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Bioactive Metabolites and Pharmacology of *Cistanche Tubulosa*- A Review

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Abstract: *Cistanche tubulosa* contained many bioactive metabolites, included phenylethanoid glycosides (cistantubuloside A, cistantubuloside B, echinacoside, cistanoside A, acteoside, isoacteoside, 2-acetylacteoside, cistanoside C and tubuloside), syringalide A 3-O-α-L-rhamnopyranoside, campneoside I, iridoids, iridoid glycosides (kankanose, kankanol, kankanoside A, B, C, D, F, H1, H2 and I), phenylethyl oligosaccharides, terpenes and lignans. The plant produced many pharmacological effects included, memory enhancing, hypolipidemic, hair promoting antimicrobial, antidiabetic, antiosteoporotic, hepatoprotective, anti-inflammatory, neuroprotection and antiparkinsonian, anti-fatigue, vasorelaxant and reproductive effects. This review will highlight the chemical constituents and pharmacological effects of *Cistanche tubulosa.*
Keywords: *Cistanche tubulosa*, chemical constituents, pharmacology

I. INTRODUCTION:

Plants generally produce many secondary metabolites which are bio-synthetically derived from primary metabolites and constitute an important source of chemicals which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives. Recent reviews showed that the medicinal plants possessed wide range of biological effects included central nervous, cardiovascular, antioxidant, endocrine and reproductive, gastro-intestinal, respiratory, antidiabetic, antimicrobial, antiparasitic, dermatological, anticancer, anti-inflammatory, antipyretic, analgesic, immunological[1-36] and many other pharmacological effects. Many bioactive compounds were isolated from *Cistanche tubulosa*, included phenylethanoid glycosides (cistantubuloside A, cistantubuloside B, echinacoside, cistanoside A, acteoside, isoacteoside, 2-acetylacteoside, cistanoside C and tubuloside), syringalide A 3-O-α-L-rhamnopyranoside, campneoside I, iridoids, iridoid glycosides (kankanose, kankanol, kankanoside A, B, C, D, F, H1, H2 and I), phenylethyl oligosaccharides, terpenes and lignans. The plant produced many pharmacological effects included, memory enhancing, hypolipidemic, hair promoting antimicrobial, antidiabetic, antiosteoporotic, hepatoprotective, anti-inflammatory, neuroprotection and antiparkinsonian, anti-fatigue, vasorelaxant and reproductive effects. This review was designed to highlight the chemical constituents and pharmacological effects of *Cistanche tubulosa.*

Plant profile:
Synonyms:
Cistanche lutea,*Cistanche tubulosa* f. *albiflora*,*Cistanche tubulosa* var. *albiflora*,*Cistanche tubulosa* var. *toment osa*[37].

Common names:
Arabic: Haluk, Tarthuth, Danum, Tarfas; **Chinese**: Rou Cong Rong; **English**: Cistanchis, Desert Hyacinth, Broomrape, yellow broomrape; **French**: Cistanque.
Family: Orobanchaceae
Distribution:
Cistanche tubulosa, an orobanchaceae parasitic plant found in Africa, Asia and Arabia[38-40].

Description:
Perennial parasitic, yellowish to yellow-brown, fleshy herb, herb, 15-40 cm tall, with a purplish tinge, simple, erect, glabrous to puberulous, often broader at base. Densely covered with triangular or lanceolate, 1-4 cm long scales. Spikes dense, showy, cylindrical 10-20 cm long. Flowers yellow, 3- 4.5 cm long. Calyx lobes 5, 1- 1.5 cm long, oblong-ovate. Corolla, funnel shaped, 4-5 cm long, bright yellow to pinkish-yellow or violet. Staminal filaments, anthers densely hairy, woolly at the base, rounded or blunt at the ends. Capsules ovoid-oblong, laterally compressed, 20-25 mm long, beaked, many-seeded, seeds pitted, dark-coloured, 1 mm long. [41].

Traditional uses:

Cistanche tubulosa is commonly used in the treatment of forgetfulness, impotence and senile constipation by traditional Chinese physicians [(42)]. It is also used as tonic, for the treatment of blood circulation-related disorders, body weakness, impotence, sterility and lumbago[(38-40,43)].

