ORYZA OIL & FAT CHEMICAL CO., LTD.

CISTANCHE TUBULOSA EXTRACT

Food and cosmetic ingredients with tonics, memory improving, anti-aging, anti-fatigue, anti-sex dysfunction, immune boosting and fat metabolism accelerating properties

■ CISTANCHE TUBULOSA EXTRACT-P25

(Water-soluble Powder、Food Grade)

■ CISTANCHE TUBULOSA EXTRACT-PC25

(Water-soluble Powder、Cosmetic Grade)

ORYZA OIL & FAT CHEMICAL CO., LTD.

Ver.2.2 YF

Improve Memory･Anti-aging,-fatigue

CISTANCHE TUBULOSA EXTRACT

1. Introduction

Cistanche Tubulosa (Schenk) R. Wight is a plant that parasitizes the roots of Tamarix. Since it has no root or chloroplast, it cannot photosynthesize (Fig. 1). Therefore, it grows by absorbing the nutrition out of plants it parasitizes. In China, Cistanche Tubulosa is known as a rare Panax ginseng found in deserts and used as a pharmaceutical agent to cure Alzheimer's disease. In Japan, Cistanche Tubulosa has been categorized as a food after its food/pharmaceutical classification was revised by the Health, Labor and Welfare Ministry in 2005. It belongs to Cistanche, Orobanchaceae, same as the parasitic plant Cistanche salsa used in Chinese herbal medicine. According to the Chinese Comprehensive Pharmaceutical Dictionary, it supplements renal function, aphrodisiac, and smoothes the intestines. The dictionary stated that it treats impotence, infertility, menstrual disorder, and psychroalgia of the back and knees. Cistanche salsa has been widely used clinically as a prescribed drug for supplementing renal function and nutritional fortification in China. However, collecting the valuable crude drug Cistanche salsa (certified as a class 2 national protected plant) has become difficult recently. Therefore, there is a growing awareness about Cistanche Tubulosa which belongs to the same family and has been reported to have similar effects and functions as Cistanche salsa.

Cistanche Tubulosa grows in the Takla Makan Desert in Hsinchiang Uighur Autonomous Region, China. It has a very strong vital energy to flower and fruit under severe desert conditions (Fig. 2). Its host tamarix grows to 6 meters tall and has small, dark pink flowers. This plant is used to protect against wind and sand (prevents the spread of yellow sand) in desert regions. Cistanche Tubulosa is now considered to be the key to greenification of deserts and prevention of global warming and the Chinese government recommends the plantation of Cistanche Tubulosa to stimulate local industries.

According to Mr. Keiichi Morishita’s report, the Hotan region, an oasis in the Takla Makan Desert, is one of the 4th longest life expectancies in the world. The percentage of elderly people that live over one-hundred years old in this region is the highest in China, over three times that of Okinawa Prefecture in Japan which is famous for its human longevity. Every 100,000 people, Okinawa has 51 people aged over 100 and Hotan has 183 such people. People of the Hotan region eat Cistanche Tubulosa daily and prepare it by slicing and then boiling with mutton in a pot or pickling it in tea or liquor in order to survive the harsh environmental conditions of the region. This habit is believed to be the key of people’s longevity in the region.

Oryza Oil & Chemical Co., Ltd. studied Cistanche Tubulosa extract jointly with Sinphar Pharmaceutical Co., Ltd. (pharmaceutical company in Taiwan) with the assistance of Peking University. Sinphar Pharmaceutical Co., Ltd. established a raw

ingredient production base and also a subsidiary 新疆天力砂生薬物有限公司 in Hotan. The company implements GAP plantation of Cistanche Tubulosa to constantly provide high-quality, pesticide-free raw material. The company's products are safe and highly stable in quality. Moreover, its products have been certified as organic foods by the government and raised in GMP facilities. Through joint research, we have discovered that Cistanche Tubulosa extract has activities to prevent aging of the brain and skin, increase sexual power, and accelerate fat metabolism in addition to its known activities. Sinphar Pharmaceutical Co., Ltd. and Peking University have also discovered that the extract has activities to improve brain functions, prevent aging or fatigue, and boost immune strength from test data accumulated in their long-term study.

Our Cistanche Tubulosa extract is of highest concentration of active components (echinacoside 25% min, acteoside 9% min). We believe that the extract can be used in a wide variety of foods and cosmetics as a new ingredient to improve brain functions, vitalize the body, and enhance beauty.

