THE CHEMICAL CONSTITUENTS AND PHARMACOLOGICAL IMPORTANCE OF CARTHAMUS TINCTORIUS - AN OVERVIEW

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ABSTRACT

The chemical groups isolated from Carthamus tinctorius were included oils, proteins, minerals, phenolics, flavonoids, alkaloids, lignans, carboxylic acids, steroids, polysaccharides, quinochalcone C-glycosides and quinone-containing chalcones. It exerted many pharmacological activities including central nervous, cardiac, vascular, anticoagulant, reproductive, gastrointestinal, antioxidant, hypolipidemic, metabolic and many other pharmacological effects. This paper will highlight the chemical constituents and pharmacological effects of Carthamus tinctorius.

Keywords: Carthamus tinctorius, chemical constituents, pharmacology.

INTRODUCTION

Plants are valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives [1-48]. The chemical groups isolated from Carthamus tinctorius were included, oils, proteins, minerals, phenolics, flavonoids, alkaloids, lignans, carboxylic acids, steroids, polysaccharides, quinochalcone C-glycosides and quinone-containing chalcones. It exerted many pharmacological activities including central nervous, cardiac, vascular, anticoagulant, reproductive, gastrointestinal, antioxidant, hypolipidemic, metabolic and many other pharmacological effects. This paper was designed to highlight the chemical constituents and pharmacological effects of Carthamus tinctorius.

HISTORY

In Egypt, dye from safflower was used to colour cotton and silk as well as ceremonial ointment used in religious ceremonies and to anoint mummies prior to binding. Safflower seeds and packets and garlands of florets have been found with 4000 year-old mummies. By the 18th century, Egyptian safflower dye was used in Italy, France and Britain to colour cheese and flavour sausages. Carthamin dye was used extensively to colour cloth until the 19th century, when cheaper aniline dyes became available. Safflower dyes were used previously in carpet-weaving industries in Eastern Europe, the Middle East and the India. Safflower has been used in the Middle East, India and Africa for purgative and alexipharmic (antidote) effects for long period [1-2]. Safflower evaluations in the United States started in 1925, but commercial production did not begin until the 1950s. Production is concentrated in the western United States and the Canadian prairie provinces. California grows about 50 percent of United States safflower. North Dakota and Montana are the next major areas of commercial production. South Dakota, Idaho, Colorado, Arizona, and Nebraska also produce the crop, but on small acreage [47-49].

Common names

Afghan: Muswar, Maswarah, Kajireh, Kariza; Arabic: Osfur, Usfar, Qurtum, Qorton; Khiri; Aramaic/Hebrew: Qurtami, Qurtema, Qurtam, Dardara, Qotzah, Moriqa; Ayurvedic: Kusumbha, Vahinshikhaa, Vastraranjaka, Kusum; Chinese: Honghua (red flower), Grass safflower, Huai safflower, Chuan safflower, Du safflower; English: Safflower, Dyer’s thistle, False saffron, Bastard saffron, Dyer’s saffron; Ethiopia: Suff; French: Carthame officinal, Faux safran, Graine de perroquet, Safran Bâtard, Safran d’Allemagne, Vermillon de Provence; German: Borstenkraut, Deutscher Saflor, Falscher Saffron, Färber-Saflor, Wilder Saflor, Türkische Saflor; Hindi: Kusumba, Kusuma, Kusum, Karadai,
Hubulkurtun, Cusumbha, Kamalotarra; **Iranian**: Kafsha, Kafshe, Kosheh, Zafaran-golu, Kouchan gule, Kahli, Golbar aftab, Brarta, Kharkhool; **Italian**: Cartamo, Zafferone, Zaffranone, Zafferano bastardo, Asfore, Grogo; **Japanese**: Benibana, Benihan; **Kannada**: Kusubi, Kusube; **Marathi**: Karadi; **Pakistani**: Khurtum; **Spanish**: Caeramo, Azafaran bastardo, Alazor, Azafran romi; **Siddha/Tamil**: Chen Durakam; **Turkey**: Aspir, Dikken, Kazhira, Cnicus, Cnecus, Cnikos, Onicus; **Unani**: Qurtum [50-56].

**Distribution**

*Carthamus tinctorius* is believed to have originated in Southern Asia and Middle East and has been cultivated in India, China, Persia, and Egypt. During the middle ages, it was introduced in Italy, France and Spain and after the discovery of America, it introduced to Mexico and Venezuela and Colombia in 1925 from the Mediterranean region [57]. Historically, the plant has been restricted to the Middle East, part of Asia and Africa, but over time it has also been adapted to the semi-arid climatic areas [49-58].

It was distributed to Afghanistan, Iran, Iraq, Jordan, Syria, Turkey, Sudan, Bangladesh, Pakistan, Japan Ethiopia, France, Germany, Italy, Spain and Latin America [58-59].

**Description**

**Flower and Fruit**: Axillary flowers grow in the leaf axils. They are initially red-yellow, later bright orange. The heads are up to 4 by 3 cm and are encircled by upper leaves. The bracts are light green and have thorny tips with a thorny appendage. The fruit is 6 to 8 cm long, obovate or pear-shaped and bluntly wedge-shaped at the base with protruding long ribs. The pappus consists of scales. **Leaves, Stem and Root**: *Carthamus tinctorius* is an annual plant, that grows up to 90 cm high. It has a thin fusiform root. The stem is erect, simple or branched at the top into stiff, glabrous, whitish-yellow and glossy branches. The leaves are long, fairly soft, and glabrous with a thornyserrate margin and tip [60].

**Traditional uses**

Traditionally this crop was grown for its flowers for colouring and flavouring foods. Flowers contain the water soluble yellow dye carthamidin (C16H20O11) and a water insoluble red dye carthamin (C35H32OH.H2O). These have been the source of yellow and red dye in the food and industries to colour cotton and silk [58]. Recently, these yellow and red pigments have been shown to be safe for cosmetic colourings such as face cream, shampoo, perfume or body lotion and hair cream. In Chinese medicine, flower petals have been used as a stimulant for blood circulation and phlegm, healing of fractures, contusions and strain and for various female maladies. It was used for the problem in mensuration to increase blood flow and, mixture of ground safflower seed and mustard oil has been used to reduce rheumatic pain [61]. The florets of *Carthamus tinctorius* have been used as a remedy for stroke, gynecological disease, coronary heart disease, angina pectoris, and hypertension in Chinese folk medicine [62]. In Korea, the safflower seed extracts have traditionally been used for the treatment of blood stasis, the promotion of bone formation and the prevention of osteoporosis [63-64].

In India and Afghanistan the tea made from safflower foliage was used to prevent the abortion in women. Male sterility and dead sperm diseases have also been treated with using safflower dicotyledons [58]. It was widely used as a traditional Thai herbal remedy for blood, heart and nerves tonics, blood detoxifier, lymph stimulator, menstruation enhancer, to relief menstruation pain, to control blood pressure and for various types of dyslipidemic syndromes [65].

Oil is used by both food producers and industry. However, Safflower is currently grown mostly for its edible oil, considered as a favourable oil for human consumption due to high quantity (70-75%) of polysaturated (linoleic acid) or mono-unsaturated fatty acid (oleic acid), which play an important role in reducing cholesterol level in blood [66-67]. In the United States, there are three major uses for safflower: oil, meal, and birdseed. The crop is divided into two categories based on oil quality: (1) high linoleic (a polysaturated fatty acid) acid varieties, these contain 75 percent linoleic acid, and (2) high oleic (a monounsaturated fatty acid) acid varieties [49].

**Parts used**: The medicinal parts are the flowers, seeds and the oil extracted from its embryos [60].

**Physicochemical properties**

Foreign organic matter: not more than 2%, total ash: not more than 18%, loss on drying: not more than 13% [68-70]. The physicochemical characteristics of *Carthamus tinctorius* seed oils: (% solvent extraction) 42.00, peroxide number 83.00, saponification value (mg/g) 141.78, iodine value (g/100g) 16.70, viscosity (mPas) 97.50, colour yellow, odour unaccepted [71].

**Chemical constituents**

The chemical groups isolated from *Carthamus tinctorius* were included, oils, proteins, minerals, phenolics, flavonoids, alkaloids, lignans, carboxylic acids, steroids, polysaccharides, quinonechalcone C-glycosides and quinone-containing chalcones [72-78].

Many factors such as genotype, ecology, morphology, physiology and agronomic practices influence the oil content and fatty acid synthesis of crops [79]. Commercial safflower varieties contained 32 to 52 percent oil. The crop was divided into two categories based on oil quality: high linoleic (a polysaturated fatty acid) acid varieties, these contain 75 percent linoleic acid,
and high oleic (monounsaturated fatty acid) acid varieties [49]. Safflower seeds oil content of the four varieties of *Carthamus tinctorius* was ranged from 28.84 to 35.38 g/100g. Safflower oils contained palmitic acid, palmitoleic acid, margaric acid, margaroleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid and behenic acid. Linoleic acid was the principal fatty acid (77.94-79.49%) followed by oleic acid as the second main fatty acid. Palmitic acid was the major saturated fatty acid (7.2-8.6%) followed by stearic acid (2-2.39%) [80-81]. However in other study, evaluation of four safflower (*Carthamus tinctorius L.*) genotypes for oil content and fatty acid composition showed that oil content varied from 22.16 to 34.39%. Among the detected fatty acids, linoleic acid (75.81-77.86%) was the predominant fatty acid followed by oleic (12.57-13.75%), palmitic (6.09-7.07%) and stearic (2.17-2.62%) acid, while trace amounts of other fatty acids were presented and the values of them did not exceed 0.81%. The oil content and fatty acid composition of oil among the genotypes were significantly different (P<0.01), indicating that synthesis of them is influenced by genotype. The lipids of safflower rich in polyunsaturated essential fatty acids, linoleic acid makes the oil nutritionally and therapeutically valuable for human consumption [82].

Carthamin, safflor yellows A and B, safflomin A, and C, isocarthamin, isocarathonidin, hydroxysafflor yellow A, tinctormine, puerarin, 3'-methoxyl-puerarin, and puerrarinapioside pigments have been isolated from *Carthamus tinctorius* petals [83-86]. The quantitative analysis of the pigments showed that yellow safflower (carthaminid) represented 29.59% and red safflower (carthamin) 0.77% [87].