Chemical constituents:

Many bioactive compounds were isolated from *Cistanche tubulosa*, included phenylethanoid glycosides (cistantubuloside A, cistantubuloside B, echinacoside, cistanoside A, acteoside, isoacteoside, 2-acetylacteoside, cistanoside C and tubuloside), syringalide A 3-O-α-L-rhamnopyranoside, campneoside I, iridoids, iridoid glycosides (kankanose, kankanol, kankanoside A, B, C, D, F, H1, H2 and I), phenylethyl oligosaccharides, terpenes and lignans [(43-48)].

Four new acylated phenylethanoid oligoglycosides, kankanosides J1, J2, K, and K2, were isolated from stems of *Cistanche tubulosa* (Orobanchaceae) together with isocampneoside I[(49)].

Kankanosides L, M, N, O and P were also isolated from fresh stems of *Cistanche tubulosa* together with eight iridoid glycosides, five acyclic monoterpene glycosides, three phenylpropanoid glycosides, and four lignan glycosides[(11)]. *Cistanche tubulosa* contents of echinacoside and acteoside were (31.8-119.9) and (7.3-25.4) mg/g respectively[(50)].

However, Xie *et al.,* isolated 16.9 mg of echinacoside and 5.1 mg of acteoside from 220 mg of *Cistanche tubulosa* extract[(51)].

The plant contained many heavy metals, the concentration of heavy metals (Fe, Zn, Ni, Co, Cu, Mn, Cd and Cr) were 63.45±0.85, 52.07±1.23, 3.04±0. 32, 2.19±0.19, 32.43±1.23, 28.97±1.23, 0.83±0.09, 0.31±0.09 and 11.72±0.88 mg/kg, respectively. However, the concentration of iron, manganese, copper, zinc and magnesium in the stem of *Cistanche tubulosa* were 242.18, 17.85, 7.96, 6.64 and 1357.50 microg/g[(51-53)].

Pharmacological effects:

Effect on memory:

The improvement of learning ability and consolidation of *Cistanche tubulosa* extract was carried out with a step down test in mice. In this method, a platform (safe area) is located on an electric wire with 36 V current and mice's learning ability and consolidation were evaluated by the time they spend on the platform and the number of electronic shocks they received. Scopolamine (which may retard learning ability) was administered before the training started, and sodium nitrite (a drug to inhibit the synthesis of protein involved in the formation of memory by inducing oxygen deficit in the brain) was administered after the training to induce learning/memory disorder. As a result, the safe area time (latency) and the number of errors (frequency that mice hit by electronic shocks) were significantly better in the *Cistanche tubulosa* extract administration group as compared to the memory consolidation dysfunction model group. *Cistanche tubulosa* extract exerted stronger activity than piracetam, a pharmaceutical agent to activate energy metabolism of brain cells. According to these results, *Cistanche tubulosa* extract significantly helped the brain to recover from scopolamine-induced learning disorder and sodium nitrite-induced memory consolidation dysfunction and it improved the learning ability and formation of memory of brain[(54)].

On the other hand, water maze test was carried out to evaluate the memory recall ability of mice. Training was conducted to create memory in mice on the routes of water maze. *Cistanche tubulosa* extract (50-400 mg/kg) were orally administered to mice every day throughout the training period, four weeks. On the last day of the training, 30% ethanol was given to mice to induce memory loss (failing to recall memorized information). The mice in the group consumed *Cistanche tubulosa* extract required shorter time to arrive destination compared to control. The rate of error was significantly lower in the group consumed *Cistanche tubulosa* extract. *Cistanche tubulosa* demonstrated stronger activity than piraceetam. Accordingly, *Cistanche tubulosa* extract improved the ability to elicit or recall memorized information[(55)].

The ameliorating effects of *Cistanche tubulosa* extract which was quantified with three phenylpropanoid glycosides was studied in Alzheimer's disease (AD)-like rat model. Amyloid β peptide 1-42 (Aβ 1-42) intracisternally infused rats by osmotic pump was used as an AD-like rat model. The major pathological makers were measured including Aβ 1-42 immunohistochemical stain, behavioral tests (inhibitory avoidance task and Morris water maze) and central neurotransmitter functions. Aβ 1-42 caused cognitive deficits, increased amyloid deposition and acetylcholine esterase activities, and decreased the levels of brain's acetylcholine and dopamine. Daily administration of *Cistanche tubulosa* extract throughout Aβ 1-42 infusion periods ameliorated the cognitive deficits, decreased amyloid deposition and reversed cholinergic and hippocampal dopaminergic dysfunction caused by Aβ 1-42 [(56)].