Fig. 1 Cistanche Tubulosa

Fig. 2 Hsinchiang Uighur Autonomous Region (GAP plantation)

2. Functional Components of Cistanche Tubulosa

The main effective ingredients of Cistanche Tubulosa extract are phenylethanoid glycosides, especially echinacoside and acteoside (Fig. 3). Although echinacoside is known as the main component of the herb Echinacea, Cistanche Tubulosa contains a higher amount of echinacoside than any other plant. Acteoside (a type of polyphenols) has an extremely strong antioxidative property which is reported to be 15 times stronger than resveratrol (polyphenols contained in grapes) and 5 times stronger than vitamin C[1]. In recent study, new compounds (kankanoside and others) have been isolated[2] and vasorelaxing activity has been reported as a pharmacological action of the new compounds, echinacoside, and acteoside[3]. Active constituents in extracts of Cistanche salsa and Cistanche Tubulosa that belong to the same family, were compared and it was clarified that Cistanche Tubulosa has more active constituents (Fig. 4). Cistanche Tubulosa extract with high quantity of active constituents performs various bioactive functions as described below.

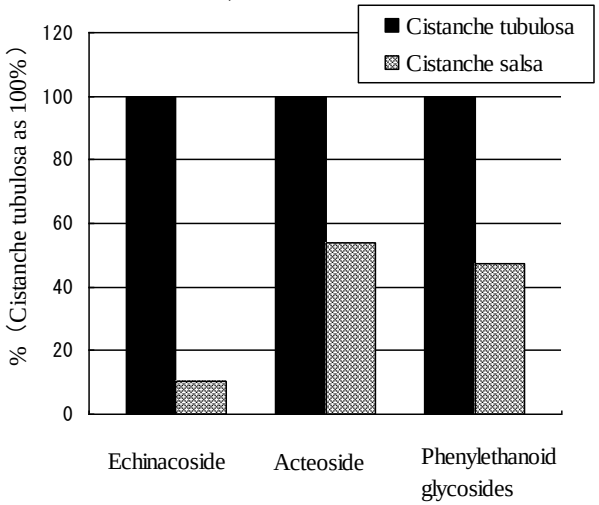
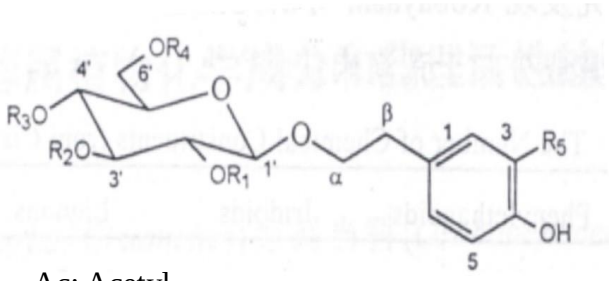


Fig. 4 Echinacoside, acteoside and phenylethanoid glycosides content of Cistanche salsa and Cistanche tubulosa

Compound Name	R1	R2	R3	R4	R5
2'-Acetylacteoside	Ac	Rha	Cf	H	OH
Acteoside	H	Rha	Cf	H	OH
Cistanoside A	H	Rha	Cf	Glc	OMe
Cistanoside C	H	Rha	Cf	H	OMe
Echinacoside	H	Rha	Cf	Glc	OH
Isoacteoside	H	Rha	H	Cf	OH
Tubuloside A	Ac	Rha	Cf	Glc	OH



Ac: Acetyl
Cf: trans-Caffeoyl
Glc: β-D-Glucopyranose
Rha: α-L-Rhamnopyranose

Fig. 3 Components of Cistanche Tubulosa Extract

References
1) Kanebo：news release, 2005.
2) Haihui Xie *et al*., *Chem. Pharm. Bull*., **54**(5), 669-675, 2006.
3) Yoshikawa M., *et al*., *Bioorg. Med. Chem*., **14**(22), 7468-7475, 2006.

Bioactivities of Cistanche Tubulosa Extract

(1) Improvement of brain function

(2) Anti-aging effect

(3) Skin beautifying effect

(4) Anti-fatigue

(5) Aphrodisiac effect

(6) Immune boosting effect

(7) Metabolism enhancing effect

(8) Antioxidant Activities

3. Physiological Function of Cistanche Tubulosa Extract

(1) Improvement of brain function

1) Improvement of learning and memory (*in vivo*)

There are three levels of mechanisms in consideration of learning and memory.
(1) Ability to acquire memory, referred to as learning ability
(2) Ability to store memory, referred to as consolidation
(3) Ability to elicit and recall information memorized

Cistanche Tubulosa extract was clarified to significantly improve the above mechanisms. The test results are described below.