Many serotonin derivatives were isolated from *Carthamus tinctorius* oil cake. Their structures were established as N-[2-(5-hydroxy-1H-indol-3-y1)ethyl] ferulamide, N-[2,5-hydroxy-1H-indol-3-y1]ethyl]p-coumaramide, N,N'-[2,2',(5,5'-dihydroxy-4,4'-bi-1H-indol-3,3'-y1)diethyl]-di-p-coumaramide, N-[2-[3-(p-coumaramido)ethyl]-5,5'-di hydroxy-4,4'-bi-1H-indol-3-y1]ethyl]ferulamide, N,N'-[2,2',(5,5'- dihydroxy-4,4'-bi-1H-indol-3,3'-y1)diethyl]-diferulamide, N-[2-[5-(beta-D-glucosyloxy)-1H-indol-3-y1]ethyl]p-coumaramide, N-[2-[5-(beta-D-glucosyloxy)-1H-indol-3-y1]ethyl]ferulamide, N-(p-coumaroyl) serotinin, N(1),N(5)-(Z)-N(10)-(E)-tris-p-coumaroyl spermidine and N-feruloylserotonin[74, 88-94].

The total phenolic contents were 126.0±2.4 mg, gallic acid equivalent/g), and the total flavonoid contents were 62.2±1.9 mg (mg, quercetin equivalent/g). Phenolic compounds identified in *Carthamus tinctorius* seed extract were included (mg/g) hydroxybenzhydrazide derivative 18.2, amino-3,4-dimethylbenzic acid 16.8, chlorogenic acid 2.4, syringic acid 0.2, p-coumaric acid 0.5, trans-Ferulic acid 3.0, gallo catechin 17.0, (+) epigallocatechin 109.6, epigallocatechin gallate 1.1, quercetin dehydrate 2.2, kaempferol 0.8, rutin hydrate 3.7, luteolin 1.6, naringin 6.0 and trans-chalcone 2.1 [95-96].

Quinocalcone compounds, saffloquinoside A, saffloquinoside B and quinocalcone C-glycosides were isolated from the florets of *Carthamus tinctorius* [85, 97]. Many Erythro-alkane-6,8-diols compounds were isolated from the flowers of *Carthamus tinctorius* [98]. A new bioactive triterpenoid saponin 3β-O-[β-D- xylopyranosyl(1 → 3)-O-β-D-galactopyranosyl]lup-12-ene-28 oic acid-28-O-α-L-rhamnopyranosyl ester compound, was isolated from the methanolic fraction of the roots of *Carthamus tinctorius* [99]. Eleven linear polycetylene glucosides, containing two C(10)-, one C(13)- and five C(14)-acetylenes, were isolated from the florets of *Carthamus tinctorius* [100]. The lignan glycoside, tracheloside, was also isolated from seeds of *Carthamus tinctorius* [101].

The nutritional composition of the flowers of safflower included total sugar 7.36-11.81%, protein10.40-12.86%, potassium 3264.00-3992.00 mg/100g, calcium 558.00-708.00 mg/100g, magnesium 142.00-207.00 mg/100g, iron 42.50-55.10 mg/100g, sodium 17.00-1043.00 mg/100g, manganese 4.34-4.70 mg/100g, zinc 2.60-2.88 mg/100g and copper 1.10-4.73 mg/100g. The composition and quantitative analysis of amino acids of the flower of safflower (% of the total amino acids) showed the presence of aspartic acid 5.09, glutamic acid 9.78 , serine 6.02, glycine 6.97, histidine 2.27, arginine 5.72 , threonine 4.78, alanine 8.66, proline 8.96, tyrosine 2.81, valine 6.77 , methion 1.49, cysteine 0.18, isoleucine 5.63, leucine 8.63, phenylalanine 5.28, lysine 5.35 and hydroxyl proline 5.63 [102-103]. The polysaccharide of *Carthamus tinctorius* were composed of xylose, fructose, galactose, glucose, arabino, rhamnose and uronic acid residues [51].

**Pharmacokinetics**

Studies were conducted to characterize the pharmacokinetics and excretion of hydroxysafflor yellow A (HSYA) in rats and dogs after administration by intravenous injection or infusion. Linear pharmacokinetics of HSYA after the intravenous administration was found at doses ranging from 3 to 24 mg/kg in rats and from 6 to 24 mg/kg in dogs. At a dose of 3 mg/kg, HSYA in urine, feces and bile was determined. For 48h after dosing, the amount of urinary excretion accounted for 52.6 ± 17.9 % (range: 31.1-78.7%) of the dose, and the amount of fecal amount accounted for 8.4±5.3% (range 1.7-16.4%) of the dose. Biliary excretion amount accounted for 1.4±1.0% (range 0.4-2.9%) of the dose for 24h after dosing. Percent plasma protein binding of HSYA ranged from 48.0 to 54.6% at 72 h [104].

The pharmacokinetics of *Carthamus tinctorius* extraction and Naodesheng tablet was studied in rats, the effect of other components on the
pharmacokinetics of hydroxysafflower yellow A was also studied. The rats were orally treated with Carthamus tinctorius extraction and Naodesheng capsule respectively. The AUC(0-t), AUC(0-infinity), C(max) and T(max) of hydroxysafflower yellow A were increased in the Naodesheng group, compared with 50 mg x kg⁻¹ C. tinctorius extract group [105].

A high-performance liquid chromatographic method was used for determination and pharmacokinetic studies of safflower yellow A, puerarin, 3’-methoxyypuerarin, and puerarinapioside in the plasma and tissues of rats that had been administered with the traditional Chinese medicine (TCM) preparation Naodesheng via the caudal vein. The calibration curves of the four components were linear in the given concentration ranges. The intra- and inter-day precisions in plasma and tissues were less than 15% and the extraction recoveries were higher than 60%. The lower limits of quantitation of four components were low enough to determine the four components. These four components exhibited kinetics that fitted a two-compartment model in rats. The elimination half-life was 1.19h for safflower yellow A, 2.69 h for puerarin, 2.94 h for 3’-methoxypuerarin, and 0.87h for puerarinapioside. Following administration of a single injection of Naodesheng, the concentration (C) of the four components in the tissues showed C (kidney)>C (lung), C (liver) > C (spleen), C (stomach), C (heart) [106].

The pharmacokinetics of hydroxysafflower yellow A (HSYA) and safflower extract were evaluated following oral administration with the same dose of HSYA 100mg/kg in both normal and acute blood stasis rats. It was found that AUC(0-t), C(max), Vd and CL of HSYA in both HSYA monomer and safflower extract in acute blood stasis rats were with significant difference (P<0.05) comparing with that in normal rats [107].

The pharmacokinetic characteristics of HSYA was studied in healthy Chinese female volunteers. The volunteers were given intravenous infusion of single doses of safflower yellow injection (containing HSYA 35, 70 and 140 mg) in separate trial periods with one week washout period. The pharmacokinetic parameters were estimated from the plasma concentration versus time data using non-compartmental methods. The C(max) values were 2.02±0.18, 7.47±0.67 and 14.48±4.71 microg/ml after the administration of single doses of 35, 70, and 140 mg of HSYA respectively. The corresponding values of AUC(0-15 h) were 6.57±1.20, 25.90±4.62 and 48.47±12.11 microg/(ml h⁻¹), and the values of t₁/₂ were 3.21±1.26, 3.33±0.68 and 2.98±0.09h. The statistical analysis showed that C(max) and AUC(0-15 h) were both linearly related to dose [108].

PHARMACOLOGICAL EFFECTS
Nervous system effect
Subcutaneous administration of 1–10 g/kg bw of an aqueous or 50% methanol extract of the flowers had central nervous system depressant effects and relaxed skeletal muscles in mice. Subcutaneous administration of 10 g/kg bw of a 50% methanol extract of the flowers inhibited pentylentetrazole-induced convulsions in mice [109].

The effect of safflower or its isolate on functionally regulating monoamine transporter was studied using in vitro screening cell lines. Safflower insoluble fraction significantly inhibited serotonin uptake in Chinese hamster ovary cells stably expressing serotonin transporter (i.e. S6 cells). The active compound was isolated as coumaroyspermidine analog N(1),N(5)-(Z)-N(10)-(E)-tri-p-coumaroyspermidine. This compound potently and selectively inhibited serotonin uptake in S6 cells or in synaptosomes, with IC₅₀ of 0.74±0.15 microM for S6 cells or 1.07±0.23 microM for synaptosomes and with a reversible competitive property for the 5HT-uptake inhibition. The potency of it for 5HT uptake was weaker than that of fluoxetine, whereas efficacy generally similar for both. Animals treated with this testing compound showed a significant decrease in synaptosomal 5HT uptake capacity [88-94].

All solvent-extracted Safflower (HH) fractions, in different degrees, markedly increased both dopamine uptake by Chinese hamster ovary (CHO) cells stably expressing dopamine transporter (DAT) and norepinephrine uptake by CHO cells expressing norepinephrine transporter (NET), and also showed that chloroform (HC), ethyl acetate (HE), and n-butyl alcohol extract strikingly depressed serotonin uptake by CHO cells expressing serotonin transporter (SERT); wherein, the potencies of ethanol extract, HC, HE, and aqueous extract to up-regulate dopamine/norepinephrine uptake and potency of HE to inhibit serotonin uptake were relatively stronger. Further investigation revealed that the enhancement of dopamine/norepinephrine uptake by HE and HE was dependent of DAT/NET activity, and the HE-induced inhibition of serotonin uptake was typical of competition [110].

The neuroprotective properties of Hydroxysafflower yellow A (HSYA) on neurotoxicity of glutamate in primary cultured rat cortical neurons along with its possible mechanism of action were examined. The excitotoxic neuronal death was attenuated markedly by HSYA treatment. HSYA decreased expression of Bax and rescued the balance of pro- and anti-apoptotic proteins. In addition, HSYA significantly reversed up-regulation of NR2B-containing NMDA receptors by exposure to NMDA, while it did not affect the expression of NR2A-containing NMDA receptors [111].

The neuroprotective efficacy of the combination of (astragali, ligusticum wallichii, angelica sinensis and Carthamus tinctorius) in mitigating brain infarction and global ischemia as well as preventing the neurodegeneration following ischemia was studied. They improved cerebral blood circulation, which refer to a
potential to alleviate the symptoms of degenerative diseases, Alzheimer's disease and Parkinson's disease [112].