The efficacy and safety of *Cistanche tubulosa* glycoside capsules (CTG capsule, Memoregain®) for treating Alzheimer's disease (AD) were studied clinically. A total of 18 patients with AD administered with Memoregain® for 48 weeks were assessed for drug efficacy by Alzheimer's disease assessment scale–cognitive

subscale (ADAS-cog), mini-mental state examination (MMSE), activities of daily living (ADLs), blessed behavioral scale, and clinical global impression (CGI) scales. The MMSE score was 14.78 ± 2.51 at baseline and 14.06 ± 4.26 at study completion. While changes in ADAS-cog score before and after 48 weeks of treatment were statistically insignificant, the score improved, deteriorated, and remained unchanged in 10, 7, and 1 patients, respectively. The ADL and CGI scores showed no significant difference from baseline. All adverse reactions were mild. After Memoregain® treatment, patients with AD showed no obvious aggravation of cognitive function, independent living ability, and overall conditions but were stable throughout the study. Comparison with other long-term medications, acetylcholinesterase inhibitors suggests that Memoregain® has a potential to be a possible treatment option for mild to moderate AD[57-58].

The body of *Cistanche tubulosa* (Schenk.) Wight, was used to make a medicinal preparation containing phenylethanoid glycosides and comprising 10-70% of echinacoside and 1-40% of acteoside by weight of the preparation. The medicinal preparation was used effectively in the prevention of senile dementia, and inhibition of aggregation of blood platelets[59].

Effect on immunity and aging:

The effect of *Cistanche tubulosa* (Scheuk) Whight acteoside (CTWA) was studied on malondialdehyde (MDA) content, telomerase activity in heart, liver and brain tissues and immune function of experimentally aging model mice. Mice were given sc 10% D-galactose 10 ml/kg, once daily for 8 weeks to establish model of aging mice. CTWA 10, 20 and 40 m/kg were given ig, respectively, from the ninth week, once daily for 2 weeks. In model untreated group, MDA content was significantly increased in heart, liver and brain, telomerase activity was significantly decreased in heart and liver, and lymphocyte proliferation, phagocytosis of peritoneal macrophages and blood IL-2 content were obviously decreased. After treatment with CTWA for 2 weeks, MDA content in heart, liver and brain was significantly decreased. In CTWA 40 mg/kg group telomerase activity in heart and brain was significantly increased, lymphocyte proliferation, phagocytosis of peritoneal macrophages and peripheral blood IL-2 content were enhanced. Accordingly, the authors concluded that CTWA may delay aging, which may be attributed to antagonizing free radical injury and enhancing the immunity of aging mice[60].

Hypolipidemic effects:

The hypocholesterolemic effect of the aqueous ethanol extract (CTE) of the roots of *Cistanche tubulosa* was evaluated in mice using gene chip and RT-PCR analysis of the livers of mice given CTE (400 mg/kg) for 14 days. The administration of CTE (400 mg/kg) for 14 days significantly suppressed serum cholesterol elevation in high cholesterol diet-fed mice. The mRNA expressions of VLDL receptor and cytochrome P450 SCC were significantly enhanced. In addition, acteoside, a major constituent of CTE, was found to enhance the mRNA expressions of apolipoprotein B, VLDL receptor, and cytochrome P450 SCC in HepG2 hepatocytes. According to these results, the authors concluded that CTE affected the mRNA expressions of molecules related to cholesterol transport and metabolism and exhibited hypocholesterolemic activity in diet-induced hypercholesterolemia mice. Acteoside was involved in the hypocholesterolemic activity of CTE[61].

Effects on hair health:

Cistanche tubulosa extract was studied in double-blinded, placebo-controlled clinical trial, to investige its efficacy in promoting hair health in patients with mild to moderate patterned hair loss. The density and diameter of hairs was compared with that in patients receiving a placebo at baseline, 8 and 16 weeks of the study. In order to determine the efficacy of treatment on dandruff and scalp inflammation, investigator's visual assessment score and patient's subjective score were also performed. A statistically significant increase in the hair density and hair diameter of the test group was recorded after 16 weeks. There were also significant outcomes regarding the investigator's visual assessment and patient's subjective score of dandruff and scalp inflammation in the test group compared to those in control group. Based on the results of this clinical study, the authors conclude that *Cistanche tubulosa* extract is a promising substances for promoting health of the scalp and hair[62].