① Improve learning ability and retentive memory (Sinphar and Peking University data)

In order to evaluate Cistanche Tubulosa extract's activities to improve learning ability and consolidation, a step down test was carried out on mice[4]. In this method, a platform (safe area) is located on an electric wire with 36 V current and mice's learning ability and consolidation are evaluated by the time they spend on the platform and the number of electronic shocks they received (Fig. 5). Mice were trained by the device. Scopolamine (an acetycholine inhibitor 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 in order 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. The levels were recovered to the same level as normal mice that were trained (normal group) (Figs. 6 and 7). Cistanche Tubulosa extract was confirmed to exert a stronger activity than piracetam (positive control), a pharmaceutical agent to activate energy metabolism of brain cells. These results clarified that Cistanche Tubulosa extract significantly helps the brain to recover from scopolamine-induced learning disorder and sodium nitrite-induced memory consolidation dysfunction and improve the learning ability and formation of memory of brain.

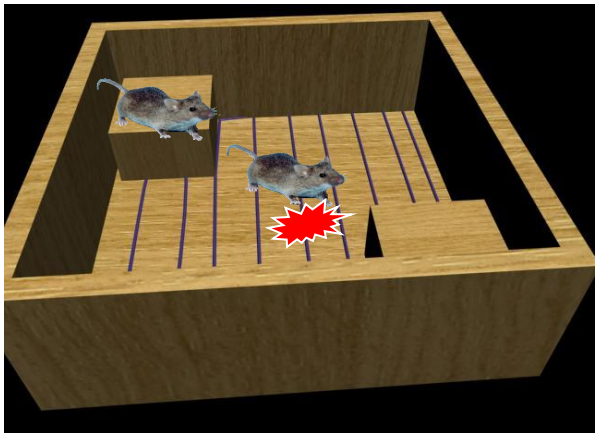


Fig. 5 Step down test

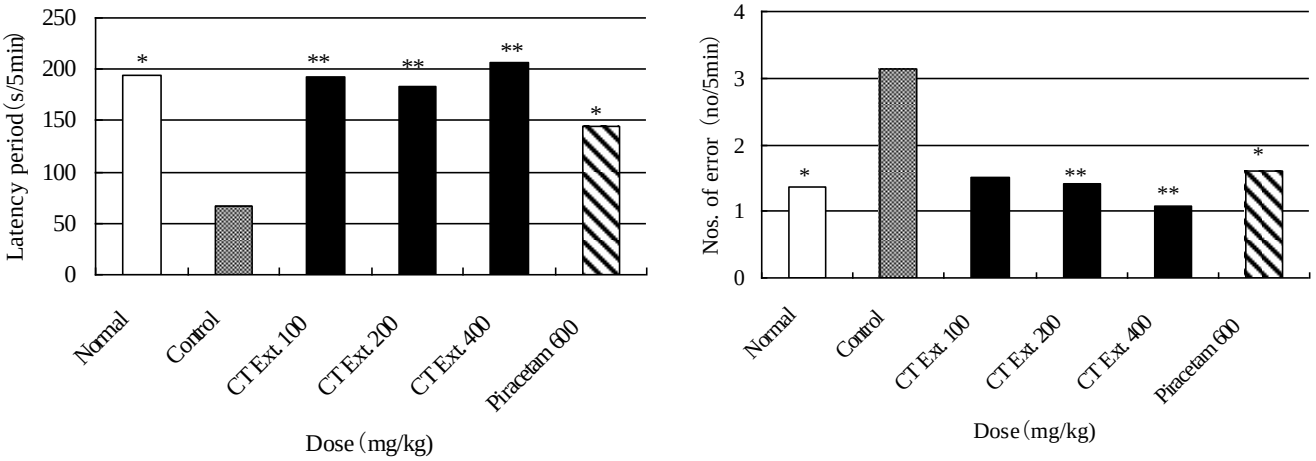


Fig. 6 The Effect of Cistanche Tubulosa Extract (CT Ext.) on Scopolamine-induced memory impaired mice model. *n*=12-15, *: $p<0.05$, **: $p<0.01$

