floret of *Carthamus tinctorius* L., Asteraceae (safflower), has been commonly used in traditional Chinese medicine to treat a wide range of ailments, such as coronary heart disease, angina pectoris, gynecologic disease, stroke, and hypertension. In China, *Carthami flos* has been used as medication for more than 2100 years, since the species was introduced by Zhang Qian in the historic "Silk Road." Traditional Chinese medicine has accumulated ponderable information on the use of *Carthami flos*, which has been documented in ancient manuscripts and summarized in recently published books, such as Chinese Pharmacopoeias, "Newly Revised Canon of Materia Medica," and "Medical Treasures of the Golden Chamber." Modern pharmacological investigations have substantiated that *Carthami flos* extracts or isolated pure components have protective effects on brain tissue, myocardial tissue, and osteoblasts, as well as antithrombotic, anti-inflammatory, and antitumor effects, which, to some degree, are tightly linked to the accounts of blood circulation promotion, blood stasis removal, and pain relief recorded in ancient Chinese documents.

Thus far, many compounds have been isolated from *Carthami flos*, including quinochalones, flavonoids, alkaloids, polyacetylene, aromatic glucosides, organic acids, *etc.* (Guo and Zhang, 1998, 2000; Zhang et al., 2004, 2005, 2006, 2009a; Asgarpanah and Kazemivash, 2013; Zhou et al., 2014). Effective compounds or active parts of *Carthami flos* have been screened for pharmacological activity *in vivo* and *in vitro*, suggesting good repercussions for human health maintenance and promotion. Some compounds have been applied to clinical treatment of coronary heart disease, chronic pulmonary heart disease, cerebrovascular diseases, orthopedic diseases, and diabetes mellitus. This paper introduces the ethnopharmacological application, pharmacological properties, modern clinical application, and side effects of *Carthami flos* in China and thereby provides support and evidence for further investigation of its use.

Ethnopharmacological use

In traditional Chinese medicine, the application of *Carthami flos* in clinical treatment can be traced back to as early as 2000 years ago. The book Medical Treasures of the Golden Chamber, written by Zhang Zhongjin, documented that, when given as a cold

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infusion, the decoction of *Carthami flos* and distillate spirit (1:10) had therapeutic effects on gynecological diseases, including induction of abortion early in pregnancy, expulsion of a retained afterbirth or stillbirth, and moderation of pain during menstrual periods. Given as a major medicament portion, *Carthami flos* has been used as a remedy for coronary heart disease, angina pectoris, hypertension, and gynecological diseases (Guo and Zhang, 1996, 1999). The leaves are used as a diuretic, an appetizer, and a cure for urinary discharge. Also, powder made from leaves of safflower combining with *Cortex Lycii Radicis* could be used to clavus after adding sesame oil to be pasty. Due to the impact of specific geographic environments, customs, and cultures, the medicinal properties and clinical applications of *Carthami flos* differ across various ethnic regions of China. In Mongolian and Tibetan medicine, *Carthami flos* is used to treat liver metabolism disorders, such as hepatomegaly, hepatic injury, xantho eyes, and heat hepatic blood (Luo, 1988). In Dai and Yi medicine, the nature of *Carthami flos* is similar to that in Chinese medicine, but its clinical applications are mainly aimed at treating infertility, galacturia, soft tissue injuries, and fractures (Lin et al., 2003; Guan, 1993). Modern medical investigations have shown the activities of *Carthami flos* in improving oxygen supply to the heart and brain, mitigating ischemic injury, and hepatoprotection, which, to some extent, provide scientific support for the traditional medicinal theory and application of *Carthami flos* (Chen et al., 2012).

Pharmacological reports

Effect on brain injury

Hydroxysafflor yellow A (HSYA) (**1**), a major active chemical ingredient of *Carthami flos*, has been widely researched in China as a treatment for cerebrovascular diseases (Li et al., 2005, 2010; Zhang et al., 2009a,b; Feng et al., 2010; Tang et al., 2010). Numerous studies have indicated a protective ability of HSYA (**1**) against brain impairment. An *in vitro* study corroborated that interference with HSYA (**1**) (0.072 mg/ml) contributed to nerve regeneration of an organotypic hippocampal slice from neonatal SD rats in normal or ischemic conditions (Qin et al., 2012a; Chart 1). HSYA (**1**) also relieved oxygen-glucose deprivation in neural stem cell injury and contributed to neurogenesis *in vitro* (Qin et al., 2012b; Chart 1). Neuron damage induced by exposure to glutamate and sodium cyanide (NaCN) in cultured fetal cortical cells was significantly inhibited by HSYA (**1**) (Zhu et al., 2003; Chart 1). In an *in vivo* study, the therapeutic effect of HSYA (**1**) on focal cerebral ischemia was investigated in a middle cerebral artery occlusion (MCAO) model. Beginning with a dose of 3 mg/kg, HSYA (**1**) suppressed thrombosis formation in MCAO rats, followed by inhibition of platelet aggregation and adjustment of PGI_2/TXA_2 (Zhu et al., 2003, 2005; Chart 1). Spinal cord ischemia–reperfusion injury in rabbits was also found to improve when treated with HSYA (10 mg/kg) (Shan et al., 2010; Chart 1). An investigation of its effect on mitochondrial permeability transition pores (mtPTP) in rat brain indicated that HSYA (**1**) (10–80 μmol/l) inhibited Ca^{2+}-induced swelling of mitochondria isolated from rat brains and generation of ROS. Taken together with the improved mitochondrial energy metabolism, the enhanced ATP levels, and the respiratory control ratio, it was extrapolated that HSYA (**1**) inhibited the opening of mtPTP by a free radical-scavenging action in the brain, which consequently may have resulted in the neuroprotective effect (Tian et al., 2008). In a study by Pan et al. (2012), HSYA (**1**) (5 mg/kg, *i.p.*) was found to attenuate brain injury induced by lymphostatic encephalopathy, which showing alleviating neurologic deficits and cell apoptosis in the rostral ventrolateral medulla (RVLM), suppressing the impaired regulatory roles of the autonomic nervous system in cardiovascular, and preventing the decrease of endothelial nitric oxide synthase (eNOS) mRNA. Additionally, pretreatment with HSYA (**1**) improved spatial memory deficits and inhibited changes in the blood–brain barrier, the SOD activity, and the malondialdehyde content in brain injury induced by $^{12}C^{6+}$ particle therapy (Gan et al., 2012). A recent work implied that HSYA (**1**) has a protective effect against cerebral I/R injury, partly by reducing apoptosis through the PI3K/Akt/GSK3b signaling pathway (Chen et al., 2013a,b; Chart 1) and decreasing nitrotyrosine formation (Sun et al., 2013). Based on these studies, it was hypothesized that the underlying mechanisms of the protective effects of HSYA on brain injury involved a reduction of lipid peroxidation, the suppression of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, the upregulation of the expression of endothelial nitric oxide synthase (eNOS) protein, and a decrease in cell apoptosis and structural damage of nervous tissues. Recent reports have suggested that HSYA (**1**) protect cortical neurons from inhibiting the phosphorylation of PPARr and the expression of NR2B-containing NMDA receptors and regulating the Bcl-2 family (Yang et al., 2010; Liu et al., 2012a,b). However, whether neuronal NOS is involved in the protective effects of HSYA (**1**) remains to be determined in a future study. An *in vitro* investigation also confirmed that HSYA showed protective effects on neurotoxicity induced by β-amyloid in PC12 cells, as evidenced by reversed changes triggered by β-amyloid, such as a decrease in cell viability, glutathione level, mitochondrial membrane potential, and the ratio of Bcl-2/Bax protein expression, along with an increase in lactate dehydrogenase, DNA fragmentation, and the levels of malondialdehyde and intracellular reactive oxygen species. This finding suggested that HSYA (**1**) was a promising candidate drug for prevention and treatment of Alzheimer's disease (Kong et al., 2013a,b).