Antimicrobial effect:

The extracts of the aerial parts of the plant showed mild antibacterial and antifungal effects against *Bacillus subtilis, Enterococcus faecalis, Pseudomonas aeruginosa, Salmonella entrica, subsp. entrica. S. typhi, Escherichia coli, methicillin resistant Staphylococcus aureus, Fusarium axyosporum, Aspergilus niger* and *Aspergilus fumigatus*[63]. Phenylethanoid glycosides, Campneosid I and Campneosid II, isolated from *Cistanche tubulosa*, have high antibacterial and antifungal activity. Campneosid I showed significant antibacterial activity against several pathogenic strains of *Streptococcus* and *Staphylococcus*[64].

Cytotoxic effects:

The extract of the aerial parts of the plant was evaluated for cytoxicity using the larvae (nauplii) of *Artemia salina* (brine shrimp) model. LD_{50} of the plant extract against *Artemia salina* was 62.95 (49.26-79.05) ppm. *Cistanche tubulosa* extract possessed the highest toxicity among eight tested plants [(63)].

Antidiabetic effect:

The effects of *Cistanche tubulosa* on glucose homeostasis and serum lipids were studied in male mice model of type 2 diabetes. Different doses of *Cistanche tubulosa* (equivalent to 120.9, 72.6 or 24.2 mg verbascoside/kg) were administered orally once daily for 45 days to male db/db mice. *Cistanche tubulosa* significantly suppressed the elevated fasting blood glucose and postprandial blood glucose levels, improved insulin resistance and dyslipidemia, and suppressed body weight loss. However, *Cistanche tubulosa* did not significantly affect serum insulin levels or hepatic and muscle glycogen levels[(65)].

Acylated phenylethanoid glycosides, echinacoside and acteoside, the principal constituents in the stems of *Cistanche tubulosa*, inhibited the increased postprandial blood glucose levels in starch-loaded mice at doses of 250-500 mg/kg po. They also significantly improved glucose tolerance in starch-loaded mice after 2 weeks of continuous administration at doses of 125 and/or 250 mg/kg/day po without producing significant changes in body weight or increasing food intake. In addition, several constituents from *Cistanche tubulosa*, including echinacoside (IC_{50} = 3.1 μM), acteoside (1.2 μM), isoacteoside (4.6 μM), 2'-acetylacteoside (0.071 μM), tubulosides A (5, 8.8 μM), B (9, 4.0 μM), syringalide A 3-O-α-L-rhamnopyranoside (10, 1.1 μM), campneoside I (13, 0.53 μM), and kankanoside J1 (14, 9.3 μM), demonstrated potent rat lens aldose reductase inhibitory activity. The potency of 2'-acetylacteoside was similar to that of epalrestat (0.072 μM), a clinical aldose reductase inhibitor[(66)].

Antiosteoporotic effect:

The antiosteoporotic effect of echinacoside (ECH) on bone metabolism was studied in the ovariectomized (OVX) rat model of osteoporosis *in vivo*. In OVX rats, the increases of body weight, serum hydroxyproline (HOP) levels, and the decreases of uterus wet weight and BMD were significantly reversed by ECH treatment. Moreover, three dosages of ECH completely corrected the increased urine concentration of calcium (Ca), inorganic phosphorus (P), and HOP observed in OVX rats. Furthermore, Micro-CT analysis results of distal femur showed that all ECH-treated groups notably enhanced bone quality compared to OVX group ($p<0.05$). The total femur BMD and biomechanical strength of tibia were significantly improved ($p<0.05$) after 12 weeks ECH administration. Histological results also showed the protective activity of ECH through promotion of bone formation and suppression of bone resorption. In addition, the ECH administration also significantly enhanced the expression of ER in the uteri according to immunohistochemical evaluation ($p<0.05$). According to these findings, the authors concluded that ECH is a new class of phytoestrogen, with remarkable antiosteoporotic activity, similar to estrogen, and especially effective for prevention osteoporosis induced by estrogen deficiency[(67)].