The neuroprotective effects of hydroxysafflor yellow A (HSYA) on cerebral ischemic injury in both in vivo and in vitro were studies. In in vivo experiment, male Wistar-Kyoto (WKY) rats with middle cerebral artery occlusion (MCAO) were evaluated for neurological deficit scores followed by the treatment with a single dose of HSYA. Furthermore, the infarction area of the brain was assessed in the brain slices. In in vitro experiment, the effect of HSYA was tested in cultured fetal cortical cells exposed to glutamate and sodium cyanide (NaCN) to identify its neuroprotection against neurons damage. The results of in vivo study showed that sublingual vein injection of HSYA at doses of 3.0 mg/kg and 6.0 mg/kg exerted significant neuroprotective effects on rats with focal cerebral ischemic injury by significantly decreasing neurological deficit scores and reducing the infarct area compared with the saline group. HSYA at a dose of 6.0 mg/kg gave a similar potency as nimodipine at a dose of 0.2 mg/kg. Sublingular vein injection of HSYA at the dose of 1.5 mg/kg showed a neuroprotective effect, however, with no significant difference when compared with the saline group. In vitro results showed that HSYA significantly inhibited neuron damage induced by exposure to glutamate and sodium cyanide (NaCN) in cultured fetal cortical cells, however, the neuroprotective action of HSYA on glutamate-mediated neuron injury was much better than that of HSYA on NaCN-induced neuron damage [113].

Free radical scavenging activity of the extracts of petals (bud, early stage, full blooming and ending stage), leaf, stem, root and seeds of Mogami-benibana (Carthamus tinctorius), the contents of the major active components of carthamin and polyphenols, and neuroprotective effect of the petal extracts and carthamin in the brain of mice and rats were examined. Water extracts of Mogami-benibana petals scavenged superoxide, hydroxyl and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and singlet oxygen. There was also a relationship between DPPH radical scavenging activity and carthamin content in the petal extracts of safflower [114].

The potential protective effect of Hydroxysafflor Yellow A (HSYA) in spinal cord ischemia/ reperfusion (I/R) injury was studied in rabbits. Neurological outcomes in HSYA group were slightly improved compared with those in I/R group. Histopathological analysis revealed that HSYA treatment attenuated I/R induced necrosis in spinal cords. Similarly, alleviated oxidative stress was indicated by decreased malondialdehyde (MDA) level and increased superoxide dismutase (SOD) activity after HSYA treatment. Moreover, HSYA also protected neurons from I/R-induced apoptosis in rabbits as seen from TUNEL results [115].

The probable attenuating effect of Hydroxysafflor yellow A (HSYA) on brain injury induced by lymphostatic encephalopathy (LE) was investigated in rats. Heart rate variability (HRV) was used as an indirect measurement of the regulatory function of the autonomic nervous system by recording the ECG signals from rats. It was shown that treatment with HSYA (5 mg/kg, ip) significantly alleviated the neurological deficits observed in rats with LE. Histological staining revealed that HSYA treatment attenuated LE-induced cell apoptosis in the rostral ventrolateral medullus (RVLM). Animals in the LE groups exhibited impaired regulatory roles of the autonomic nervous system in cardiovascular function, which was suppressed by pretreatment with HSYA. Additionally, HSYA administration significantly prevented the decrease of endothelial nitric oxide synthase (eNOS) mRNA and protein expression in the RVLM of rats with LE. Accordingly, HSYA might provide neuroprotection against LE-induced brain injury and the associated functional alterations, which is likely regulated by the nitric oxide pathway [116].

The therapeutic effects of hydroxysafflor yellow A (HSYA) on focal cerebral ischemic injury in rats and its related mechanisms have been investigated. Focal cerebral ischemia in rats were made by inserting a monofilament suture into internal carotid artery to block the origin of the middle cerebral artery and administrated by HSYA via sublingual vein injection in doses of 1.5, 3.0, 6.0 mg /kg at 30 min after the onset of ischemia, in comparison with the potency of nimodipine at a dose of 0.2 mg/kg. Then, 24 h later, the evaluation for neurological deficit scores of the rats were recorded and postmortem infarct areas were determined. HSYA dose-dependently improved the neurological deficit scores and reduced the cerebral infarct area, and HSYA bore a similarity in potency of the therapeutic effects on focal cerebral ischemia to nimodipine. The inhibition rates of thrombosis formation by HSYA at the designated doses were 20.3%, 43.6% and 54.2%, respectively, compared with saline-treated group. Inhibitory activities of HSYA were observed on ADP-induced platelets aggregation in a dose-dependent manner, and the maximum inhibition of aggregation of HSYA was 41.8%. HSYA provided a suppressive effect on production of TXA2 without significant effect on plasma PGI2 concentrations. Blood rheological parameters were markedly improved by HSYA, such as whole blood viscosity, plasma viscosity, deformability and aggregation of erythrocyte, but no significant effect for HSYA on homatocrit was found [117].

The effects of Carthamus tinctorius was evaluated on bcl-2, caspase-3 expression of apoptosis of neurons. The middle cerebral artery of rats was occluded for 2h by inserting an intraluminal monofilament, and reperfusion was then instituted for 4h or 22h. All treated groups at different times decreased the volume of
infarction (P<0.05), while large-dose group showed more distinct decrease than other groups (P<0.05). All treated groups at different times increased bcl-2 and decreased caspase-3 expression as well, while, large-dose group showed more distinct effect (P<0.05) [118].

The effect of Hydrosyssafflor yellow A (HSYA) on mitochondrial permeability transition pores (mPTP) was studied in the rat brain. HSYA at 10-80 micromol/l inhibited Ca$^{2+}$- and H$_2$O$_2$-induced swelling of mitochondria isolated from rat brains. The addition of Ca$^{2+}$ generated reactive oxygen species (ROS) in isolated mitochondria, the effect which inhibited by HSYA (10-80 micromol/l). At the same time, HSYA significantly improved mitochondrial energy metabolism, enhanced ATP levels and the respiratory control ratio [119].

**Cardiac effect**

The anti-myocardial ischemia effects of a purified extract of *C. tinctorius* (ECT) was studied both *in vivo* and *in vitro*. An animal model of myocardial ischemia injury was induced by left anterior descending coronary artery occlusion in adult rats. Pretreatment with ECT (100, 200, 400, 600 mg/kg body wt.) protected the heart from ischemia injury by limiting infarct size and improving cardiac function. In the *in vitro* experiment, neonatal rat ventricular myocytes were incubated to test the direct cytoprotective effect of ECT against H$_2$O$_2$ exposure. Pretreatment with 100-400 microg/ml ECT prior to H$_2$O$_2$ exposure significantly increased cell viability as revealed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. ECT also markedly attenuated H$_2$O$_2$-induced cardiomyocyte apoptosis, as detected by Annexin V and PI double labeling with flow cytometry. ECT pretreatment significantly inhibited H$_2$O$_2$-induced ROS increase. The cardioprotective effects of ECT in myocardial ischemia operate partially through reducing oxidative stress induced damage and apoptosis. The protection is achieved by scavenging of ROS and mediating the PI3K signaling pathway [120].

The protective effects of *Carthamus tinctorius* injection (CTI) (2.5 and 0.625 g/kg, respectively, ip for 5 days) on isoprenaline-induced acute myocardial ischemia (AMI) was evaluated in rats, the underlying mechanisms were also studied. Results showed that CTI (2.5 and 0.625 g/kg) significantly inhibited the typical ECG S-T segment elevation, reduced concentration of IL-6 and TNF-α in serum, suppressed overexpression of Bax protein and also inhibited the reduction of BCI-2 expression and markedly depressed the Bax/Bcl-2 ratio. These findings demonstrate that CTI is cardioprotective against AMI in rats and is likely to related to decrease inflammatory response mediated by TNF-α and IL-6, down-regulate protein level of Bax and up-regulate that of Bcl-2 in the heart tissue [121].

The effects of safflower injection (SI) in protecting heart, on energy charge and anti-apoptosis gen bcl-2 in cardiac tissue were investigated by Rats’ Langendorff isolated heart infused model. As compared with the control, SI improved the functions of cardiac contraction and dilation, increasing coronary blood flow, and strengthening the bcl-2 protein expression [122].

The protective effects of N-(p-Coumaroyl) serotonin (C) and N-feruloylsertotonin (F) were investigated in perfused guinea-pig Langendorff hearts subjected to ischemia and reperfusion. Changes in cellular levels of high phosphorous energy, NO and Ca$^{2+}$ in the heart together with simultaneous recordings of left ventricular developed pressure (LVDP) were monitored by nitric oxide (NO) electrode, fluorometry and 31P-NMR. The rate of recovery of LVDP from ischemia by reperfusion was 30.8% in the control, while in the presence of C or F a gradual increase to 63.2 or 61.0% was observed. Changes of transient NO signals (TNO) released from heart tissue in one contraction (LVDP) was observed to be upside-down with respect to transient fura-2-Ca$^{2+}$ signals (TCa) and transient O$_2$ signals detected with a pO$_2$ electrode. At the final stage of ischemia, the intracellular concentration of Ca$^{2+}$ and the release of NO increased with no twitching and remained at a high steady level. The addition of C increased the NO level at the end of ischemia compared with the control, but Ca$^{2+}$ during ischemia decreased. On reperfusion, the increased diastolic level of TCa and TNO returned rapidly to the control level with the recovery of LVDP. By *in vitro* EPR, C and F were found to directly quench the activity of active radicals. Accordingly, the antioxidant effects of both derivatives isolated from safflower play an important role in ischemia-reperfusion hearts in close relation with NO [123].

The effect of Flos Carthami FC(ETH) ethanolic extract on LPS-induced apoptosis in H9c2 cardiomyoblast cells was studied. FC(ETH) (62.5 microg/mL) inhibited LPS-induced apoptosis by suppressing JNK1/2 activity, which resulted in the reduction of both IkappacB degradation and NF kappaB activation. In addition, FC(ETH) led to activation of anti-apoptotic proteins, Bcl-2 and Bcl-xL, the stabilization of the mitochondria membrane and the down-regulation of extrinsic and intrinsic pro-apoptotic proteins, such as TNF alpha, active caspases-8, t-Bid, Bax, active caspases-9, and -3. The ability of *Carthamus tinctorius* to suppress JNK activity and inhibit LPS-induced TNF alpha activation and apoptosis in H9c2 cardiomyoblast cells could potentially serve as a cardio-protective agent against LPS-induced apoptosis [124].

The effects of safflor yellow A (SYA) was evaluated on cultured rat cardiomyocytes exposed to anoxia/reoxygenation (A/R). The A/R exposure markedly decreased the viability of cardiomyocytes, suppressed the activities of SOD, GSH, CAT, GSH-Px, and Bcl-2 protein.
expression. Meanwhile, the A/R exposure markedly increased the release of LDH, CK, MDA production in the cardiomyocytes, increased the rate of apoptosis, caspase 3 activity and Bax protein expression. Pretreatment with SYA (40, 60 and 80 nmol/l) concentration-dependently blocked the A/R-induced changes in the cardiomyocytes. Pretreatment of the cardiomyocytes with the SYA (80 nmol/l) produced protective effects that were comparable to those caused by N-acetylcysteine (NAC, 200 μmol/l) [125].