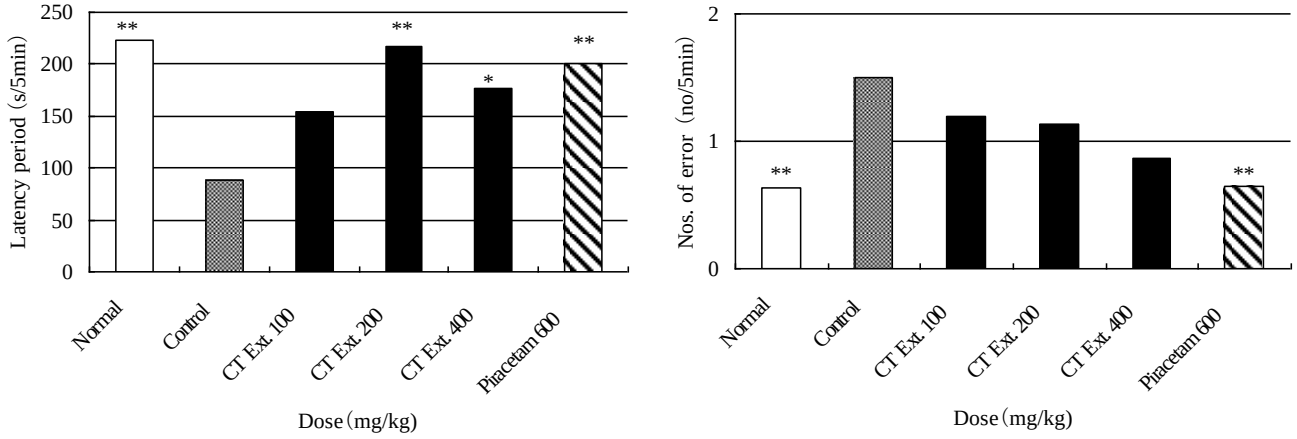


Fig. 7 Effect of Cistanche Tubulosa Extract (CT Ext.) on sodium nitrite-induced memory dysfunction mice model. *n*=14-15, *: $p<0.05$, **: $p<0.01$

4) Cong G., *et al.*, Effects of CTG on memory consolidation dysfunction of mice. *Traditional Chinese Drug Research and Clinical Pharmacology*., **16**(3), 162-164, 2005.

② Ability to recall memorized information（data of Sinphar and Peking University）

Water maze test was conducted to evaluate the memory recall ability of mice four times daily for duration of 1 week (Fig. 8). Training was conducted to create memory in mice on the routes of water maze. Test samples were orally administered to mice everyday throughout the training period. On the last day of the training, 30% ethanol was given to mice to induce memory loss (failing to recall memorized information). Result from water maze test revealed that mice in group consuming Cistanche Tubulosa Extract required shorter time to arrive destination compared to control (Fig. 9). In addition, the rate of error (getting to the wrong point before arriving at correct destination is regarded as 1 error) was significantly lower in group consuming Cistanche Tubulosa Extract (Table 1). It was shown that Cistanche Tubulosa demonstrated stronger activity than piraceetam (positive control). It is indicative that Cistanche Tubulosa Extract helps improving the ability to elicit or recall memorized information.