Echinacoside (ECH), isolated from *Cistanche tubulosa* (Schrenk) R. Wight stems, was subjected to *in vitro* experiments to investigate its bioactivities on proliferation, differentiation and mineralization of the osteoblastic cell line MC3T3-E1. ECH caused a significant increase in cell proliferation, ALP activity, COL I contents, OCN levels and an enhancement of mineralization in osteoblasts at the concentration range from 0.01 to 10nmol/l ($p<0.05$). In addition, the ratio of OPG/RANKL was also enhanced by ECH. Accordingly, ECH can promote bone regeneration in cultured osteoblastic MC3T3-E1 cells, which might be done by elevating the OPG/RANKL ratio[(68)].

Hepatoprotective effect:

The methanolic extract from fresh stems of *Cistanche tubulosa* possessed hepatoprotective effects against D-galactosamine (D-GalN)/lipopolysaccharide (LPS)-induced liver injury in mice. Among the isolated compounds, echinacoside, acteoside, isoacteoside, acetylacteoside, and tubuloside A, inhibited D-GalN-induced death of hepatocytes. These five compounds, and cistantubuloside B also reduced TNF-alpha-induced cytotoxicity in L929 cells[(69)].

The hypocholesterolemic effect of the aqueous ethanol extract (CTE) from the roots of *Cistanche tubulosa* was evaluated using gene chip and RT-PCR analysis of the livers of mice given CTE (400 mg/kg) for 14 days. The administration of CTE (400 mg/kg) for 14 days significantly suppressed serum cholesterol elevation in high cholesterol diet-fed mice. The mRNA expressions of VLDL receptor and cytochrome P450 SCC were significantly enhanced. In addition, acteoside, a major constituent of CTE, was found to enhance the mRNA expressions of apolipoprotein B, VLDL receptor, and cytochrome P450 SCC in HepG2 hepatocytes[(58)].

Three among acylated phenylethanoid oligoglycosides isolated from stems of *Cistanche tubulosa* were found to inhibit D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes[49].

Effect on inflammatory mediators:

The anti-inflammatory effects of fuciodan and *Cistanche tubulosa* extract were investigated in *in vitro* macrophage culture system and *in vivo* carrageenan-induced air pouch inflammation model. Although, fucoidan was inactive, but *in vivo* air pouch inflammation model, carrageenan-induced vascular exudation and increased nitric oxide and prostaglandin E_2 concentrations in the exudates were synergistically suppressed by co-administration of fucoidan and *Cistanche tubulosa* extract. Moreover, tissue inflammation was substantially attenuated by the combinational therapy. However, there was no synergistic effect against the inflammatory cell infiltration, although fucoidan and *Cistanche tubulosa* extract each markedly reduced the cell numbers. The authors concluded that fucoidan blocked infiltration of inflammatory cells, while *Cistanche tubulosa* extract inhibited activation of the cells, and that their combinational treatment could be a promising candidate for the relief of various types of inflammation[70].

The efficacy of echinacoside ECH-enriched extract of *Cistanche tubulosa* was evaluated in the treatment of dextran sulphate sodium (DSS)-induced colitis. Oral administration of ECH extract significantly suppressed the development of acute colitis, indicated by lowering disease activity index ($p<0.0001$) and preventing colonic damage ($p=0.0336$). Histological examinations showed that ECH extract treatment protected intestinal epithelium from inflammatory injury ($p = 0.0249$) but had less effect on inflammatory cellular infiltration ($p=0.1753$). The beneficial effect of ECH extract treatment was associated with upregulation of transforming growth factor (TGF)-β1, as well as an increase in the number of Ki67(+) proliferating cells in diseased colons ($p < 0.0001$). In cultured MODE-K cells, the addition of ECH extract enhanced *in vitro* wound healing that depended on TGF-β1 expression[71].

Cistanche tubulosa dialysate (CTD) prepared using a 3,500-Da molecular weight cut-off dialysis membrane, enhanced IgM production in B-cell line BALL-1 and IgG production in B-cell line HMy-2, induced cell proliferation in BALL-1 and T-cell line Jurkat, and oppositely inhibited cell proliferation in B-cell line Namalwa[72].