The effects and the proper dosage of Panax notoginseng (EPN) combined with Carthamus tinctorius (ECT) to strengthen their cardio-protective effects were investigated. Meanwhile, their potential anti-oxidative stress and anti-inflammation effect were assessed. Rats were orally given individual EPN 50, 100mg/kg, ECT 100, 200mg/kg, and different combinations between them. Myocardial infarction was produced by occlusion of the left anterior descending coronary artery for 24h. Infarct area was determined with 2,3,5-triphenyltetrazolium chloride (TTC) staining. The biomarkers related to myocardial ischemia injury were determined. Simultaneously, hemodynamic parameters were monitored as left ventricular systolic pressure (LVSP), LV end-diastolic pressure (LVEDP) and maximal rate of increase and decrease of left ventricular pressure (dP/dt(max)). The oxidative stress indicators and inflammatory factors were also evaluated. The results showed that EPN or ECT significantly reduced infarc size, improved cardiac function, decreased levels of creatine kinase (CK) and lactate dehydrogenase (LDH) (all P<0.05 vs. control ). EPN or ECT alone also restrained the oxidative stress related to myocardial ischemia injury as evidenced by decreased malondialdehyde (MDA) and elevated superoxide dismutase (SOD) activity (all P<0.05 vs. control). However, this cardio-protective effect was further strengthened by their combinations. Among all the combinations, EPN 50mg/kg plus ECT 200mg/kg showed predominant potential to reduce infarct size (22.21±1.72%, P<0.05 vs. each single, respectively), preserve cardiac function (P<0.05 vs. ECT 200mg/kg for LVEDP and -dP/dt(max)) after myocardial ischemia injury in rats. This heart protection was confirmed with the lowered cardiac troponin I (cTnl) (P<0.05 vs. ECT 200mg/kg and EPN 50mg/kg, respectively). EPN 50mg/kg plus ECT 200mg/kg markedly increased SOD and GSH-Px activity (475.30±23.60U/ml, P<0.05 vs. each single, respectively), while elevated SOD activity and enhance caspase 3 activity in VECs, as a result, enhance apoptosis of VECs. SYB was able to eliminate the effect of Ang-II on VECs via regulating Ca2+, mitochondrial structure and function and inhibiting apoptosis [128].

To observe the effect of Safflower Injection (SI) on mesenteric microvascular motion in vivo in rabbits, and to explore the effect of nitric oxide (NO) in the process to further investigate the action mechanism of activating blood to remove stasis of SI. The vasomotion was induced by noradrenaline (NA) in vivo, then the changes of vasomotion after injecting SI and N(G)-monomethyl-L-arginine (L-NMMA, a NO synthase inhibitor) were measured. L-NMMA injection alone can inhibit the NA induced vasomotion in vasoconstriction state, while SI injection alone can inhibit it in vasodilation state. SI could abolish the effect of L-NMMA on vasomotion but L-NMMA did not influence the effect of SI on vasomotion [129].

The mechanism of safflor effect on renal ischemia/reperfusion (I/R) injury in rats was studied. After rat’s I/R injury model was established and after three treatment doses (high, middle and low doses), renal function was assessed by measuring serum creatinine, blood urea nitrogen, urine osmotic pressure and urine osmotic pressure/blood osmotic pressure. The apoptosis rate in I/R renal tissue was measured by TUNEL method and caspase-3 concentration was measured by immune-histochemistry. Reperfusion of the ischemic kidney induced marked renal dysfunction. Saffor injection significantly inhibited the reperfusion-associated increase in apoptosis rate and caspase-3 protein absorbance value. Moreover, the renal dysfunction at all treatment groups was markedly ameliorated by Saffor injection. (P<0.01). Accordingly, the protective effect of Saffor injection may be related to the inhibition of cell apoptosis and caspase-3 gene expression following renal I/R [130].

The vasodilatation effects of hydroxysafflor yellow A (HSYA) on pulmonary artery (PA) were explored by an assay of tension study on rat pulmonary artery (PA) rings. Results suggest that HSYA possessed vascular relaxation effects on rat PA by activating the KV channel in pulmonary vascular smooth muscle cells (PVSMCs) [131]. Intravenous injection of the HSYA
significantly reduced MAP and HR in both normotensive rats and SHR in a dose-dependent manner. HSYA reduced left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), the maximum rate of increase of left ventricular pressure (+dp/dt(max)) and heart rate (HR) in a dose-dependent manner. HSYA had no remarkable effect on the maximum rate of decrease of left ventricular pressure (-dp/dt(max)); BK(Ca) and K(ATP) blocker can weakened the inhibitory effect of HSYA on heart rate and HR, but K(V) and K(ACh) blocker did not significantly weaken the HSYA effects [132].

The therapeutic and preventive effects of Safflower Injection (Al) in vascular crisis after free flap transplantation was studied clinically. Sixty patients undergoing free flap transplantation were randomly assigned to the treatment group and control group, thirty in each. Free flap transplantation was performed on all patients, and medication was given 0.5h before flap vascular anastomosis, 1-7 days after surgery. Twenty ml Al was intravenously dripped to patients in the treatment group after adding in 250 ml 5% glucose injection, while Dextran-40 was intravenously dripped to patients in the control group. The medication was conducted once per day. The hemorheology and four indices of blood coagulation [prothrombin time, international normalized ratio, activated partial thromboplastin time, fibrinogen] were compared between the two groups before operation (TO), during operation (T1), 24 h after operation (T2), three days after operation (T3), and seven days after operation (T4). Meanwhile, flaps were observed and adverse reaction recorded. The clinical efficacy and safety were compared. Better result was obtained in the treatment group when compared their clinical efficacy (86.67% vs 60.00%, P<0.05). The whole blood high and low viscosity, plasma viscosity, red blood cell volume, RBC aggregation index all decreased, and RBC deformed index increased in the two groups at T4, showing statistical difference when compared with those at T3 (P<0.05, P<0.01). There was no statistical significance in the four indices of blood coagulation when compared with any time point in the same group (P>0.05). There was no statistical significance in hemorheology and the four indices of blood coagulation between the two groups at the same time point (P>0.05). The adverse reaction rate in the treatment group was lower than that in the control group, showing statistical difference (13.33% vs 30.00%, P<0.05) [133].

The vascular effect of N-(p-coumaroyl)serotonin (CS) and N-feruloylserotonin (FS), was evaluated. Both CS and FS (each 10 to 100 µM) relaxed rat femoral arteries, which were pre-contracted by 10^{-5} M phenylephrine or 50 mM KCl, independently of their endothelium. Both CS and FS also concentration-dependently inhibited the increase of cytosolic free Ca^{2+} concentration that was induced by KCl or 5-hydroxytryptamine in cultured rat vascular smooth muscle cells (VSMCs). The effects of CS and FS was also examined on platelet-derived growth factor (PDGF)-BB-evoked proliferation and migration of the VSMCs. Both CS and FS inhibited PDGF-BB-evoked proliferation and migration of the VSMCs in a concentration-dependent manner. They also inhibited PDGF-BB-induced phosphorylation of PDGF receptor β and ERK1/2, and Ca^{2+} release from sarcoplasmic reticulum in the VSMCs in a concentration-dependent fashion. These result explain a part of anti-atherogenic mechanism that underlies their ability to improve vascular distensibility and to inhibit aortic hyperplasia[134].

The effects of Safflower (Chinese Traditional Medicine) on the intestine ultrastructure characteristics during intestine ischemia/ reperfusion injury (I/RI) was studied in rabbits. The intestine ultrastructure was badly injured in untreated ischemia/reperfusion group. Mitochondria and intestinal mucosal cells were swollen and endoplasmic reticulum expanded, however, in the Safflower injection group the ultrastructural injury of the ischemia greatly ameliorated[135].

The effects of long-term supplementation with Safflower seed extract (SSE) on arterial stiffness in human subjects were evaluated in a double blind clinical trial. 77 males (35-65 years) and 15 postmenopausal females (55-65 years) with high-normal blood pressure or mild hypertension who were not undergoing treatment received SSE (70 mg/day as serotonin derivatives) or placebo for 12 weeks, and pulse wave measurements, ie, second derivative of photoplethysmogram (SDPTG), augmentation index, and brachial-ankle pulse wave velocity (baPWV) were conducted at baseline, and at weeks 4, 8, and 12. Vascular age estimated by SDPTG aging index, improved in the SSE-supplemented group when compared with the placebo group at four (P=0.0368) and 12 weeks (P=0.0927). The trend of augmentation index reduction (P=0.072 versus baseline) was observed in the SSE-supplemented group, but reduction of baPWV by SSE supplementation was not observed. The SSE-supplemented group also showed a trend towards a lower malondialdehyde-modified-LDL autoantibody titer at 12 weeks from baseline [136].

The effects of defatted safflower seed extract and its phenolic constituents, serotonin derivatives, on atherosclerosis were studied. Ethanol-ethyl acetate extract of safflower seeds (SSE) inhibited low-density lipoprotein (LDL) oxidation induced in vitro by an azo-containing free-radical initiator V70 or copper ions. Two serotonin derivatives [N-p-coumaroylserotonin (CS) and N-feruloylserotonin (FS)] and their glucosides were identified as the major phenolic constituents of the extract. The study revealed that a majority of the antioxidative activity of SSE was attributable to the serotonin derivatives. Orally administered CS and FS suppressed CuSO_{4} induced plasma oxidation ex vivo.
Long-term (15 week) dietary supplementation of SSE (1.0 wt %/wt) and synthetic serotonin derivatives (0.2-0.4%) significantly reduced the atherosclerotic lesion area in the aortic sinus of apolipoprotein E-deficient mice (29.2-79.7% reduction). The plasma level of both lipid peroxides and anti-oxidized LDL autoantibody titers decreased concomitantly with the reduction of lesion formation [137].