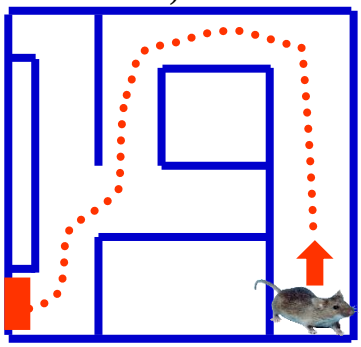


Fig. 8 Water maze test

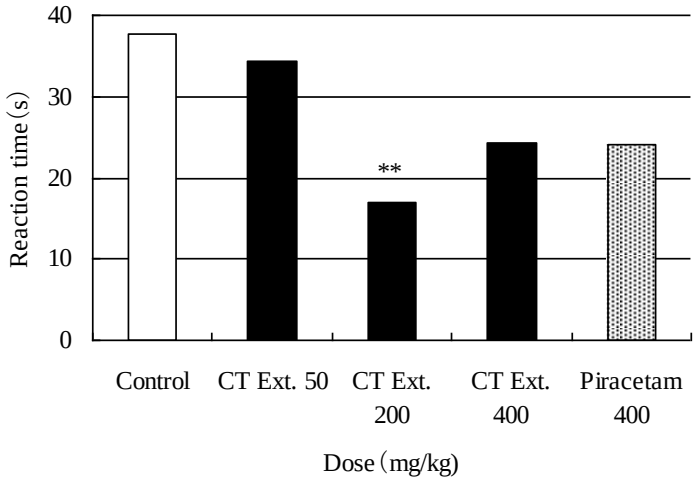


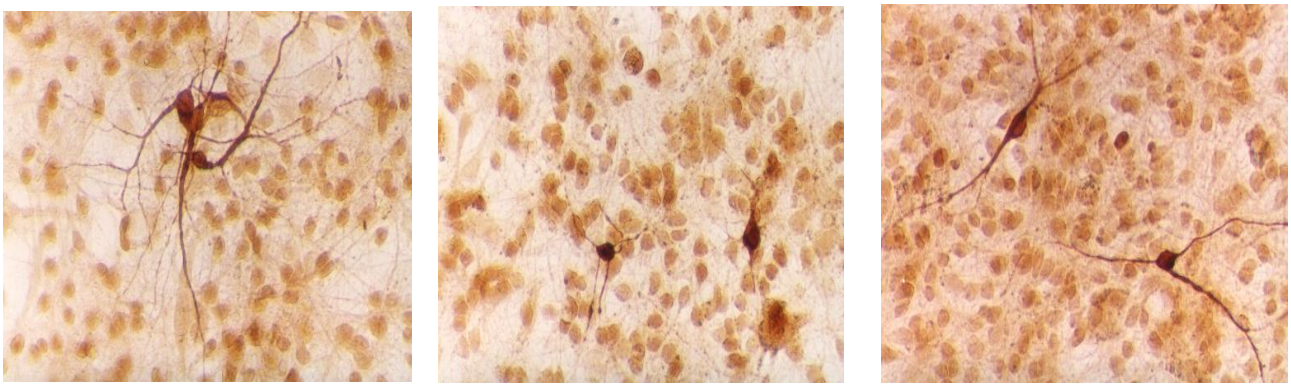
Fig. 9 Effect of Cistanche Tubulosa Extract (CT Ext.) on memory recall impairment. n=10-12, **: p<0.01

Table 1 The Effect of Cistanche tubulosa Extraact (CT Ext.) on alcohol-induced memory recall impaired mice model. **: p<0.01

Group	Dose (mg/kg)	Before ethanol		After ethanol	
		Reaction time (s)	Error number／mice	Reaction time (s)	Error number／mice
Control	—	7.73±0.75	0/0	37.78±15.90	62/10
CT Ext.	50	7.46±0.13	0/0	34.40±21.71	47/8
CT Ext.	200	7.14±0.18	0/0	16.99±9.06**	8/3**
CT Ext.	400	7.91±0.19	0/0	24.38±27.84	46/7
Piracetam	400	8.00±0.46	0/0	24.08±32.52	54/6

2) Anti-apoptosis activity (*in vitro*, data of Sinphar and Peking University)

Anti-apoptosis activity of Cistanche salsa, a congener of Cistanche Tubulosa, on brain neurons was evaluated[5]. Primary midbrain neurons taken from rat embryo (14 to 16 days old) were cultured to create midbrain neuron apoptosis models using a neurotoxin 1-methyl-4-phenylpyridium ion (MPP^+, 50 μmol/L). Then, Cistanche salsa extract (phenylethanoid glycoside content: 50 μg/mL) was given to the apoptosis models and their brain neurons were studied using a microscope over time. As a result, Cistanche salsa extract was shown to prevent brain neuron apoptosis. Moreover, brain neuron source grew better and axons (a long projection of neuron, that conduct electrical impulses away from the neuron's body) were longer in group treated with Cistanche Tubulosa Extract compared with controlled (MPP^+ treatment group). As illustrated in Fig. 10, axons in group treated with Cistanche Tubulosa Extract projected to a length similar to those in the normal group. These results indicated that Cistanche salsa extract improves brain functions by restoring damaged brain neurons. Belonging to the same family as Cistanche salsa, it is suggestive that Cistanche Tubulosa is believed to exert similar anti-apoptosis effect with higher content of bioactive components.



Normal | Apoptosis induced by MPP^+ | MPP^++ Phenylethanoid glycosides

Fig. 10 The anti-apoptosis effects of phenylethanoid glycosides on mesencephalic neurons

5) Research on anti-apoptosis mechanism of phenylethanoid glycosides in *Cistanche salsa* in middle brain neurons. *Chinese Pharmacology Communication.*, **19**(4), 50-51, 2002.