Investigations on the protective effects of extracts or other compounds from *Carthami flos* on the nervous system have also been done. In an *in vitro* study, Zhao et al. (2009a) found that all solvents extracted from *Carthami flos*, which contain extracts of chloroform, ethyl acetate, and *n*-butyl alcohol (1, 10, and 100 μg/ml, respectively), markedly enhanced both dopamine uptake by Chinese hamster ovary (CHO) cells stably expressing the dopamine transporter (DAT) and norepinephrine uptake by CHO cells expressing the norepinephrine transporter (NET), and simultaneously depressed serotonin uptake by CHO cells expressing the serotonin transporter (SERT), indicating that extracts from *Carthami flos* would improve neuropsychologic disorders through regulating the monoamine-transporter activity. Further, an *in vitro* investigation showed that N^1,N^5-(*Z*)-N^{10}-(*E*)-tri-*p*-coumaroylspermidine (**2**) potently and selectively inhibited serotonin uptake in S6 cells or in synaptosomes with a reversible competitive property for 5HT uptake inhibition, mirroring its effect of improving neuropsychologic disorders through regulating serotoninergic transmission (Zhao et al., 2009b; Chart 1). Carthamin (10 mg/kg) (**3**) significantly decreased the formation of malondialdehyde in mouse cerebrum and of thiobarbituric acid reactive substances and 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the cerebral cortex of rats subjected to an injection of $FeCl_3$ solution into the sensory motor cortex inhibited glutamate-induced (Hiramatsu et al., 2009; Chart 1). Nicotiflorin (kaempferol-3-*O*-rutinoside) (**4**), a natural flavonoid extracted from *Carthami flos*, showed neuroprotection in focal cerebral ischemia *in vitro* and *in vivo*, which might be attributed to the upregulation of endothelial nitric oxide synthase (eNOS) activity (Li et al., 2006a,b; Chart 1). A study by Huang et al. (2007) showed that kaempferol-3-*O*-rutinoside (**4**) (30, 60, and 120 mg/kg) significantly attenuated the increase of lactic acid and malondialdehyde (MDA) contents and the decrease in LDH, Na^+K^+ATPase, $Ca^{2+}Mg^{2+}$ATPase, and superoxide dismutase (SOD) activity in multi-infarct dementia model rats, indicating its protective effects on reducing the memory dysfunction,

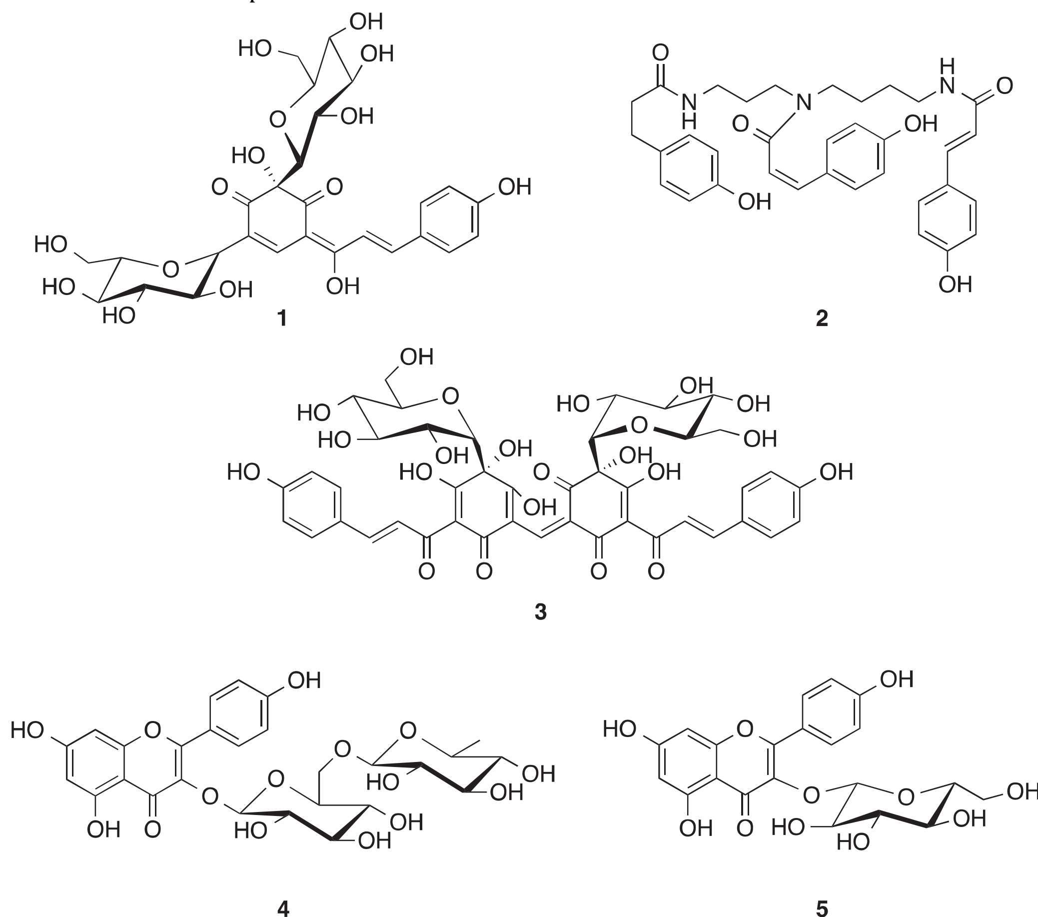
Chart 1
Pharmacological activities of compounds/extract from *Carthami flos*.