The modifying effect of hydroxysafflor yellow A (HSYA) on vascular endothelial cells (EC) induced by hypoxia and its mechanisms were evaluated. HSYA upregulated the bcl-2/bax ratio, which is downregulated under hypoxia, increased VEGF protein concentration and VEGF mRNA expression and enhanced HIF-1 alpha protein accumulation and its transcriptional activity [138]. The mechanism of regulating HIF-1alpha expression by hydroxysafflor yellow A (HSYA) in Eahy 926 cell line under 1% O2 hypoxia was studied. Eahy 926 cells were incubated with HSYA (100, 10 and 1 micromol x 10⁶) under hypoxia for the indicated time after treatment. HSYA at 100 micromol x 10⁶ increased Eahy 926 cells proliferation rate under hypoxia. HIF-1alpha mRNA and protein expression were up-regulated in the presence of HSYA. VHL, p53 mRNA and protein expression decreased significantly after 8 hours of treatment under hypoxia. Accordingly, HSYA protected Eahy 926 cells from hypoxia, and up-regulated HIF-1alpha expression partially via its inhibition of VHL and p53 expression [139].

The effect of Safflower injection was evaluated on pulmonary hypertension in rat during chronic hypoxia and hypercapnia. mPAP, weight ratio of right ventricle (RV) to left ventricle plus septum (LV + S) were much higher in rats of hypoxic hypercapnic group than those of control group. The concentration of TXB₂ and the ratio of TXB₂/6-keto-PGF₁α were significantly higher in rats of hypoxic hypercapnic group than those of control group and hypoxic hypercapnia + Safflower injection group. The results of light microscopy showed that WA/TA (vessel wall area/total area), SMC (the density of medial smooth muscle cell) and PAMT (the thickness of medial smooth cell layer) were significantly higher in rats of hypoxic hypercapnic group than those of control group and hypoxic hypercapnia + Safflower injection group. The results of electron microscopy showed proliferation of medial smooth muscle cells and collagen fibers of pulmonary arterioles in rats of hypoxic hypercapnic group, and Safflower injection reversed these changes [140].

The effect of Hydroxysafflor yellow A (HSYA) on human umbilical vein endothelial cells (HUVECs) under hypoxia was investigated. HSYA inhibited cell apoptosis and cell cycle G1 arrest induced by hypoxia. HSYA treatment increased the Bcl-2/Bax ratio of protein and mRNA, reduced p53 protein expression in cell nucleus. In addition, HSYA enhanced the NO content of cell supernatant under hypoxia, accompanied with upregulating eNOS mRNA expression and protein level. The results demonstrate that HSYA could protect HUVECs from hypoxia induced injuries by inhibiting cell apoptosis and cell cycle arrest [141].

The effects of The carthamines yellow (CY) was studied on a blood stasis model, which was obtained by placing rats in ice-cold water during the time interval between two injections of epinephrine. The results demonstrated that CY significantly decreased the whole blood viscosity, plasma viscosity, and erythrocyte aggregation index, which were increased in the blood stasis model. Hematocrit and platelet aggregation were reduced, while prothrombin time was delayed with increasing doses of CY [142].

Safflower yellow inhibited the PAF induced washed platelet aggregation and 5-HT release in a dose dependent manner. When the PAF was 2.0×10⁻⁵ mol/l, the inhibition rate of platelet aggregation was 26.2%, 41.3%, 58.1%, 81.2%, and the inhibition rate of 5-HT release was 3.7%, 11.9%, 29.9% and 54.4% after treatment with safflower yellow at 0.21, 0.42, 0.85 and 1.69 g/l, respectively. Accordingly, safflower yellow can inhibit the PAF induced platelet aggregation, 5-HT release by platelets and elevation of free calcium in platelets [143]. Intraperitoneal administration of 30 mg of an aqueous extract of the flowers to mice reduced platelet aggregation induced by adenosine diphosphate (ADP) by 65% in γ-irradiated animals [144].

**Antioxidant effect**

Antioxidative activities of serotonin derivatives isolated from safflower oil were measured by ferric thiocyanate method and DPPH method and the compounds showed storage antioxidative activity [91].

Carthamus red isolated from safflower (Carthamus tinctorius), was evaluated for antioxidant and hepatoprotective activity. An *in vivo* study against CCl₄-induced liver injury was conducted and compared with that of silymarin, a known hepatoprotective drug. Carthamus red did not show any toxicity and mortality up to 2000 mg/kg dose, and it showed strong antioxidant ability *in vitro*. In the *in vivo* study, carthamus red treatment lowered the serum levels of ALT, AST, ALP and total protein in liver damage rat models. Meanwhile, Nrf2, GSTα and NQO1 expressions were up-regulated at the protein level. Additionally, the activities of antioxidant enzymes and level of GSH were elevated by carthamus red, while the content of TBARS, which is an oxidative stress marker, was lessened. Histological examination showed that the condition of liver damage was mitigated [145].

**Carthamus tinctorius** L. seed extract (CSE) exhibited remarkable radical scavenging activities, FRAP (ferric reducing antioxidant power) and reducing power in a dose-dependent manner. Moreover, the oxygen radical absorbance capacity (ORAC) value of CSE (0.1
Antioxidative activities of serotonin derivatives isolated from safflower (Carthamus tinctorius L.) oil cake were measured by two methods. Five of serotonin derivatives were found to have relatively strong antioxidative activity [89].

Carthamus tinctorius flavonoids were evaluated against 2-deoxyribose degradation and rat liver microsomal lipid peroxidation induced by hydroxyl radicals generated via a Fenton-type reaction. Among the Carthamus tinctorius flavonoids, luteolin-acetyl-glucoside and quercetin-acetyl-glucoside showed potent antioxidative activities against 2-deoxyribose degradation and lipid peroxidation in rat liver microsomes. Luteolin, quercetin, and their corresponding glycosides also exhibited strong antioxidative activity, while acacetin glucuronide and apigenin-6,8-di-C-glucoside were relatively less active [96].

The in vitro antioxidative activities of extracts of C. tinctorius (ECT) and the main antioxidant components of ECT were determined by HPLC. The results show that flavonoids were the main components of ECT and were active in scavenging OH\(^-\) and O\(^2-\) and DPPH, in a dose-dependent manner [146].

Free radical scavenging activity of the extracts of petals (bud, early stage, full blooming and ending stage), leaf, stem, root and seeds of Mogami-benihana (safflower, Carthamus tinctorius Linne) was evaluated. The scavenging activities of the extract of safflower petals with various colors showed antioxidant activity. There was also a relationship between DPPH radical scavenging activity and carthamin content in the petal extracts of safflower [114].

In studying the antioxidative effects of water extract of Carthamus tinctorius on ox-LDL induced injury in rat cardiac microvascular endothelial cell and detecting oxygen derived free radicals (OFR) to explore the antioxidant mechanisms. It appeared that water extract of C. tinctorius increased the CMEC survival rate, reduced LDH, MDA and XOD levels, and improved SOD, GSH-Px and NOS activity, while in the cell suspension ROS signal decreased significantly [147].

The potential protective effects of C. tinctorius flower extract (CFE) against reactive oxygen species (ROS) induced osteoblast dysfunction were investigated using osteoblastic MC3T3-E1 cells. The osteoblast function was assessed by measuring alkaline phosphatase activity, collagen content, calcium deposition, and RANKL production, and the oxidative stress was assessed by measuring intracellular lipid peroxidation, and protein oxidation in osteoblastic MC3T3-E1 cells. A significant reduction in the alkaline phosphatase activity, collagen, and calcium deposition and an increase in the production of receptor activator of nuclear factor-kB ligand (RANKL) were observed after 0.3 mM H\(_2\)O\(_2\) addition. The H\(_2\)O\(_2\)-induced alterations were prevented by pre-incubating the osteoblasts with 2-10 microg/ml CFE for 48 h. When the oxidative stress was induced by H\(_2\)O\(_2\), the increased production of protein carbonyl and malondialdehyde was also reduced at the same CFE concentration [148].

The protective effect of safflower yellow B (SYB) was investigated on the acute oxidative injury induced by H\(_2\)O\(_2\) in PC12 cells. The results showed that exposure of the cells to H\(_2\)O\(_2\) significantly decreased the cell viability, SOD and GSH-Px activities and Bcl-2 expression, and increased LDH release, superoxide anion and MDA generations, caspase 3 activity and Bax expressions. Pretreatment of the cells with SYB was able to remarkably antagonize the H\(_2\)O\(_2\)-induced changes in dose-dependent way. SYB is able to protect PC12 cells from H\(_2\)O\(_2\)-induced injury and apoptosis via antioxidant and anti-apoptotic mechanisms [149].

**Hypolipidemic effect**

The effect of the extracts from safflower was investigated on cholesterol metabolism in high cholesterol fed rats. After treatment for 14 and 30 days, a significant reduction in total cholesterol and total cholesterol/HDL-cholesterol and a significant induction in HDL-cholesterol were observed in the hypercholesterolemic rats treated with the dichloromethane extract. Higher expression of SRBI and ABCA1 in the liver of the control group was observed after 4 weeks whereas no significant difference in the expression level of SRBI and ABCA1 was found in groups treated with extract after 2 and 4 weeks. The authors suggested that the expression of SRBI and ABCA1 mRNA may not be regulated by the crude extract of safflower, which may not in part explain the decrease in HDL-cholesterol and gene encoding enzymes of the cholesterol biosynthetic pathway [150].

The inhibitory effects of defatted safflower seed extract (SSE) and serotonin derivatives (N-p-coumaroylserotonin and N-feruloyl serotonin, CS+FS), were evaluated on hypercholesterolemia and atherosclerosis, using Pulse wave velocity (PWV) in Kurosawa and Kusanagi-hypercholesterolemic rabbits. The atherosclerotic lesioned area in the aorta was significantly reduced in the SSE and CS+FS groups, without significant changes in serum cholesterol and triglyceride levels among the three groups after supplementation. Local PWV (LPWV) in the middle thoracic and distal abdominal aortas was significantly smaller in the SSE and CS+FS groups than in the control group. PWV in the entire aorta was also significantly lower in the SSE and CS+FS groups, compared with that in the control group. Pressure-strain elastic modulus, an index of wall distensibility, was significantly lower in the middle thoracic and middle abdominal aortas in the SSE and CS+FS groups than in the control group. Wall thickness was also significantly smaller in the middle thoracic aorta
in the SSE and CS+FS groups compared with that in the control group [93].

**Antidiabetic effect**

The antidiabetic effect of *Carthamus tinctorius* was studied on fasting blood glucose and insulin levels in alloxan induced diabetic rabbits. Diabetic animals were treated with *Carthamus tinctorius* extract at doses of 200 and 300 mg/kg body weight. Extract were given orally for 30 days and the values for blood glucose levels were observed after 15th and 30th day of treatment. While insulin levels were checked at the end of the study. Animals were also observed for any gross toxicity during the study. Results revealed that *Carthamus tinctorius* exerted significant hypoglycemic effect at 200 mg/kg and 300 mg/kg doses as compared to diabetic control group. Insulin levels were significantly increased in *Carthamus tinctorius* treated groups as compared to diabetic control [151].