The effect of acteoside extracted from *Cistanche tubulosa* (Schrenk) R. Wight was studied on the basophilic cell-mediated allergic reaction. The effect of acteoside on β-hexosaminidase release and intracellular Ca^{2+}/l level from rat basophilic leukemia (RBL-2H3) cells was determined. Histamine, tumor necrosis factor (TNF)- α, and interleukin (IL)-4 on human basophilic (KU812) cells were also determined. The effect of acteoside on basophilic cell viability was studied using the 3-[4,5-dimethylthiazolyl]-2,5-diphenyltetrazolium bromide (MTT) assay. The results indicated that 0.1-10.0 μg/ml acteoside inhibited the release of β-hexosaminidase and Ca^{2+}/l influx from IgE-mediated RBL-2H3 cells. Moreover, acteoside inhibited histamine release, TNF- α, and IL-4 production in a dose-dependent manner from calcium ionophore A23187 plus phorbol 12-myristate 13-acetate (PMA) or compound 48/80-stimulated KU812 cells. The authors concluded that acteoside inhibited basophilic cell-derived immediate-type and delayed-type allergic reactions[73].

Neuroprotection and antiparkinsonian effects:

Campneosid II, isolated from *Cistanche tubulosa* has strong protective effect on neurons against neurotoxin-induced 1-Methyl-4-phenylpyridinum (MPP) apoptosis[64]. Total glycosides obtained from Cistanches herba have been demonstrated to have neuroprotective effects on dopaminergic neurons of substantia nigra in a chronically intoxicated MPTP mice model of Parkinson's disease. Treatment with 400 mg/kg of total glycosides significantly improved the altered neurobehavioral pattern of MPTP-intoxicated mice and inhibited the reduction of nigral dopaminergic neurons and the expression of TH in the striatum[74].

Acteoside extracted from Cistanches herba has neuroprotective effects against rotenone-induced damage to SH-SY5Y cells. Pretreatment of SH-SY5Y cells with acteoside (10, 20, or 40 mg/l) for 6 h significantly reduces the release of lactate dehydrogenase induced by rotenone (0.5 μM/l). Pretreatment of SH-SY5Y cells with acteoside at the same dose range for 6 h, decreased the cleavage of parkin induced by 0.5 μM/l of rotenone, dose dependently, decreased α-syn-positive SH-SY5Y cells, and stopped the dimerization of α-syn[75].

Acteoside was also studied to investigate its neuroprotective effects in MPTP models of PD. Pretreatment with acteoside at 10 and 30 mg/kg significantly improved MPTP-induced behavioral deficits in C57BL/6 mice. Acteoside also increased the dopaminergic neurons and content of DA[76].

3.5 and 7.0 mg/kg of echinacoside prevented the 6-OHDA-induced extracellular loss of monoamine neurotransmitters, including DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA), in rat striatum. Alleviation of MPTP-induced behavioral deficits in C57BL/6 mice by pretreatment of echinacoside might result from a decrease in the biliverdin reductase B level. Acteoside selectively suppressed AP-1 activation, which essential for iNOS induction in the LPS-treated macrophages. Prior treatment with

echinacoside to MPTP-intoxicated mice increased levels of striatal DA and its metabolite, reduced behavioral deficits, cell death, and caused a significant rise in TH expression as compared to mice treated with MPTP alone. Pretreatment with echinacoside markedly reduced MPP^+-induced activations of caspase-3 and caspase-8 in cerebellar granule neurons. Echinacoside increased neurochemical and behavioral outcomes in MPTP mice models of PD and inhibited caspase-3 and caspase-8 activation in cerebellar granule neurons. Oral administration of echinacoside (30 mg/kg/day for 14 days) to MPTP-induced sub-acute mice model of PD, significantly overcame the reduction of striatal fibers, nigral dopaminergic neurons, dopamine transporter, and dopamine in MPTP-lesioned animals. In comparison with vehicle-treated mice, echinacoside treatment increased mRNA and protein expression of glial cell-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor in MPTP-lesioned mice. echinacoside treatment also decreased the increased apoptotic cells and mRNA/protein ratio of Bax/Bcl-2 in MPTP-lesioned mice. It was also improved motor deficits produced by MPTP[77-80].

Anti-fatigue effect:

5 and 10 g/kg of *Cistanche tubulosa* extract were orally administered to mice for seven days. One hour after the final administration, mice were placed in a 250 ml bottle containing sodium carbonate and the survival time under oxygen deficient condition was measured. The survival time of oxygen deficient mice receiving *Cistanche tubulosa* extract was significantly longer. The prolonged survival time was concentration dependent. On the other hand, *Cistanche tubulosa* extract was orally given to mice for seven days. One hour after the final administration, a forced swimming test was conducted with 5% increased in weight of total body weight of mice. The total time spent when the mice went under water till their breathing stopped was measured. Mice receiving *Cistanche tubulosa* extract demonstrated a prolonged tolerance time in a forced swimming test. The effect was concentration-dependent. *Cistanche tubulosa* extract exhibited anti-fatigue effect with increased tolerance on ischemic condition[55].