Biological activities	Extract/compound (effective dose)	Test model	Remarks	Reference
Protection on brain injury	HSYA (**1**) (0.036 and 0.072 mg/ml)	Organotypic hippocampal slices from neonatal SD rats (*in vitro*)	Increase organotypic hippocampal cells from neonatal SD rats (DAPI+, BrdU+ and Nestin+) with arranged in cords and migrated to the codex as compared to control (normal saline)	Qin et al. (2012a)
	HSYA (**1**) (0.036 and 0.072 mg/ml)	Oxygen-glucose deprivation in hippocampal slice cultures from rats (*in vitro*)	Reduce the proliferation of neural stem cell compared to normal saline	Qin et al. (2012b)
	HSYA (**1**) (10–80 μmol/l)	Ca^{2+}-induced and H_2O_2-induced swelling of mitochondria isolated from rat brains (*in vitro*)	Inhibited swelling and reactive oxygen species of mitochondria, improve mitochondrial energy metabolism and enhanced ATP levels and the respiratory control ratio compared to 50 μmol/l Ca^{2+}-treated group	Tian et al. (2008)
	HSYA (**1**) (3.0 and 6.0 mg/kg, sublingular vein injection)	Male Wistar–Kyoto rats with middle cerebral artery occlusion (MCAO) (*in vivo*)	Decrease neurological deficit scores and reducing infarct area compared to the saline group and dosage of 6.0 mg/kg show a similar potency as nimodipine (0.2 mg/kg)	Zhu et al. (2003)
	HSYA (**1**) (3.0 and 6.0 mg/kg, sublingular vein injection)	MCAO rats (*in vivo*)	Produce a dose-dependent reduction in infarct area (60% and 85% respectively), increase the ratio of 6-Keto-PGF1a and TXB2, reduce thrombotic weight	Zhu et al. (2005)
	HSYA (**1**) (10.0 mg/kg, ear intravenous injection)	Rabbits with ischemia/reperfusion (I/R) injury (*in vivo*)	Attenuate I/R induced necrosis in spinal cords, alleviate oxidative stress as indicated by decreased malondialdehyde (MDA) level and increased superoxide dismutase (SOD) activity and protected neurons from I/R-induced apoptosis in rabbits	Shan et al. (2010)
	HSYA (**1**) (5 mg/kg, i.p.)	Rats with lymphostatic encephalopathy-induced brain injury (*in vivo*)	Alleviate the neurological deficits, attenuate cell apoptosis in the rostral ventrolateral medullus compared to saline group	Pan et al. (2012)
	HSYA (**1**) (5,10 and 20 mg/kg, intraperitoneal injection)	Mouse with brain injury induced by $^{12}C^{6+}$ particle therapy (*in vivo*)	Dose-dependently improve the spatiomemory deficits and increase SOD activity and reduce malondialdehyde content in brain tissue	Gan et al. (2012)
	HSYA (**1**) (4 and 8 mg/kg, tail-vein injection)	MCAO rats (*in vivo*)	Diminish the number of apoptotic cells and increase the Bcl-2/Bax ratio as well as the phosphorylations of Akt and GSK3b	Chen et al. (2013a,b)
	N^1,N^5-(*Z*)-N^{10}-(*E*)-tri-*p*-coumaroylspermidine (**2**) (0.04–100 μM)	Chinese hamster ovary cells (*in vitro*)	Inhibit serotonin uptake in S6 cells (IC_{50} = 0.74 ± 0.15 μM) and in synaptosomes (IC_{50} = 1.07 ± 0.23 μM)	Zhao et al. (2009b)
	Carthamin (**3**) (10 mg/kg)	Rats with epileptic foci induced by iron (*in vivo*)	Inhibit 8-hydroxy-2′-deoxyguanosine in the cerebral cortex of rats	Hiramatsu et al. (2009)
	Kempferol-3-O-rutinoside (**4**) (2.5, 5 and 10 mg/kg, tail vein)	Rats with permanent focal cerebral ischemia (*in vivo*)	Dose-dependently reduce brain infarct volume and neurological deficits compared with nimodipin (positive control)	Li et al. (2006a)
	Kempferol-3-O-rutinoside (**4**) (25–100 μg/ml)	Cultured neurons suffered hypoxia (*in vitro*)	Attenuate cell death and reduce lactate dehydrogenase release	Li et al. (2006a)
Anti-myocardial ischemia effect	Ethanolic extract (62.5 and 125 μg/ml)	H9c2 cardiomyoblast cells (*in vitro*)	Reduce IkB degradation and NFkB activation, activate of anti-apoptotic proteins, Bcl-2 and Bcl-xL, stabilization the mitochondria membrane and the down-regulation of extrinsic and intrinsic pro-apoptotic proteins, such as TNFα, active caspases-8,9 and 3 t-Bid, Bax, compared to LPS	Tien et al. (2010)
	HSYA (**1**) (4 and 8 mg/kg, tail vein)	Rats with coronary artery ligation (*in vivo*)	Reduction of myocardial infarction size, superoxide dismutase activity, endothelial nitric oxide synthase activity and nitric oxide content, and inhibit elevation of creatine kinase activity and malondialdehyde content	Wang et al. (2009a,b)
	HSYA (**1**) (0.1–3 mg/kg, intravenous injection)	Rats with pentobarbitone-anesthetized normotensive and spontaneously hypertensive (*in vivo*)	Dose-dependently reduce heart rate, mean arterial pressure, left ventricular systolic pressure, left ventricular end-diastolic pressure	Nie et al. (2012)
	N-(p-coumaroyl) serotonin (**6**) (5×10^{-7} M)	Model of perfused guinea-pig Langendorff hearts subjected to ischemia and reperfusion (*in vitro*)	Increase the NO level at the end of ischemia and show 63.2% recovery rate of left ventricular developed pressure compared to drug-free control (30.8%)	Hotta et al. (2002)
	N-feruroylserotonin (5×10^{-7} M) (7)	Similar to N-(p-coumaroyl) serotonin	Show 61.0% recovery rate of left ventricular developed pressure and quench the activity of active radicals	Hotta et al. (2002)

Chart 1 (*Continued*)

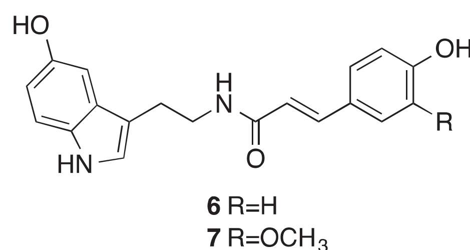
Biological activities	Extract/compound (effective dose)	Test model	Remarks	Reference
Antithrombotic effect	Aqueous extract (1 and 0.7 g/kg, oral)	(a) Rats arterial thrombosis model (*in vivo*) (b) Rats venous (Wessler) thrombosis model (*in vivo*) (c) Mouses with collagen/epinephrine-induced pulmonary embolism (*in vivo*) (d) Mouses tail bleeding (*in vivo*)	(a) Show a mild thrombosis inhibition but without difference with control when plus clopidogrel or not (b) Inhibit thrombus formation from 16.1 to 7.9 mg and led to a significant decrease in venous thrombus weight when added clopidogrel and also augment thrombin time and prothrombin time (c) Increase the number of non-paralyzed animals to 33.3% and surviving rates to 53.3% (d) Prolonged the bleeding time when added clopidogrel	Li and Wang (2010)
	Carthamins yellow (100 and 200 mg/kg, oral)	Rats with blood stasis (*in vivo*)	Decrease the whole blood viscosity, plasma viscosity, erythrocyte aggregation index, hematocrit and platelet aggregation when compared with aspirin	Li et al. (2009)
	HSYA (**1**) (1×10^{-4} M)	Human EC line (EAhy926) (*in vitro*)	Attenuate cell cycle arrest and inhibit cell apoptosis in a concentration-dependent manner and increase the bcl-2/bax ratio, VEGF protein concentration, VEGF mRNA expression, HIF-1a protein accumulation and its transcriptional activity	Ji et al. (2008)
	HSYA (**1**) (1×10^{-6}, 1×10^{-5} and 1×10^{-4} M)	Human umbilical vein endothelial cells (HUVEC) (*in vitro*)	Inhibit cell apoptosis and cell cycle G1 arrest induced by hypoxia Increase the Bcl-2/Bax ratio of protein and NO content of cell supernatant Reduce p53 protein expression in cell nucleus	Ji et al. (2009)
Anti-inflammatory	Safflor yellow (25 and 50 mg/kg, intraperitoneal)	Rats of pulmonary fibrosis induced by bleomycin (*in vivo*)	(a) Alleviate the loss in bodyweight, the increase of hydroxyproline content in the lung tissues and pathologic changes of pulmonary fibrosis (b) Prevent the increase of a α-SMA positive cells and TGF-b b1 expression	Wang et al. (2011a,b)
	Safflor yellow (0.05, 0.25 and 1.25 mg/ml)	Human embryo lung fibroblast (*in vitro*)	Dose-dependently inhibit the elevation of α-SMA expression and the morphological change	Wang et al. (2011a,b)
	Methanol extract (10, 50 and 100 μg/ml)	RAW 264.7 macrophages (*in vitro*)	The increase HO-1 protein expression, the reduced production of NO, PGE_2, iNOS and COX-2, and the inhibition of (TNF)-α-mediated VCAM-1 expression and NF-κB luciferase activity	Jun et al. (2011)
	HSYA (**1**) (1, 4 and 16 μmol/l)	Human alveolar epithelial A549 cells (*in vitro*)	(a) Inhibit the expression of TLR-4, Myd88 ICAM-1, TNFα, IL-1β and IL-6 (b) Inhibit the phosphorylation of p38 MAPK, the adhesion of leukocytes to A549 cells and decrease NF-κB p65 nuclear translocation	Song et al. (2013)
	HSYA (**1**) (5×10^{-6}, 10×10^{-6} and 20×10^{-6} mol/l)	Umbilical vein endothelial Eahy 926 cells (*in vitro*)	Inhibit the increased expression of TLR-4, IL-6, IL-1β and TNFα	Zhu et al. (2012)
	HSYA (6, 15 and 37.5 mg/kg, i.v.)	Mice with LPS-induced pulmonary inflammatory injury (*in vivo*)	(a) Ameliorate pulmonary edema and inflammatory cell infiltration (b) Suppress p38 MAPK, NF-κB p65 activation and alter inflammatory cytokine expression (TNF-α, IL-1β, IL-6 and IL-10)	Sun et al. (2010)
	HSYA (**1**) (26.7, 40 and 60 mg/kg/day, intraperitoneal injection)	Mice with bleomycin-induced pulmonary injury (*in vivo*)	Attenuate the loss in body weight, the increase of myeloperoxidase activity and pathologic changes of pulmonary inflammation (c) Attenuate the increased expression of TNF-α, IL-1β and TGF-β1, the increased activation of NF-κB and phosphorylation of p38 MAPK	Wu et al. (2012a,b)
Hepatoprotective effect	HSYA (**1**) (5 mg/kg/day for 12 weeks, oral)	Rats with CCl_4-induced hepatic fibrosis (*in vivo*)	The decrease in fibrosis and expression of α-SMA protein, MEF-2C gene, Tβ-RI, Tβ-RII, MEKK3, MEK5, and phosphorylation of ERk5	Zhang et al. (2011, 2012a,b,c)
	HSYA (**1**) (10 mg/kg, intraperitoneal injection)	Rats with hepatic fibrosis induced by oxidative stress (*in vivo*)	(a) Increase the activities of antioxidant enzymes and reduce α-SMA level. (b) Up-regulating the expression of PPARr and MMP-2, and down regulating the expression of TGF-β1 and TIMP-1	Wan et al. (2013)
	Carthamus red (5, 10 and 20 mg/kg, oral)	Rats with CCl_4-induced hepatic fibrosis (*in vivo*)	(a) Lower the serum levels of ALT, AST, ALP and total protein in liver damage rat models (b) Up-regulate Nrf2, GSTα and NQO1 expressions were at the protein level (c) Elevate the activities of antioxidant enzymes and level of GSH and lessen the content of TBARS compared to silymarin	Wu et al. (2013)