The chemical components isolated from safflower seed (*Carthamus tinctorius* L.) were evaluated as α-glucosidase inhibitors. The compounds appeared as active α-glucosidase inhibitors were serotonin derivatives (e.g. N-p-coumaroyl serotonin and N-feruloyl serotonin). These compounds showed a potent inhibitory activity, the 50% inhibitory concentration values were calculated as 47.2 μm and 99.8 μm respectively, while that of the reference drugs acarbose and 1-deoxynojirimycin were estimated as 907.5 μm and 278.0 μm, respectively. Regarding the structure of the serotonin derivative, the existence of the hydroxyl group at 5-position in the serotonin moiety and the linkage of cinnamic acid and serotonin were essential for α-glucosidase inhibitory activities. The authors suggested that these results are helpful for the proper use of safflower seed as traditional medicine for the treatment of diabetes, moreover, it could serve to develop medicinal preparations as supplements and functional foods for diabetics [152].

**Protective effect against bone loss**

Safflower seed has been reported to have a protective effect against bone loss diseases. However, the precise molecular mechanisms underlying the inhibitory effect of safflower seed in osteoclast differentiation remain unclear. The probable inhibitory action of safflower seed extract (SSE) on the receptor activator of nuclear factor κB ligand (RANKL)-induced osteoclastogenesis in cultured mouse-derived bone marrow macrophages (BMMs) was investigated. SSE significantly inhibited the formation of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells in BMMs without cytotoxicity. The gene expressions of nuclear factor of activated T-cells (NFATc1) and TRAP, which are genetic markers of osteoclast differentiation, were substantially decreased by SSE in a dose-dependent manner. Also, SSE diminished RANKL-mediated intracellular reactive oxygen species (ROS) generation on osteoclastogenesis in a dose-dependent manner. The SSE, thereafter suppressed RANKL-induced p38 mitogen-activated protein kinase and IkBα kinase signalling activities which were activated by ROS generation for osteoclastogenesis. Additionally, SSE was found to decrease RANKL-induced actin ring formation, which is required for bone resorption activity [153].

Anti-bone resorption properties of the Korean herbal formulation, Honghwain (HHI; *Carthamus tinctorius* L. seed) was biochemically investigated. HHI inhibited in vitro and in vivo bone resorption by inhibition of phosphorylation of peptide substrates. HHI dose-dependently reduced the hypercalcemia induced in mice by IL-1β and partly prevented bone loss and microarchitectural changes in young ovariectomized rats, the protective effect on bone was exerted via the inhibition of bone resorption. The results indicated that the synergy between IL-1β, TNF-α. IL-6 on PGE2 production is due to an enhanced gene expression of COX-2 and that tyrosine kinase(s) are involved in the signal transduction of COX-2 in mouse calvarial osteoblasts [154].

The production of PGE2 is inhibited by 20-100 microg/ml HHI in nontransformed osteoblastic cells (MC3T3-E1 cells), indicating that HHI inhibited PGE2 production. The effect of HHI on the proliferation and osteoblastic differentiation in MC3T3-E1 was also studied. HHI dose-dependently increased DNA synthesis (significant at 20-100 microg/ml), and increased alkaline phosphatase (ALP) and prolyl hydroxylase activities of MC3T3-E1 cells (20-100 microg/ml), while anti-estrogen tamoxifen eliminated the stimulation of proliferation and ALP activity of MC3T3-E1 which was induced by HHI. The results indicated that HHI directly stimulates cell proliferation and differentiation of osteoblasts. Also, when the effects of HHI was examined on osteoblastic differentiation in MC3T3-E1, HHI enhanced ALP activity and mineralization in a dose- and time-dependent fashion. This stimulatory effect of the HHI was observed at relatively low doses (significant at 20-100 microg/ml and maximal at 100 microg/ml). Northern blot analysis showed that the HHI (60 microg/ml) increased bone morphogenetic protein-2 as well as ALP mRNA concentrations in MC3T3-E1 cells. HHI (100 microg/ml) slightly increased type I collagen mRNA abundance throughout the culture period, whereas it markedly inhibited the gene expression of collagenase-1 between days 15 and 20 of culture. The results also indicated that HHI has anabolic effect on bone through the promotion of osteoblastic differentiation, suggesting that it could be used for the treatment of common metabolic bone diseases [155].

The effects of Safflower (*Carthamus tinctorius*) seed oil (SSO) on osteoporosis induced-ovariectomized rats were investigated. Animals were administered SSO
oraly (1 ml/kg) daily for 30 days. IGF-I, IGF-II, IGBP-3 and BALP levels were significantly increased (p<0.05). The results showed that the safflower seeds have possible roles in the improvement of osteoporosis induced-ovariectomized rats [156].

The bone nodule formation, calcium uptake, alkaline phosphatase activity, and intracellular concentration of calcium ion Ca2+ was studied in murine osteoblastic cells of the MC3T3-E1 line, that were cultured on modified Eagle's minimal essential medium alone (controls) or with addition of 0.1% crude extract of safflower seed or 0.1% aqueous fraction of safflower seed. Fluorescence spectrometry measurement of Ca2+ showed significantly accelerated rates of osteoblast differentiation with 0.1% crude extract of safflower seed (3 microl of crude extract in 8 x 10⁴ cells) and with 0.1% aqueous fraction of safflower seed (2 microl of aqueous fraction in 8 x 10⁴ cells) compared to the control group [157].

**Antitumor effect**

The in vitro effects of dichloromethane, methanol and hexane extracts of *Carthamus tinctorius* on caspase-dependent anti-tumor activity against human colon carcinoma SW620 cell lines were investigated. In addition, the immunomodulatory activity of each solvent extract was examined. Only dichloromethane extract of *C. tinctorius* exhibited inhibitory effect on growth of SW620 cells with IC₅₀ of 0.15 mg/ml, in comparison to the Hep2 (0.5 mg/ml) and control BHK cells (0.6 mg/ml). Moreover, it was associated with up-regulation of caspase 3, 7 and 9 and down regulation of Bcl2 transcripts in treated SW620 cell. The dichloromethane extract showed the highest stimulatory effect on the lymphocyte proliferation with an increase of 8±1.6 fold, followed by the methanol and hexane extract with increases of 12±1.1 and 14±1.6 fold, respectively [158].

The effects of *Carthamus tinctorius* (CT) on the dendritic cell (DC)-based vaccine in cancer treatment, cytokine secretion of mouse splenic T lymphocytes and the maturation of DCs in response to CT were analyzed. To assess the antitumor activity of CT extract on mouse CD117+ (c-kit)-derived DCs pulsed with JC mammal tumor antigens, the JC tumor was challenged by the CT-treated DC vaccine in vivo. CT stimulated IFN-γ and IL-10 secretion of splenic T lymphocytes and enhanced the maturation of DCs by enhancing immunological molecule expression. When DC vaccine was pulsed with tumor antigens along with CT extract, the levels of TNF-α and IL-1β were dramatically increased with a dose dependent response and more immunologic and co-stimulatory molecules were expressed on the DC surface. In addition, CT treated tumor lysate-pulsed DC vaccine reduced the tumor weight in tumor-bearing mice by 15.3% more than tumor lysate-pulsed DC vaccine without CT treatment. CT polarized cytokine secretion toward the Th1 pathway and also increased the population of cytotoxic T lymphocytes *ex vivo* [159].

The drug resistance index of the total extract of Carthami Flos (CF) and the dried flower of safflower, in MDR KB-V1 cells and its synergistic effects with other chemotherapeutic agents were studied. SRB cell viability assays were used to quantify growth inhibition after exposure to single drug and in combinations with other chemotherapeutic agents using the median effect principle. The combination indexes were then calculated according to the classic isobologram equation. The results revealed that CF showed a drug resistance index of 0.096. In combination with other chemotherapeutic agents, it enhanced their chemo-sensitivities by 2.8 to 4.0 folds and gave a general synergism in cytotoxic effect. The results indicate that CF could be a potential alternative adjuvant antitumour herbal medicine representing a promising approach to the treatment of some malignant and MDR cancers in the future [160].

A compound (Zhu-xiang) from herbal extracts containing ginseng and *Carthamus tinctorius* was used to treat the MDA-MB-231 breast cancer cell and normal human mammary gland cell lines. The Zhu-xiang showed significantly inhibition in cell proliferation and the inhibition was dose dependent. The inhibitory effect of Zhu-xiang was significantly greater than that of commonly used cytotoxic drugs. The inhibitory effect was a result of the induction of apoptosis, which was concentration- and time-dependent. DNA histograms indicate that the compound caused accumulation of cells mainly in the S phase. The viability of cells in breast solid tumours was measured by ATP bioluminescence assay to determine the drug-induced cytotoxicity of Zhu-xiang. The three different concentrations of Zhu-xiang all exhibited the ability to inhibit proliferation in solid tumour [161].

The mixture of erythro-alkane-6,8-diols from the flowers of *C. tinctorius* markedly suppressed the promoting effect of TPA (12-0-Tetracanoylphorbol-13-acetate) on skin tumor formation in mice following initiation with 7,12-dimethylbenz[a]anthracene [98]. The anti-tumor activity of safflower polysaccharide (SPS) was examined in vivo and in vitro. The transplanted tumor model of LA795 lung cancer was established with T739 mouse and safflower polysaccharide (SPS) 40mg/kg was administered ip for 10 days and the tumor weight and the cytotoxicity of CTL cells, NK cells were detected. The Anti-tumor activity of SPS on three types of tumor cells in vitro was observed with trypan blue exclusion staining. SPS can significantly inhibit the growth of S180 Sarcoma in mice with an inhibitory rate of 51.33% (P<0.01). It can also inhibit the growth of LA795 lung cancer in mice and the tumor volume was reduced obviously for 3.29 mm³ (P<0.05). It can remarkably enhance the cytotoxicity of splenic CTL cells, NK cells in tumor-bearing (P<0.05) [162].
**Immunological effects**

The polysaccharide of *Carthamus tinctorius* modulated immune function in mice [51]. Safflower yellow (SY) produced declines in both nonspecific and specific immune functions. Administration of safflower yellow (SY) ip 50-450 mg/kg/day for 6-8 days in mice decreased serum lysozyme concentration and phagocytosing functions of both peritoneal macrophages and peripheral leukocytes; diminished the production of plaque forming cells, specific rosette forming cells, and antibody production; inhibited delayed type hypersensitivity reaction and the activation of T suppressor cells elicited by supraoptimal immunization. *In vitro* experiments showed inhibitory effects on [3H]TdR incorporation during human peripheral T- and B-lymphocyte proliferation by SY 0.03-3.0. 0.1-2.0 mg/ml respectively, murine mixed lymphocyte culture response and the production of interleukin-2 by SY 0.1-2.5 mg/ml. In conclusion, SY produced declines in both nonspecific and specific immune functions [163].