3) Rescue human fibroblasts (*in vitro*)

Echinacoside, an active component of Cistanche Tubulosa, has been reported to rescue human fibroblasts (SHSY5Y) from TNFα-induced apoptosis[6]. After incubation of SHSY5Y cells ($1x10^4$ cell/well), echinacoside (1, 10 and 100 μg/mL) and TNFα (100 ng/mL) were added 36 hours prior to each evaluation analysis. Cell viability was determined by the MTT assay method. The reactive oxygen species (ROS) level in cells was analyzed by a staining method using a fluorescence dye 2,7-dichlorodihydrofluorescein diacetate (H_2DCFDA: When this reagent is reacted in cells, fluorescence develops. Quantitatively, the intensity of fluorescence reflects the level of reactive oxygen species in the cell.) Caspase activity (a family of cysteine protease which play essential roles in apoptosis, necrosis and inflammation) was examined using a kit. As illustrated in Fig. 11, result revealed a dose-dependent inhibition of apoptosis in group treated with Echinacoside as the cell viability rate was higher compared with group treated with TNFα. Similarly, Echinacoside was shown to reduce the amount reactive oxygen species in the experiment (Fig. 12). Moreover, it was clearly demonstrated to prevent the activation of an apoptosis effector caspase-3 (activated by apoptosis initiator caspase, it cleave other protein substrate within the cells to trigger apoptosis) in a concentration-dependent manner (Fig. 13). These results clarified that echinacoside protects damaged fibroblasts by regulating the reactive oxygen species level in fibroblasts and the activation of caspase-3. It is suggestive that Cistanche Tubulosa Extract may exert similar effect due to its high content of echinacoside.

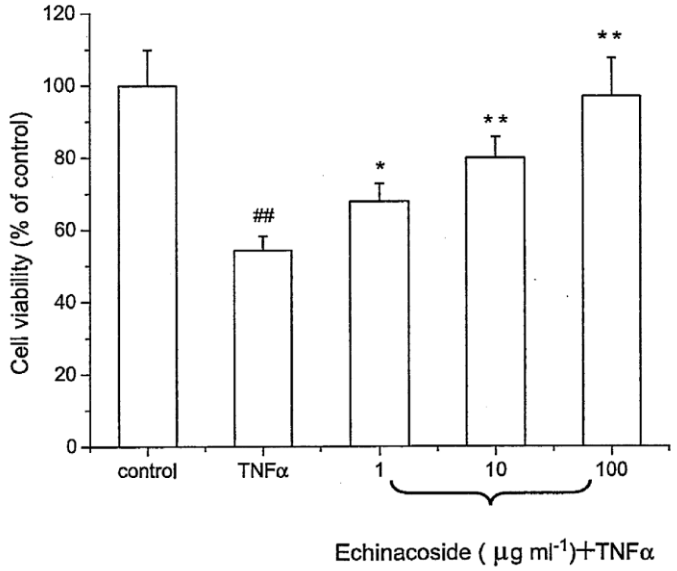
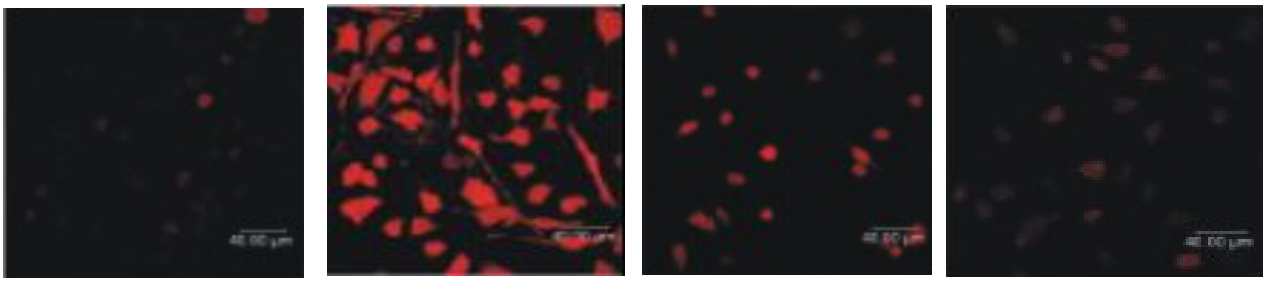


Fig. 11 The Effect of echinacoside on TNFα-induced decreased in SHSY5Y cell viability. n=8, *: p<0.05, **: p<0.01 as compared to TNFα, ##: p<0.01 as compared to control cells