energy metabolism failure, and oxidative stress that are involved in multi-infarct dementia. Additionally, postischemic treatment with kaempferol-3-*O*-glucoside (7.5 mg/kg) (**5**) showed a neuroprotective effect in focal cerebral ischemia–reperfusion rats. The mechanism of these kaempferol flavonoid effects was attributed to anti-neuroinflammatory activity by inhibiting the activation of STAT3 and NF-kB p65, including independent and dependent pathways of IkB degradation and the subsequent expression of pro-inflammatory mediators (Yu et al., 2013). Nevertheless, more pharmacological evaluations in animal models relating to neurological disorders need to be considered in future studies to explain the underlying mechanisms of these compounds.



Effects on myocardial ischemia

Ethanolic extract of *Carthami flos* (62.5 μg/ml) has the ability to suppress JNK activity and inhibit LPS-induced TNFα activation and apoptosis in H9c2 cardiomyoblast cells (Tien et al., 2010; Chart 1). In an *in vivo* study, the protective effects of a purified extract from *Carthami flos* (100, 200, 400, and 600 mg/kg body wt.) on myocardial ischemia was assessed in a model of myocardial ischemia injury induced by left anterior descending coronary artery (LAD) occlusion, which resulted in reduced infarct size and improved cardiac function (Han et al., 2009). Further investigations have reported that this cardioprotective effect of *Carthami flos* extract (200 mg/kg) was not only supported by decreased levels of creatine kinase (CK) and LDH but, further, could be strengthened by adding *Panax notoginseng* (Burk) F.H. Chen (EPN) extract (50 mg/kg) (Han et al., 2013a,b). HSYA (**1**) (4 or 8 mg/kg) also showed a cardioprotective effect, as evidenced by the reduced myocardial infarction size in rats with acute myocardial ischemia induced by LAD ligation (Wang et al., 2009a,b). Whether the protective effect of HSYA (**1**) on myocardial ischemia injury could be enhanced by combination with EPN remains inconclusive (Fu et al., 2011). Contrarily, extracellular Ca^{2+} influx through receptor-operated Ca^{2+}channels and potential-dependent Ca^{2+}channels could be blocked by crude drug of *Carthami flos* (Liu et al., 2005). A further finding showed that HSYA (**1**) markedly reduced Ca^{2+} influx on cardiac cells, as well as decreased the contractile force and heart rate in rats. Such an effect may implicate the activation of BK_{Ca} and K_{ATP} channels (Nie et al., 2012; Chart 1). Whether HSYA (**1**) could lower the peripheral resistance remains under study. Other compounds, such as *N*-(*p*-coumaroyl) serotonin (**6**) and *N*-feruroylserotonin (**7**), showed cardioprotective effects on isolated guinea pig Langendorff hearts subjected to normothermic global ischemia and subsequent reperfusion, speculated that this was in close association with the synthesis of high phosphorous energy, ATP, which was constituted an important part of the regulatory mechanisms involved in myocardial ischemic injury (Hotta et al., 2002). As evidenced by these findings, the mechanisms responsible for the cardioprotective effects may be partially achieved by scavenging of ROS, mediating the PI3K signaling pathway, and regulating superoxide dismutase activity and endothelial nitric oxide synthase activity.



Antithrombotic effect

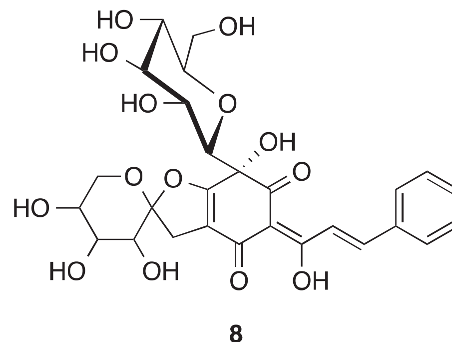
The aqueous extracts of *Carthami flos* have been shown to be more efficient than clopidogrel against venous thrombosis and pulmonary embolism, with similar functions to carthamus yellow (Li and Wang, 2010; Li et al., 2009; Chart 1). An HSYA (**1**) activity of enhancing the survival of vascular endothelial cells under hypoxia has also been found, which may be correlated with its effect on upregulation of the HIF-1a-VEGF pathway and regulation of Bcl-2/Bax (Ji et al., 2008; Chart 1). A further investigation showed that HSYA (**1**) could protect human umbilical vein endothelial cells (HUVECs) from hypoxia-induced injury by inhibiting cell apoptosis and cell cycle arrest, partly indicating the molecular mechanism of HSYA (**1**) in the treatment of ischemic heart disease (Ji et al., 2009; Chart 1). Contrarily, carthamin (**3**) (10 mg/l) could repair the blocked HUVEC migration and refinement of the f-actin structure caused by modeled microgravity (MMG), which would provide a new alternative for intervention in cardiovascular dysfunction apart from HSYA (**1**).