Vasorelaxant effect:

The vasorelaxant activity of echinacoside, a phenylethanoid glycoside isolated from *Cistanche tubulosa*, and its possible underlying mechanism on isolated rat thoracic aortic rings pre-contracted with phenylephrine (PE, 1 microM) and KCl (60 mM) was investigated. Echinacoside (30-300 microM) exhibited an acute relaxation in endothelium-intact rings in a concentration-dependent manner, while this relaxation was significantly inhibited in endothelium-denuded condition and in the presence of the endothelial nitric oxide synthase (eNOS) inhibitor, N(W)-nitro-L-arginine methyl ester (L-NNA, 100 microM), an unselective soluble guanylate cyclase blocker, methylene blue (10 microM) and the selective sGC inhibitor 1 H-[1, 2, 4] oxadiazolo[4,3- A]quinoxalin-1-one (ODQ, 1 microM); in addition, atropine (1 microM), a selective muscarinic receptor antagonist, partially affected the relaxation. However, the cyclooxygenase inhibitor indomethacin (5 microM) had no influence on the relaxant action. Echinacoside enhanced the cyclic guanosine monophosphate (cGMP) production in aortic rings contracted with PE. The authors concluded that echinacoside mediates the endothelium-dependent vasodilator action in rat thoracic aortic rings through nitric oxide (NO)-cGMP pathway[81].

The methanolic extract from the dried stems of *Cistanche tubulosa* showed inhibitory effect on contractions induced by noradrenaline in isolated rat aortic strips. From the extract, new phenylethanoid oligoglycoside constituents, kankanosides F and G, and an acylated oligosugar, kankanose, were isolated together with 14 known compounds. Kankanoside F, kankanose, echinacoside, acteoside, and cistanoside F, showed vasorelaxant activity[43].

Effect on reproductive system:

The effect of ethanol extract of *Cistanche tubulosa* (Schenk) R. Wight stem (CTE) was studied on hormone levels and testicular steroidogenic enzymes in rats. It appeared that the administration of CTE (0.4 and 0.8 g/kg) increased sperm count (2.3 and 2.7 folds) and sperm motility (1.3 and 1.4 folds) and decreased the abnormal sperm (0.76 and 0.6 folds) respectively. The serum level of progesterone and testosterone in rats was also increased by CTE administration ($p<0.05$). Results of immunohistochemistry and western blot analysis confirmed that the expression of CYP11A1, CYP17A1, and CYP3A4 was enhanced by CTE ($p<0.05$)[82].

The weights of seminal vesicle and prostate gland of castrated young rats were significantly increased by administration of alcohol soluble extract from the decoction of *Cistanche tubulosa*. The phagocytic function of intra-abdominal macrophage in mice was activated by the decoction of *Cistanche tubulosa* [83].

Side effects and toxicity:

LD_{50} of *Cistanche tubulosa* extract was deduced to be >26.4 g/kg in both male and female mice. *Cistanche tubulosa* extract orally administered to male and female mice and kept for 8 days caused no

abnormalities and fatal event and autopsy abnormalities at 26.4 g/kg. Ames test showed no difference of the colony counting in TA97, TA98, TA100 and TA102 strains with *Cistanche tubulosa* extract (8-5000 μg/plate). Micronucleus test of polychromatic erythrocyte in mice marrow showed that *Cistanche tubulosa* extract (2.5-10 g/kg) caused no damage to bone marrow cells. The teratogenic test showed that *Cistanche tubulosa* extract (2.5-10 g/kg) has no teratogenesis to mice spermatozoon. In subacute test, *Cistanche tubulosa* extract was orally administered to male and female rats. No abnormalities and fatal event and autopsy abnormalities were observed at 0.65-1.30 g/kg for 30 days. In long term toxicity, *Cistanche tubulosa* extract was orally administered to male and female rats at 1.65 g/kg and kept for 180 days. No abnormalities and fatal event and autopsy abnormalities were recorded [(55)].

Dose:

The recommended daily dosage for *Cistanche tubulosa* extract is 100-400 mg/day[(55)].

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