N-(p-coumaroyl)serotonin and N-(p-coumaroyl) tryptamine, active ingredients in CT, were shown to strongly inhibit the production of proinflammatory cytokines (IL-1α, IL-1β, IL-6, IL-8 and TNF-α) from lipopolysaccharide-stimulated human monocytes [46]. HSYA treatment increased adhesion potency (HSYA dose 1.0 x 10^5 mol x L^-1), free calcium concentration (HSYA dose 3.1 x 10^5 mol x L^-1), TNF-alpha and IL-6 mRNA expression elevation (HSYA dose 5.2 x 10^5 mol x L^-1) induced by LPS. HSYA also inhibited NF-kappaB p65 subgroup nuclear translocation (HSYA dose 5.2 x 10^-5 mol x L^-1) [164].

**Antimicrobial effects**

The antibacterial activity of methanol extract of *Carthamus tinctorius* was evaluated against *H. pylori*. The inhibition zone of methanol extract of *Carthamus tinctorius* at concentration 2 mg/disc against *H. pylori* clinical isolates was 18.77±0.56mm, while, MIC and MBC for the same extract were 691.25 691.25 μg/ml respectively [165].

An ethanol extract of the flowers inhibited the growth of *Staphylococcus aureus* in vitro at a concentration of 0.5 mg/plate, but was not effective against *Escherichia coli* [166]. A 95% ethanol extract of the flowers inhibited the growth of *Bacillus subtilis*, *Candida albicans*, *Salmonella typhosa* and *Staphylococcus aureus* in vitro at a concentration of 100 μg/plate, but was not effective against *E. coli* and *Shigella dysenteriae* [167].

The antiviral activity of *Carthamus tinctorius* L. (CT) was examined against gamma herpes virus infection. The results showed that treatment with CT extracts disrupted KSHV latency in the viral-infected host cells. n-Hexane and ethanol fractions of CT extracts critically affected at least two stages of the KHSV life-cycle by abnormally inducing KSHV lytic reactivation and by severely preventing KSHV virion release from the viral host cells. In addition to the effects on KSHV itself, CT extract treatments induced cellular modifications by dysregulating cell-cycle and producing strong cytotoxicity [168]. A hot aqueous extract of the flowers inhibited replication of poliomyelitis virus type 1 *in vitro* [169].

**Anti-inflammatory antipyretic and analgesic effects**

Intragastric administration of 30 mg/kg bw of a 50% methanol extract of the flowers inhibited inflammation as measured by footpad oedema induced by carrageenan, serotonin, bradykinin, histamine or prostaglandin in mice. Intragastric administration of 30 g/kg bw of a 50% methanol extract of the flowers to mice also reduced writhing induced by acetic acid [124]. Subcutaneous administration of 10 g/kg bw of an aqueous or 50% methanol extract of the flowers inhibited carrageenan-induced footpad oedema in mice. Subcutaneous administration of 10.0 g/kg bw of an aqueous extract of the flowers to mice did not reduce pain perception as measured in the hot-plate test. Subcutaneous administration of 1.0–3.0 g/kg bw of a 50% methanol extract of the flowers to mice reduced writhing induced by acetic acid [109].

The effects of Hydroxysafflor yellow A (HSYA) on lipopolysaccharide (LPS)-induced inflammatory signal transduction in human alveolar epithelial A549 cells was studied. A549 cells stimulated with LPS were incubated with three doses of HSYA (1, 4 and 16μmol/l). HSYA suppressed the expression of TLR-4, Myd88, ICAM-1, TNFα, IL-1β and IL-6 at the mRNA and protein level, and inhibited the adhesion of leukocytes to A549 cells. HSYA treatment also decreased NF-kB p65 nuclear translocation and inhibited the phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK) [171].

The effects of dried safflower petals aqueous extracts (SFA) and Carthamus yellow (CY) were investigated on lipopolysaccharide (LPS)-induced inflammation using RAW264.7 macrophages. Treatment with SFA (1-1000 microg/ml and CY (1-2000 microg/ml does not cause cytotoxicity. SFA and CY inhibited LPS-stimulated nitric oxide (NO), prostaglandin E2 (PGE_2), and interleukin 1β (IL-1β) release, through attenuation of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expression. Furthermore, SFA and CY suppressed the LPS-induced phosphorylation of nuclear factor-kB, which was associated with the inhibition of IκB-α degradation [172].

N-(p-Coumaroyl)serotonin (CS) inhibited proinflammatory cytokine generation from human monocytes *in vitro*. CS augmented the proliferation of normal human and mouse fibroblast cells. The cells continued to proliferate in the presence of CS and form a transformed cell-like focus without transformation. CS, however, does not augment the proliferation of other cell
types, either normal or tumor cells. CS augmented the proliferation of fibroblasts in synergy with basic fibroblast growth factor (bFGF) or epidermal growth factor (EGF), but not with acidic FGF(aFGF) or platelet-derived growth factor (PDGF) [173].

The inhibitory effect of HSYA was studied on the inflammatory signal transduction pathway related factors which were induced by permanent cerebral ischemia in rats. The result showed that intravenous injection of HSYA (10 mg/kg) to rats after cerebral occlusion, the p65 translocation activity and the phosphorylation of IkappaB-alpha were significantly inhibited. At the same time, HSYA suppressed p65 binding activity and the transcriptional level of pro-inflammatory cytokines including TNF-alpha, IL-1beta and IL-6, and promoted the mRNA expression of anti-inflammatory cytokine IL-10. The authors suggested that the anti-cerebral ischemic mechanism of HSYA may be due to its inhibition of NF-kappaB activity and the mRNA expression of cytokines in the inflammatory transduction pathway [174].

Alkane diols inhibited the inflammation induced by 12-O-Tetradecanoylphorbol-13-acetate (TPA, 1 microgram/mice ear). The 50% inhibitory dose of these compounds for TPA-induced inflammation was 0.5-0.7 mg/ear [175]. A new bioactive triterpenoid saponin 3beta-O-[beta-D-xlylopyranosyl(1→3)-O-beta-D-galacto pyranosyl]-1up-12-ene-28ioc acid-28-O-alpha-L-rhamno pyranosyl ester, isolated from the methanolic fraction of the roots of *Carthamus tinctorius*, showed anti-inflammatory activity [99]. All the polyacetylene glucosides compounds isolated from the florets of *Carthamus tinctorius* (11 compounds) were also tested for antinflammatory and inhibitory activities against LPS-induced NO production in murine macrophages, they showed weak activities at concentrations of 1×10^{-5}M [100]. The mechanism of anti-inflammatory effect of the methanol extracts of *Carthamus tinctorius* (MEC) was investigated. The results showed that the expression of HO-1 protein by MEC in macrophages was increased in a concentration- and time dependent manner. Treatment with MEC significantly inhibited upregulation of both iNOS and COX-2 in LPS-activated macrophages and consequently reduced production of NO and PGE2. The reduced expression of iNOS and COX-2 by MEC was reversed by siHO-1 RNA transfection. In addition, NF-E2-related factor (Nrf2) was translocated from cytosol to nucleus by MEC. The binding of NF-E2-B as well as NF-E2 luciferase activity was also significantly diminished by MEC. Tumor necrosis factor (TNF)-α-mediated VCAM-1 expression in endothelial cell was significantly inhibited by MEC [176].

Intragastric administration of 500 mg/kg body weight (bw) of a 95% ethanol extract of Flos Carthami reduced the responsiveness of mice as measured in the hot-plate test, indicating an analgesic effect, and also decreased yeast-induced fevers [177].

**Hepatoprotective effect**

Hepatoprotective activity of methanolic extract of leaves of *Carthamus tinctorius* (MECT) was investigated against hepatotoxicity produced by administering a combination of two anti-tubercular drugs isoniazid and rifampicin for 24 days by oral route in rats. MECT were administered at two graded dose (200 and 300 mg/kg po) 45 min after anti-tubercular challenge for 24 days. MECT, in all doses caused significant decrease in AST, ALT, ALP, and total bilirubin levels and elevated the level of GSH [178]. The potential protective effect of HSYA was investigated in liver fibrosis induced by carbon tetrachloride (CCL4)-induced in rats. HSYA was given in a daily dose of 5 mg/kg intraperitoneally with concurrent CCL4. CCL4 treatment induced micronodular liver fibrosis with a pronounced deposition of collagen fibers. HSYA significantly reduced liver fibrosis. It down regulates α-smooth muscle actin (SMA), collagen α type I, matrix metalloproteinases (MMP)-9, and tissue inhibitors of metalloproteinases (TIMP)-1 gene expression. This was accompanied by a decreased expression of transforming growth factor (TGF)-β1 and phosphorylation [179].

The effect of Safflower injection on the lipid peroxidation level and expression of heme oxygenase-1 of the rat liver with chronic hypoxia and hypercapnia was studied in rats. The activity of SOD of the liver in Safflower injection group was significantly higher than those in chronic hypoxia and hypercapnia for four weeks group, and the content of MDA was significantly lower. In chronic hypoxia and hypercapnia for four weeks group, there were multiple dispersed immunoreactivity cells in liver, the immunoreactivity cells were significantly decreased in Safflower injection group. Histological study revealed that there were many hepatocytes with obvious adipose degeneration. Hepatic pathological damage in Safflower injection group was slighter than that in chronic hypoxia and hypercapnia for four weeks group [180].

**Lung protective effects**

The protective effect of Hydroxysafflor yellow A (HSYA) was studied on inflammatory phase of bleomycin-induced pulmonary injury in mice. Three doses of HSYA (26.7, 40, 60 mg/kg/d) were intraperitoneally injected to mice consecutively for 1 week after bleomycin administration. It was found that HSYA attenuated the loss in body weight, the increase of myeloperoxidase activity and pathologic changes of pulmonary inflammation caused by bleomycin. Treatment with HSYA also alleviated bleomycin-induced increase of mRNA level of tumor necrosis factor (TNF)-α, interleukin (IL)-1β and transforming growth factor
(TGF)-β1 in lung homogenates. Moreover HSYA inhibited the increased activation of nuclear factor (NF)-κB and phosphorylation of p38 mitogen-activated protein kinases (MAPK) in lung tissue [181]. 