Anti-inflammatory effect

In a study by Wang et al. (2010), *Carthami flos* aqueous extract and carthamus yellow (CY) were examined regarding their effects on LPS-induced inflammatory response in a murine macrophage cell line RAW264.7 model. According to the results, aqueous extract from *Carthami flos* (1–1000 μg/ml) and CY (1–2000 μg/ml) suppressed the production of NO, PGE2, and IL-1β and decreased the iNOS and cyclooxygenase-2 (COX-2) protein expression levels in LPS-induced RAW264.7 macrophages. Based on the inhibition of cytosol IκB-α protein degradation and phospho-NF-κB protein expression, it was theorized that aqueous extract from *Carthami flos* and CY may inhibit LPS-stimulated expressions of the iNOS and COX-2 genes through the inactivation of NF-κB. As an effective part of the aqueous extract of *Carthami flos*, safflor yellow (SY) has shown an inhibitory effect on pulmonary fibrosis *in vivo* and *in vitro*, supported by suppressing the expression of a-smooth muscle actin (a-SMA) (Wang et al., 2011a,b; Chart 1). Regarding the anti-inflammatory action of methanol extracts of safflower (MEC), it has been reported that MEC triggered heme oxygenase-1 expression through Nrf2 (NF-E2-related factor) translocation and NF-kB activity inhibition. This potential molecular mechanism has provided other clues on the molecular mechanism underlying the anti-inflammatory action of aqueous extract from *Carthami flos* and CY (Jun et al., 2011; Chart 1). Contrarily, polysaccharides from *Carthami flos* were found to have immunomodulating activities that effectively activated the NF-kB signaling pathway through TLR4 and induced the production of various cytokines (IL-1, IL-6, IL-12, and IFN-γ) by peritoneal macrophages (Ando et al., 2002).

Recent investigations on HSYA (**1**) have focused on the treatment of acute lung injury (ALI). The effects of HSYA (**1**) on ALI have been evaluated *in vitro* (human alveolar epithelial A549 cells and umbilical vein endothelial cells (Eahy 926 cells)) and *in vivo* (LPS-induced and BLM-induced ALI mice), which all manifested that HSYA (**1**) ameliorated acute lung injury by suppressing both p38 MAPK (mitogen-activated protein kinase) phosphorylation and NF-κB activation, subsequently leading to a dramatic reduction in inflammatory cell infiltration and pro-inflammatory cytokine expression in lung tissue, as well as pulmonary edema and respiratory dysfunction (Sun et al., 2010; Song et al., 2013; Zhu et al., 2012; Wu et al., 2012a,b; Chart 1). Due to the high water solubility of HSYA (**1**), these findings imply that HSYA (**1**) may target the cell membrane and then interfere with the interplay of receptors and their specific ligands (such as microbial ligands, pro-inflammatory cytokines, growth factors, *etc.*) to regulate downstream signal transduction pathways so as to exert its effects. However, the concrete mechanism by which HSYA alters intracellular signaling still warrants further studies.

Other compounds have anti-inflammatory effects as well. Compared with ginkgolide B (IC_{50} 5.45 × 10^{-6} mol/l), saffloquinoside A (**8**) (10^{-5} mol/l) exhibited 54.3% inhibitory rate on the release of β-glucuronidase from rat polymorphonuclear neutrophils (PMN), which was induced by the platelet-activating factor (PAF), suggesting its anti-inflammatory activity (Jiang et al., 2010).



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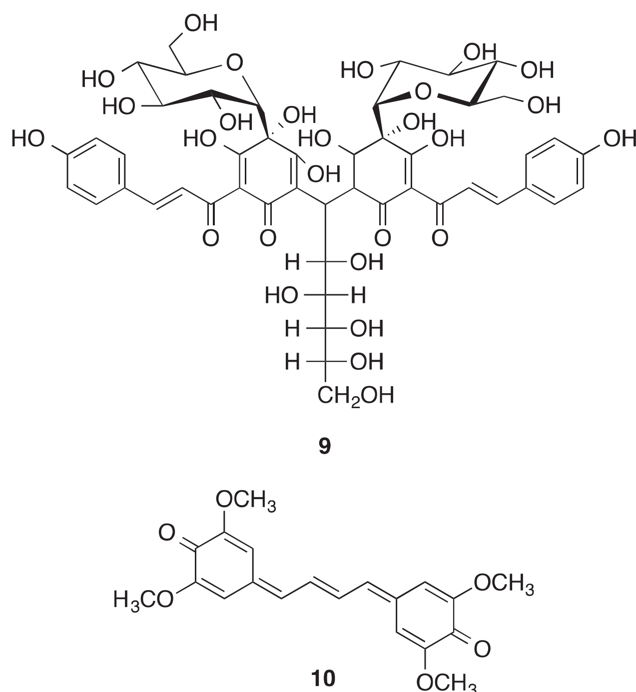
Antitumor effect

Carthami flos has been applied in cancer adjuvant therapy in traditional medicine. Modern pharmacological experiments have confirmed the antitumor activity of *Carthami flos in vitro* and *in vivo*. Herbal extract of *Carthami flos* (40 mg/ml) has antiproliferative and proapoptotic effects on hepatic stellate cells, which may act by regulating the gene expression of Fas and Bcl2 pathways (Chor et al., 2005). Further *in vitro* experiments have shown that safflor yellow B (**9**) (1, 10, and 100 nmol/l) protected pheochromocytoma (PC12) cells from H_2O_2-induced injury and apoptosis through antioxidant and antiapoptotic mechanisms that are linked to suppressing caspase-3 activity and Bax expression and increasing Bcl-2 synthesis (Wang et al., 2009a,b). Another *in vitro* study by Zhang et al. (2012a,b,c) reported that polysaccharide S of *Carthami flos* (0, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, and 1.28 g/l) could restrain the proliferation of SMMC-7721 and enhance the apoptosis of SMMC-7721 in a dose- and time-dependent manner, as shown by the increased Bax expression and the decreased Bcl-2 expression and mitochondrial membrane potential. However, HSYA (**1**) (0.028 g/l) inhibited the growth of a transplanted BGC-823 tumor through inhibition of tumor vascularization (Xi et al., 2012). Carthamin (**3**) dose-dependently (10^{-5}, 10^{-4}, and 10^{-3} mol/l) induced the K562 leukemic cells to the haemoglobin end cells, the mechanism behind which remains unknown (Wu et al., 2012a,b). *In vivo*, Chang et al. (2011) showed *in vivo* the antitumor activities of *Carthami flos*-treated dendritic cell vaccine in JC (mouse mammary adenocarcinoma) tumor-bearing mice, relevant to the polarization toward Th1 cytokines and the increase in cytotoxic T lymphocytes. This finding further supports the report regarding the effectiveness of *Carthami flos* in breast cancer (Loo et al., 2004). As determined from these studies, the mechanism responsible for the antitumor activity of *Carthami flos* may occur partly by suppressing caspase-3 activity and Bax expression and by increasing Bcl-2 synthesis, as well as by inhibiting tumor vascularization. Inhibition of human tyrosinase activity increased with increasing concentrations of kinobeon A (**10**) with the use of L-tyrosine or L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate (Kanehira et al., 2003a,b). Recent work has uncovered that carthamus yellow reduced the activity of mushroom tyrosinase in a dose-dependent manner (IC_{50} 1.01 ± 0.03 mg/ml) and showed a mode of competitive inhibition with a Ki of 0.607 mg/ml. Moreover, carthamus yellow clearly decreased the melanin

production of B16F10 melanoma cells at concentrations of 4.0 mg/ml, indicating no cytotoxicity (Chen et al., 2013a,b). As derived from these studies, *Carthami flos* has the potential to become a useful skin-whitening agent or a potent natural tyrosinase inhibitor in the future.