Control | Treatment with TNFα | Echinacoside 10 μg/mL | Echinacoside 100 μg/mL

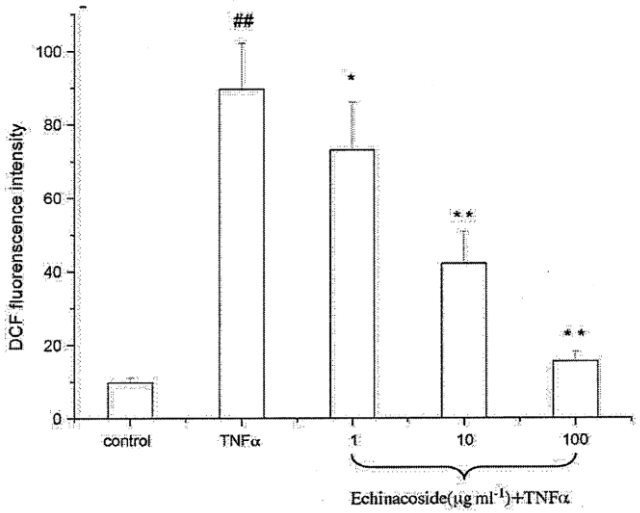


Fig. 12 Effect of echinacoside on level of intracellular reactive oxygen species in TNFα-induced SHSY5Y cells. n=8, *: p<0.05, **: p<0.01 as compared to TNFα, ##: p<0.01 as compared to control cells

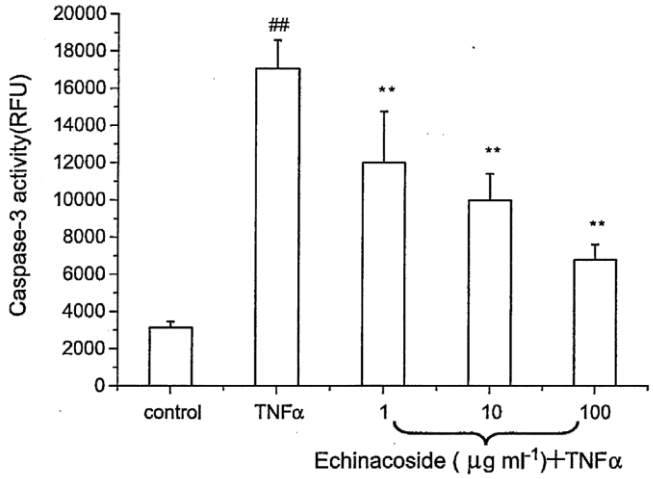


Fig. 13 Effect of echinacoside on TNFα-induced increase of caspase-3 activity. n=8, **: p<0.01 as compared to TNFα, ##: p<0.01 as compared to control cells

6) Min D., *et al.*, Echinacoside rescues the SHSY5Y neuronal cells from TNFα-induced apoptosis. *European Journal of Pharmacology*., **505**, 11-18, 2004.

4）Prevent aging of the brain (*in vitro*, Oryza data)

In order to study the anti-ageing effect of Cistanche Tubulosa on brain, its effect on the proliferation of human fibroblasts (SK-N-SH) was examined and evaluated by the MTT assay method. Microscopic observation was carried out to view the effect of Cistanche Tubulosa Ext. on neurite growth. As shown in Fig. 14, Cistanche tubulosa Ext. demonstrated dose-dependent increase in the proliferation of fibrobalsts. Moreover, microscope illustration showed that neurite growth and the formation of cell networks were accelerated in group treated with Cistanche Tubulosa extract compared to the control (Fig. 15). These results indicate that Cistanche Tubulosa extract accelerates the proliferation of fibroblasts and may promote the production of neurons by accelerating the neurite growth. Thus, Cistanche Tubulosa Extract is believed to prevent ageing of the brain such as dementia (dementia is caused by denaturation and desquamation of cranial nerves) while improve brain functions. Echinacoside and acteoside, the main bioactive components of the extract, were shown to exert mitogenic activity. Especially, echinacoside demonstrated significant effect, even in low concentrations (Fig. 16) and accelerated neurite outgrowth (Fig. 17). These results indicated that echinacoside and acteoside are involved in Cistanche Tubulosa extract's mitogenic activity and activity to accelerate the growth of neurite.