The effects of Safflor yellow (SY) was studied on rats of pulmonary fibrosis induced by bleomycin (BLM) and on differentiation of lung fibroblast into myofibroblast stimulated by transforming growth factor-β1 (TGF-β1). Two dose SY (intraperitoneal, 25, 50 mg/kg/d) were administered to rats treated by BLM consecutively for four weeks. SY alleviated the loss in body weight, the increase of hydroxyproline content in the lung tissues and pathologic changes of pulmonary fibrosis caused by BLM instillation. SY also prevented the increase of α-SMA positive cells and TGF-β1 expression induced by BLM. These effects were more significant with the using of high dose of SY. Moreover, SY (0.05, 0.25, 1.25 mg/ml) inhibited the expression of α-SMA during differentiation of lung fibroblast into myofibroblast stimulated by TGF-β1 [182].

The attenuated effect of Hydroxysafflor yellow A (HSYA) on acute lung injury (ALI) induced by lipopolysaccharide (LPS) administration was studied in mice. HSYA administration significantly attenuated inflammatory cell infiltration and alleviated pulmonary edema induced by LPS. Moreover, HSYA decreased NF-κB p65 nuclear translocation, inhibited proinflammatory cytokine TNF-α, IL-1β and IL-6 mRNA expression and promoted antiinflammatory cytokine IL-10 gene expression following LPS injection. Pulmonary p38 MAPK phosphorylation was upregulated 4 h after LPS treatment, which could be suppressed by pretreatment with HSYA [183]. The pharmacological effect and mechanism of action of hydroxysafflor yellow A (HSYA) on acute lung injury (ALI) was studied in rats. HSYA alleviated pulmonary edema, reduce acidosis, keep PaO2 from descending, inhibit inflammatory cell infiltration, inhibit rat lung TNF-alpha and ICAM-1 mRNA expression and plasma IL-6 and IL-1beta level elevation [184].

Anti-uroolithiasis

The effects of Flos carthami (FC) (600 and 1200 mg/day, by gastric gavages), was evaluated on calcium oxalate (CaOx) formation in ethylene glycol (EG)-fed rats. 24-h urine and blood samples were analyzed at the beginning and end of the experiment. Kidney tissue was histopathologically examined using a polarized light microscope, and crystal deposits were evaluated by a semi-quantitative scoring method; these scores were significantly lower in the FC groups (600 and 1200 mg/day) than in the placebo group [185].

Effect on tendon injury-repair

The effects of safflower yellow (SY) on pathologic changes in tendon, expression of basic fibroblast growth factor (bFGF) and collagen type I, and on the process of tendon injury-repair were investigated. The adhesion to surrounding tissues and tensile strength gradually increased after the injury and repair in control (no-SY) tendons, and were significantly greater by the sixth weeks than any other time. In the SY tendons, adhesion was significantly lower, and tensile strength significantly higher than in (no-SY) tendons at the same post-injury-suture time points. An inflammatory reaction was observed in the injury-repair areas of the tendon by the end of first week post-injury-suture, and reached its peak by the end of second week. The inflammatory reaction was significantly less in SY tendons than in controls. Immunostaining for bFGF in the tendon injury-repair areas by the end of first week, and the number of bFGF positive cells reached a peak by the end of second week, with a greater abundance in SY than control tendons from the second to sixth week. Expression of collagen type I protein was observed in the injury-repair areas as well, coincident with bFGF, and was remarkably higher in SY than in controls. The results indicated that SY promoted the repair of injured tendon by up-regulating expression of bFGF and collagen type I protein [186].

Reproductive effect

The effects of aqueous extract of Carthamus tinctorius was tested on mouse spermatogenesis. Histopathological criteria such as epithelial vacuolization, sloughing of germ and detachment were significantly decreased in Carthamus tinctorius L. treated mice (p<0.001). Carthamus tinctorius extract induced formation of multinucleated giant cells in the germinal epithelium. It also caused a significant decrease in seminiferous tubule diameter, seminiferous epithelium height and maturation arrest (p<0.001). Accordingly, Carthamus tinctorius extract has toxic effects on mouse testicular tissue, and it was recommended to be used with caution with reproductive problem [187].

In order to evaluate the safety of the flowers of Carthamus tinctorius, the teratogenic effects of carthami flos on the central nervous system development in mice was investigated. Furthermore, its cytotoxic effect on the rat nervous cell culture was studied. The pregnant mice were treated with different dosage regimens of aqueous carthami flos extract during 0-8 days of gestation. Embryos were then isolated at the 13th gestation day and evaluated for macroscopic, microscopic and morphometric characteristics. The results showed that in higher doses (1.6 and 2 mg/kg/day) the embryos were absorbed, whereas with lower dose (1.2 mg/kg/day) changes in external, internal and longitudinal diameters, open neuropore, changes in cellular orientation and cellular degeneration were observed [188].

The lignan glycoside, tracheloside, was tested as an anti-estrogenic principle against cultured Ishikawa
cells. Tracheloside significantly decreased the activity of alkaline phosphatase (AP), an estrogen-inducible marker enzyme, with an IC$_{50}$ value of 0.31 microg/ml, a level of inhibition comparable to that of tamoxifen (IC$_{50}$=0.43 microg/ml) [101]. The decoction of *Carthamus tinctorius* exerted stimulating action on the uterus of mouse in *vitro*. The stimulating action of *Carthamus tinctorius* has been found related to the stimulating effects on H1-receptor and alpha-adrenergic receptor of uterus [189]. On the other hand, intraperitoneal administration of a hot aqueous extract of the *Carthamus tinctorius* flowers increased uterine contractions in pregnant female rats [169].

**Gastrointestinal effects**

Inhibition zone of the methanol extract of *Carthamus tinctorius* at concentration of 2 mg/disc against *H. pylori* clinical isolates was 18.77±0.56mm, while, MIC and MBC for the same extract were 691.25 and 691.25 μg/ml, respectively [165]. 200 and 400mg/kg of *Carthamus tinctorius* extract with carbachol protected rat from gastric ulceration after pylorus ligation. The doses were significantly decreased volume of gastric secretion, free acidity, mEq/dl of gastric secretion, total acidity, mEq/dl of gastric secretion and ulcer index. They significantly increased the PH of gastric juice and gave 78 and 83% gastric protection respectively [190].

**Maintenance of skin and hair promoting Effect**

It appeared that *Carthamus tinctorius* was sufficiently characterized for the maintenance of skin and hair when used as safflower seed oil 314 and 50 mg/day respectively [191]. The potential of hydroxysafflor yellow A-rich *C. tinctorius* extract (CTE) was examined on hair growth both in *vitro* and in *vivo*. The effect of CTE on cell proliferation and hair growth-associated gene expression in dermal papilla cells and keratinocytes (HaCaT) was determined. In addition, hair follicles from mouse neonates were isolated and cultured in media supplemented with CTE. Moreover, CTE was applied topically on the hair-shaved skin of female C57BL/6 mice, and the histological profile of the skin was investigated. *C. tinctorius* floret ethanolic extract promoted the proliferation of both dermal papilla cells and HaCaT and significantly stimulated hair growth-promoting genes, including vascular endothelial growth factor and keratinocyte growth factor. In contrast, CTE suppressed the expression of transforming growth factor-β1 that is the hair loss-related gene. Furthermore, CTE treatment resulted in a significant increase in the length of cultured hair follicles and stimulated the growth of hair with local effects in mice [192].

**Other effects and uses**

The pigments extracted from the plant were utilized as a natural color in ice cream to replace the synthetic color. The chemical characteristics of ice cream were significantly increased (P<0.05) with the addition of carthamin extract in ice cream. The sensory evaluation of the ice cream fortified with carthamin was carried out by the panel of 10 trained judges using 9-point Hedonic scale. The addition of carthamin (0.06 ml) in the ice cream was found to score higher overall acceptability. Furthermore addition of the safflower yellow 0.09 ml scored less for color of the ice cream [193].

Safflower (*Carthamus tinctorius*) seeds were evaluated for melanogenesis inhibitory activity and its active principles were identified following activity-guided isolation. The 80% aqueous methanol extract and ethyl acetate fraction from safflower seeds showed a significant inhibition for mushroom tyrosinase. Three active compounds, N-feruloylserotonin, N-(p-coumaroyl)serotonin, and acacetin, were isolated from the ethyl acetate fraction as the active principles. Compared with arbutin (IC$_{50}$=0.223 mM), the IC$_{50}$ values were 0.074, and 0.779 mM for N-feruloylserotonin, N-(p-coumaroyl)serotonin, and acacetin, respectively. It was also found that N-feruloylserotonin and N-(p-coumaroyl)serotonin strongly inhibited the melanin production of *Streptomyces bikiniensis* and B16 melanoma cells in comparison with a known melanogenesis inhibitor, arbutin [74].

**Adverse effects and contraindications**

No health hazards or side effects are known in conjunction with the proper administration of designated therapeutic dosages [60].

The intraperitoneal median lethal dose (LD$_{50}$) of a decoction of the flowers in mice was 1.2 g/kg bw. The intravenous LD$_{50}$ of 50% ethanol extract of the flowers in mice was 5.3 g/kg bw. The intravenous LD$_{50}$ in mice was 2.35 g/kg bw, and oral LD$_{50}$ was >8.0 g/kg. Intraperitoneal administration of 12.5 g/kg of a decoction of the flowers per day for 2 days to mice cause death. Chronic administration of 0.015–1.5 g/kg bw of carthamin in the diet per day for 3 months had no toxic effects on the heart, liver, kidneys or gastrointestinal tract of young rats. However, in human, it increases menstrual flow and cause dizziness, skin eruptions and transient urticaria [194]. As a result of its uterine contraction stimulatory effect, it should not be administered during pregnancy. It was also contraindicated in haemorrhagic diseases, peptic ulcers and excessive menstruation [60, 169, 194].

**Daily Dosage:** The average daily dose is 3-9 g of the Flos Carthami as an infusion or decoction [51, 60, 69].

**CONCLUSION**

*Carthamus tinctorius* is a plant with wide range of chemical constituents which exerted many pharmacological effects. There is a great promise for development of novel drugs from *Carthamus tinctorius* to treat human diseases as a result of its effectiveness and safety.
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