Effect on osteoblasts

In a study by Choi et al. (2010), extract of *Carthami flos* (2–10 μg/ml) was shown to inhibit osteoclastogenesis by modulating the receptor activator of nuclear factor-kB ligand (RANKL) signaling in MC3T3-E1 cells. Recently, evaluations of the effects of other compounds on bone have been carried out. Liu et al. (2011a,b) found that SY (1.6 mg/ml) promoted the repair of injured tendon in Leghorn chicken, manifested by the enhanced expression of bFGF and collagen type I protein. Also, HSYA (**1**) downregulated the expression of TLR4 mRNA callus osteoblasts (Lu and Tu, 2012). These results may have therapeutic implications in treating osteoporosis and other bone erosive diseases, such as rheumatoid arthritis or metastasis associated with bone loss.

Other effects

HSYA (**1**) has been found to alleviate carbon tetrachloride (CCl_4)-induced liver fibrosis in rats, in part through inhibition of hepatic stellate cell (HSC) activation and MAP kinase extracellular regulated kinase 5 (Erk5) signaling (Zhang et al., 2011, 2012a,b,c; Chart 1), suggesting that HSYA (**1**) can target fibrogenic pathways and therefore may be a potential therapy for hepatic fibrosis. A further work has reflected that the protective effect of HSYA (**1**) on hepatic fibrosis induced by oxidative stress requires the activation of PPARr (Wan et al., 2013; Chart 1). Regarding other compounds, carthamus red has been reported to have a hepatoprotective effect in rats with CCl_4-induced liver damage, which might be mediated by induction of antioxidant defense through increased activation of the Nrf2 pathway (Wu et al., 2013; Chart 1).

Compared with glibenclamide, hydroalcoholic extract of *Carthami flos* (200 mg/kg) has an antidiabetic activity in alloxan-induced diabetic rats, as shown by their decreased fasting blood sugar, triacylglyceride, cholesterol, LDL-C, and VLDL-C levels, as well increased insulin levels (Asgary et al., 2012). Diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defenses (Cuerda et al., 2011; Kuroki et al., 2003; Maritim et al., 2003). Previous studies have disclosed *Carthami flos* constituted a good source of antioxidant compounds with free radical-scavenging potential (Choi et al., 2010; Zhao et al., 2012; Hiramatsu et al., 2009; Kanehira et al., 2003a,b; Kambayashi et al., 2005), suggesting that it could be useful in the prevention of diseases in which free radicals are implicated. Considering their effects on these lipid components and their antioxidant activity, *Carthami flos* and its active compounds can be assumed to be potential hypolipidemic agents that could yield considerable advantages for both the diabetic condition and the associated atherosclerosis or hyperlipidemic conditions. Recently, an *in vitro* study further corroborated that HSYA (**1**) (10–100 μmol/l) ameliorated methylglyoxal-induced injury in cultured human brain microvascular endothelial cells, as shown by the decreased expression of caspase-3 and the accumulation of advanced glycation end products. These results are reminiscent of the potential of HSYA as a novel strategy for protecting against vascular complications associated with diabetes (Li et al., 2013).

Liu et al. (2012a,b) reported that administration of *Carthami flos* to ethylene glycol (EG)-fed rats led to a significant reduction in CaO_x crystal formation, indicating its antilithic effect. Meanwhile, safflower yellow (50 mg/l) can protect human renal tubular epithelial cell lines (HK-2 cells) from damage by inhibiting apoptosis induced by aristolochic acid, which may be affected by suppressing the activation of caspase-3. These findings suggest that safflower yellow may be beneficial to the treatment of aristolochic acid nephropathy.

An *in vivo* study by Lu et al. (2008) showed that menoprogen, a herbal formula consisting of *Lycii fructus*, *Rehmanniae radix*, *Mori fructus*, and *Carthami flos*, significantly increased the levels of serum estradiol and progesterone but reduced the levels of follicle-stimulating and luteinizing hormones in rats.

The 5a-reductase inhibitory and hair growth-promoting activities of *Carthami flos* ethanolic extract were tested in C57BL/6 mice, which resulted in a finasteride equivalent 5a-reductase inhibitory activity (FEA) value of 24.30 ± 1.64 mg per 1 g crude extract (Kumara et al., 2012). This finding may lead to new alternative medicines for hair loss prevention and treatment.

An *in vitro* study of the anti-gammaherpesvirus activity of *n*-hexane and EtOH fractions of *Carthami flos* extracts (iSLK-BAC16 and iSLK-puro cells) by Lee et al. (2013) indicated that *n*-hexane and EtOH fractions of *Carthami flos* extracts critically influenced two stages of the Kaposi's sarcoma herpesvirus (KHSV) life cycle by abnormally inducing KSHV lytic reactivation and severely preventing KSHV virion release from the viral host cells. Simultaneously, the mechanism of dysregulation of KSHV replication by *Carthami flos* extracts may be mediated by dysregulating the cell cycle and producing strong cytotoxicity. Based on this finding, studies to gather more *in vitro* and *in vivo* evidence on the anti-gamma herpesvirus activities of *Carthami flos* extracts and the causes of cellular cytotoxicity are needed. *In vivo*, *Carthami flos* extract (200 and 400 mg/kg) showed similar reductions in the volume, free acidity, and total acidity of gastric secretion induced by carbachol, such as cimetidine and verapamil, indicating its antiulcerogenic effect (Mandade et al., 2012). As reported by Liu et al. (2005), *Carthami flos* has a natural calcium channel blocking activity, which may have contributed to its antiulcerogenic effect. Further studies to evaluate the exact mechanism of this effect are suggested. Recently, the photoprotective activity of topical HSYA (**1**) (100 and 200 μg per mouse) was investigated in a UV-induced photoaging mice model. The results showed clear recovery of UV-induced skin damage,

which could possibly be attributed to the antioxidative property of HSYA (**1**) and activated by promoting endogenous collagen synthesis (Kong et al., 2013a,b).

Clinical applications

Treatment of coronary heart disease

In a study by Huang (2013; Chart 2), patients with coronary heart disease (CHD) treated with safflower injection showed a decrease in hemodynamic parameters, containing the whole blood, high shear viscosity, shear whole blood viscosity, whole blood viscosity and plasma viscosity, which were lower than those of the control group treated with *Salvia miltiorrhiza* injection, suggesting the clinical efficacy of safflower injection in coronary heart disease. In another study, safflower injection was found to improve the clinical symptoms of angina pectoris and the electrocardiogram of CHD patients compared with groups treated only with conventional western medicine (Su and Chai, 2011; Chart 2). The major active ingredient of *Carthami flos*, safflor yellow, has also been used to treat coronary heart disease. A study by Liu et al. (2011a,b) found that safflor yellow could reduce the endothelin, matrix metalloproteinase-9, and high-sensitivity C-reactive protein of different patients with coronary artery disease (Chart 2). Other studies have shown that safflor yellow could decrease neuropeptide Y, which was important to improve the pathogenetic condition of patients (Liu et al., 2008; Chart 2).

Treatment of chronic pulmonary heart disease

The use of safflower injection to treat heart failure in patients with chronic pulmonary heart disease (CPHD) resulted in improved heart function, as proved by the ameliorative blood gas parameters compared with the control group ($p < 0.05$) (Liao, 2012; Chart 2). contrarily, recent studies on SY and SY injection have indicated their curative function in pulmonary heart disease. An investigation by Chen et al. (2014) showed the efficacy of SY in improving ST segment depression ($p < 0.01$) and decreasing the levels of TnI, CK-MB, and CRP ($p < 0.05$), thereby protecting myocardial injury from CPHD (Chart 2). Meanwhile, the pulmonary function, as well as blood viscosity and rheology, of patients with CPHD was found to be ameliorated after treatment with SY injection (Ren and Mei, 2014; Chart 2).

Treatment of cerebrovascular diseases

Extracts and compounds from safflower have been applied with beneficial effects in the clinical treatment of cerebrovascular diseases. Systemic evaluations of the clinical effect and safety of safflower injection in the treatment of acute ischemic stroke have indicated that such injection is helpful in improving neurologic functional deficits and has a good safety profile, as shown by the meta-analysis results on total effective rates, the relative risk 99% credible region (99% CI), the number of treatments needed (99% CI), and the weight mean deviation (99% CI), which are 1.19 (1.10, 1.28), 7.14 (5.00, 12.5), and −0.62 (−1.10, −0.15), respectively (Ma et al., 2012). The latent mechanism was partially involved in decreasing the serum ratio of IL-6/IL-10 and suppressing the lipid peroxidation by increasing the level of superoxide dismutase, choramphenicol acetyltransferase, and malondialdehyde, which were supported by clinical evidence from Luo et al. (2014; Chart 2). Also, injections of safflower yellow and HSYA have been reported to be more effective in clinical treatment of acute ischemic stroke compared with Ginkgo leaf extract and dipyridamole injection (Xiao and Hu, 2011; Xing et al., 2008; Chart 2).

Treatment of orthopedic diseases

Recently, clinical observations of safflower injection in orthopedic diseases have been gradually carried out, with good echo. Safflower injection for patients with acute gouty arthritis showed equal efficacy to treatment with colchicine, suggesting that safflower injection may be a new alternative treatment for acute gouty arthritis patients due to its low toxicity (Li et al., 2011; Chart 2). A clinical report by Sui et al. (2011) pointed to the application of safflower injection in isolated limb replantation, which resulted in 95% survival of replanted fingers with no adverse effect, exceeding the 86% survival rate in the control group. An observation of its prevention of postoperative tendon adhesion in flexor tendon injury has also been conducted, which resulted in 60 patients being healed in stage I and showing an advantage over the dickon biological film group in terms of total active motion of the tendon after four weeks. No obvious discrepancy in power to grasp was observed, which was reminiscent of the apotropaic effect of safflower injection on postoperative tendon adhesion in flexor tendon injury (Choi et al., 2013).

Treatment of diabetes mellitus and its complications

In a clinical report by Wei (2011; Chart 2), safflower injection was extrapolated to effectively postpone the development of renal failure caused by diabetic nephropathy on the basis of routine treatment. Similarly, the application of safflower yellow injection in diabetic nephropathy obtained beneficial results in palliating urine protein and lowering the serum creatinine level of patients (Yang et al., 2011; Chart 2; Qiu et al., 2013). Other complications of diabetes mellitus that could be treated with safflower yellow injection have been reported. Diabetic peripheral neuropathy, a frequent chronic complication of diabetes mellitus, could be ameliorated by safflower yellow injection, which may be implicated in amelioration of microcirculation in patients (Yang and Dai, 2010; Chart 2). In a recent clinical observation, safflower yellow injection was found to decrease the incidence of delayed graft function and thereby improve recovery of graft function after renal transplantation (Pang et al., 2014; Chart 2).

Side effects

An *in vivo* study of the acute toxicity of carthamus red showed no toxicity and mortality for doses of up to 2000 mg/kg (Wu et al., 2013). However, some side effects have been reported in animal and human models. After being given orally (by gavage method) to mice at a dose of 200 mg/kg for 35 consecutive days, *Carthami flos* extract engendered the formation of multinucleated giant cells in the germinal epithelium and resulted in a marked decrease in epithelial vacuolization, germ sloughing and detachment, seminiferous tubule diameter, seminiferous epithelium height, and maturation arrest, suggesting its ability to change the testis histologic structure and cause spermatogenetic failure (Mirhoseini et al., 2012; Bahmanpour et al., 2012). Therefore, precautions should be taken when using *Carthami flos* extract on men who are infertile or have reproductive disorders. Tests of active systemic anaphylaxis and passive cutaneous anaphylaxis by *Carthami flos* injection have shown positive reactions in guinea pigs and SD rats, respectively, indicating its sensitization to allergic reaction (Zhang et al., 2012a,b,c). An essential requirement for future studies is the exploration of the recessive sensibilizing substance in *Carthami flos* injection. With the increasing clinical application of *Carthami flos*, side effects have been gradually reported, such as inducing angle-closure glaucoma, throat inflammation and rhinorrhagia (Deng, 2012). Additionally, daily intraperitoneal injection of HSYA at a

Chart 2
Summary of clinical trials.

Author (Date)	Design duration	Condition	No. of participants randomized (age)	Intervention	Comparison	Main results between groups	Adverse events	Authors conclusion
Huang (2013)	40 days	Coronary heart disease (CHD)	122 (57.33 ± 15.02)	Injected with 20 ml safflower injection with 0.5% 250 ml glucose injection once a day	Salvia injection	The hemodynamic parameters, were lower than Salvia injection ($p < 0.05$)	None reported	Safflower injection has efficacy in CHD and could improve heart function
Su and Chai (2011)	2 weeks	CHD	100; 50 per group (42–75 years)	Usual western medicine treatment and Intravenous with 40 ml safflower injection with 0.5% 250 ml glucose injection	Usual western medicine treatment	The clinical symptoms and electrocardiogram results in treated group were better than the group only treated with western medicine ($p < 0.05$)	None reported	Safflower injection could make usual western medicine treatment better in aspect of controlling angina pectoris of CHD
Liu et al. (2008)	9 days	Unstable angina pectoris	72; 36 treated with safflor yellow; 36 treated with nitroglycerin (61.7 ± 12.2)	Intravenous with 80 mg safflor yellow once a day	Intravenous with 10 mg nitroglycerin	Pretherapy neuropepite Y level was (228.5 ± 29.8)pg/ml in safflor yellow group, (237.6 ± 27.9) pg/ml in nitroglycerin group. Neuropepite Y level of post-treatment was (149.5 ± 24.3) pg/ml in safflor yellow group, (181.8 ± 23.7) pg/ml in nitroglycerin group	None reported	Neuropepite Y could be a index in observing change of unstable angina pectoris. Safflor yellow could improve oxygen supply in myocardium
Miao et al. (2010)	14 days	CHD with heart-blood stagnation syndrome	439; 330 in test group; 109 in control group (18–65)	Test group received safflor yellow injection 5 ml (250 mg) in 0.9% NaCl 250 ml	Given safflower injection 20 ml in 0.9% NaCl 250 ml	The total effective rate on angina pectoris and electrocardiogram analyzed by per protocol analysis was 91.6% and 67.3% respectively in test group, which was 69.2% and 61.2% respectively in control group	Adverse events 5 patients(1.5%) in test group while no adverse in control group	Safflor yellow injection at a dose of 5 ml is effective and safe for the treatment of angina pectoris with heart-blood stagnation syndrome in patients with CHD
Liu et al. (2011a)	3 weeks	CHD	127, 62 in group treated with safflor yellow. 65 in group treated with usual western medicine (68.58 ± 10.12)	Intravenous with 80 mg safflor yellow 100 mg/day	Usual western medicine treatment	Safflor yellow treatment resulted in decrease of plasma of endothelin, matrix metalloproteinase-9 and high sensitivity C reactive protein (hs-CRP) as usual treatment	None reported	Safflor yellow is beneficial to CHD
Liao (2012)	30 days	Chronic pulmonary heart disease (CPHD)	68, 34 per group (58.78 ± 1.74)	Usual treatment with injection of 30 ml safflower injection in 0.5% glucose injection 250 ml once a day	Usual treatment	The parameters of blood gas in the observation group were significantly better than the control group ($p < 0.05$)	None reported	Safflower injection has clinical value in CPHD
Chen et al. (2014)	10 days	CPHD	72, 36 per group (58 ± 7)	Usual treatment with 100 mg safflor yellow in 0.9% NaCl 250 ml, 30–40 drop/min	Usual treatment (7 ml 10%KCl, 4 IU insulin, ATP, CoA and so on in 0.9% NaCl 250 ml	The improvement of ST segment depression in treatment group was much more obvious than that in control group. The level of troponin, phosphocreatine kinase and hs-CRP decreased much more than that in control group	None reported	Safflor yellow displayed a good protective effect on CPHD

Chart 2 (*Continued*)

Author (Date)	Design duration	Condition	No. of participants randomized (age)	Intervention	Comparison	Main results between groups	Adverse events	Authors conclusion
Ren and Mei (2014)	14 days	Chronic obstructive pulmonary disease (COPD)	69, 35 in treated group, 34 in control group (63.8 ± 5.3)	Usual treatment and injecting 150 mg safflor yellow powder in 0.9% NaCl 250 ml once a day	Usual treatment	The *N*-terminal pro-brain natriuretic (NT-proBNP)was reduced to 438.39 pg/ml from 2335.38 in treated group while it was reduced to 738.27 pg/ml from 2178.35 in control group. Hemorheology indexes and pulmonary ventilation function were improved	None reported	Safflor yellow pigment injection combined with western medicine can beneficial to COPD
Luo et al. (2014)	14 days	Acute cerebral infarction (ACI)	51, 25 in treated group, 27 in control group (35–40)	Intravenous with safflower injection (20 ml/day) and 20% mannitol, citicoline, taken aspirin orally	Injected with 20% mannitol, citicoline and taken aspirin orally	On 14th day, neurological severity scores (NSS) of treatment group was lower than that of control group ($p = 0.040$). Also, it was resulted in serum IL-6 level decreased and IL-10 level increased. Positive correlation was observed between IL-6/IL-10 value and NSS in treatment group ($r = 0.997$, $p = 0.048$), but mot in control group ($r = 0.962$, $p = 0.177$)	None reported	Early application of safflower injection is beneficial for young patients with ACI. The mechanism might be related with lowing IL-6/IL-10 value through decreasing IL-6 level and enhancing IL-10 level
Xing et al. (2008)	15 days	Ischemic apoplexy	60, 30 per group (65 ± 3.1)	IV drip of safflor yellow 100 mg in 0.9% NaCl 250 ml	20 ml Ginkgo leaf extract and dipyridamole injection in 0.9% NaCl 250 ml	The effective rate in safflor yellow group was 93.3% while 63.3% in control group. Safflor yellow showed a remarkable effect for qi vacuity bloodstasis	None reported	Safflor yellow has favorable results in treatment of ischemic apoplexy
Li et al. (2011)	5 days	Acute gouty arthritis	120, 80 in safflower injection group, 40 in colchicine group (30–58)	IV drip of safflower injection in 0.9% NaCl 250 ml once a day	Taken 1 mg colchicine orally three times a day	Blood uric acid in both groups decreased than pretherapy	None reported in safflower injection group but serious gastrointestinal reaction happened in 29 patients of control group	Safflower injection has curative effect in acute gouty arthritis

Wei (2011)	28 days	Diabetic nephropathy (DN)	93, 47 in treated group, 46 in control group (57.2 ± 9.8)	IV drip of safflower injection 20 ml once a day and usual treatment	Usual treatment	The index of fasting serum glucose, blood urea nitrogen, serum creatinine and urine protein decreased much more than control group	None reported	Safflower injection could effectively postponement the development of renal failure caused by diabetic nephropathy
Pang et al. (2014)	7 days	Renal transplantation	382, 231 in group treated with safflor yellow. 151 in group with conventional treatment (38.0 ± 13.1)	Intravenous injection of safflor yellow twice a day (10 ml per time) and conventional treatment	Conventional treatment	The incidence of delayed graft function was lower in observation group (5.63%)than that in control group (11.26%), which is same as the level of serum creatinine, the renal segmental and lobular artery resistance indexes	None reported	Safflor yellow could descend the incidence of delayed graft function and ameliorate the recovery of graft function after renal transplantation
Xiong and Dong (2014)	14 days	Diabetic retinopathy (DR)	168, 92 in treatment group, 76 in control group (60.45 ± 6.10)	Conventional treatment with additional 100 ml safflor yellow injection once a day	Conventional treatment	The total effective rates of treatment group and control group were 91.3% and 76.32% respectively. The increased serum endostatin and decreased vascular endothelial growth factor were much more in treated group	None reported	Safflor yellow injection can be used to DR in type 2 diabetes patients and thereby delay the progression of DR
Yang et al. (2011)	14 days	DN	72, 38 in treatment group, 34 in control group (59.0 ± 7.7)	IV drip of safflor yellow 100 mg in 0.9% NaCl 250 ml once a day	IV drip of 20 ml composite salvia in 0.9% NaCl 250 ml once a day	24 h Urine protein serum creatinine were decreased both in prophase and clinical phase than control group	None reported	Safflor yellow injection was beneficial to DN treatment
Yang and Dai (2010)	One month	Diabetic peripheral neuropathy (DPN)	84, 42 per group (48.94 ± 7.15)	Conventional treatment with injecting 100 mg safflor yellow to zusanli acupoint and sanyinjiao acupoint	Conventional treatment with additional 100 mg safflor yellow injection once a day	The total effective rates of treatment group and control group were 84.47% and 62.46% respectively. The treated group of sensory threshold, motor nerve conduction velocity and sensory conduction velocity outweigh that in control group	None reported	Acupoint injection of safflor yellow could improve nerve conduction velocity and decreased sensory threshold, which may be concerned with ameliorating microcirculation of DPN patients and nerve ischemia

dosage of 180 mg/kg for ninety days resulted in slight nephrotoxicity in SD rats (Liu et al., 2004), whereas α-terpineol induced fatty liver (Choi et al., 2013). A clinical observation also reported *Carthami flos* as a new cause of occupational asthma, as shown by bronchial challenges or brochial provocation tests (Compes et al., 2006). As mentioned previously, further evaluations of the systemic toxicity and safety of *Carthami flos* are needed.

Conclusion

The available pharmacological studies on crude extracts or identified compounds of *Carthami flos* provide pragmatic support for some traditional therapeutic claims. However, there are a number of issues that need to be addressed. First, the extensive pharmacological investigations on quinochalcone glycosides have predominantly focused on HSYA. Its advantages in brain tissue, myocardial tissue, diabetes mellitus, and hypertension have been reported, and different preparations have been applied in clinical practice in China. However, the underlying molecular mechanisms of action of HSYA have not been sufficiently clarified. Consequently, more rigorous experiments on *in vitro* and *in vivo* systems, as well as in human models, are required. Moreover, how to exploit other quinochalcone glycosides remains a subject of continuing study. Second, more systemic evaluations of *Carthami flos* in clinical applications, including treatment of coronary heart disease, chronic pulmonary heart disease, cerebrovascular diseases, orthopedic diseases, and diabetes mellitus, need to be carried out. Third, in spite of the distinguished activities of *Carthami flos* in some diseases, the side effects of its use should not be ignored, such as spermatogenetic failure, allergic reaction, and nephrotoxicity. Thereby, systemic toxicity and safety evaluations regarding *Carthami flos* are necessary. In summary, the challenge for the future of *Carthami flos* lies in confirming the mechanism underlying its effects and in providing brawinest clinical support.

Author's contribution

YHT and YRX contributed with data collection and writing of the manuscript. DDG contributed with data collection and format of the manuscript. LNS and MLG suggested the manuscript outline and guided the writing of the manuscript and data analysis. All the authors contributed to the critical reading of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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