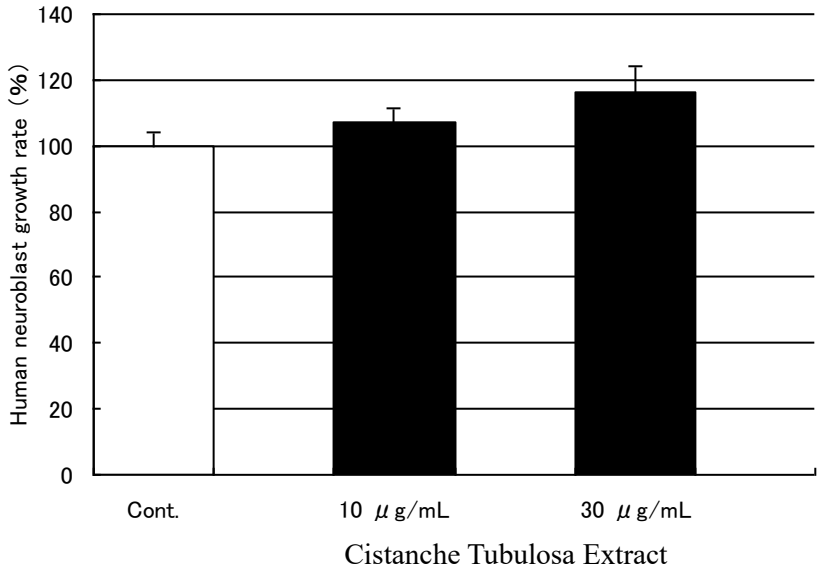
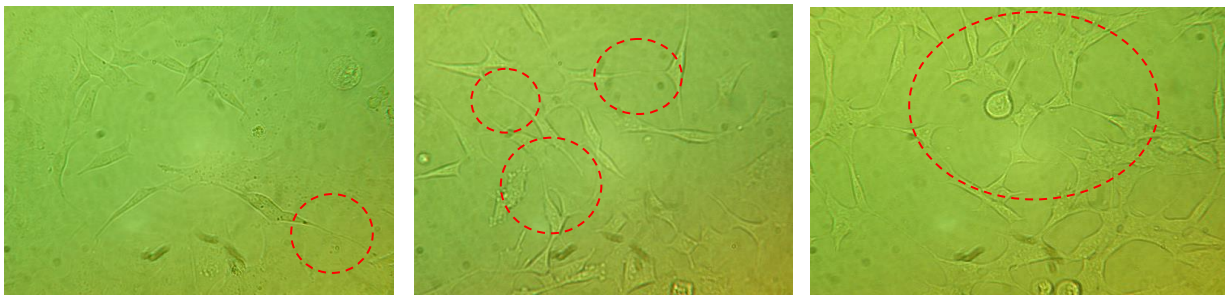


Fig. 14 The Effect of Cistanche Tubulosa extract on the proliferation of fibroblasts（% of Control, Mean±S.D., *n*=5）



Control

Cistanche Tubulosa Extract 10 μ g/mL

Cistanche Tubulosa Extract 30 μ g/mL

Fig. 15 The Effect of Cistanche Tubulosa extract on growth of neurite (microscopic illustrations, ×400)

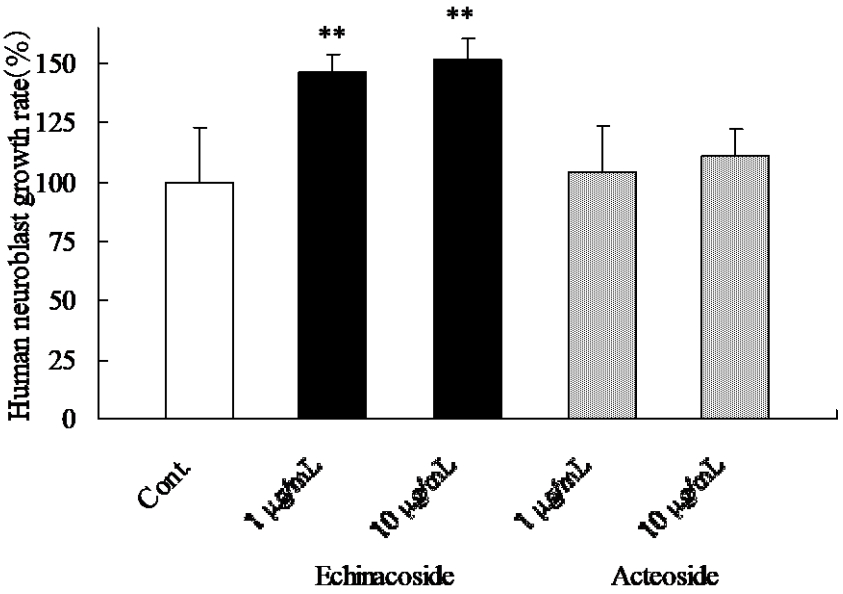


Fig. 16 Effect of echinacoside and acteoside on the proliferation of fibroblasts (% of Control, Mean±S.D., n=5 **: p<0.01)

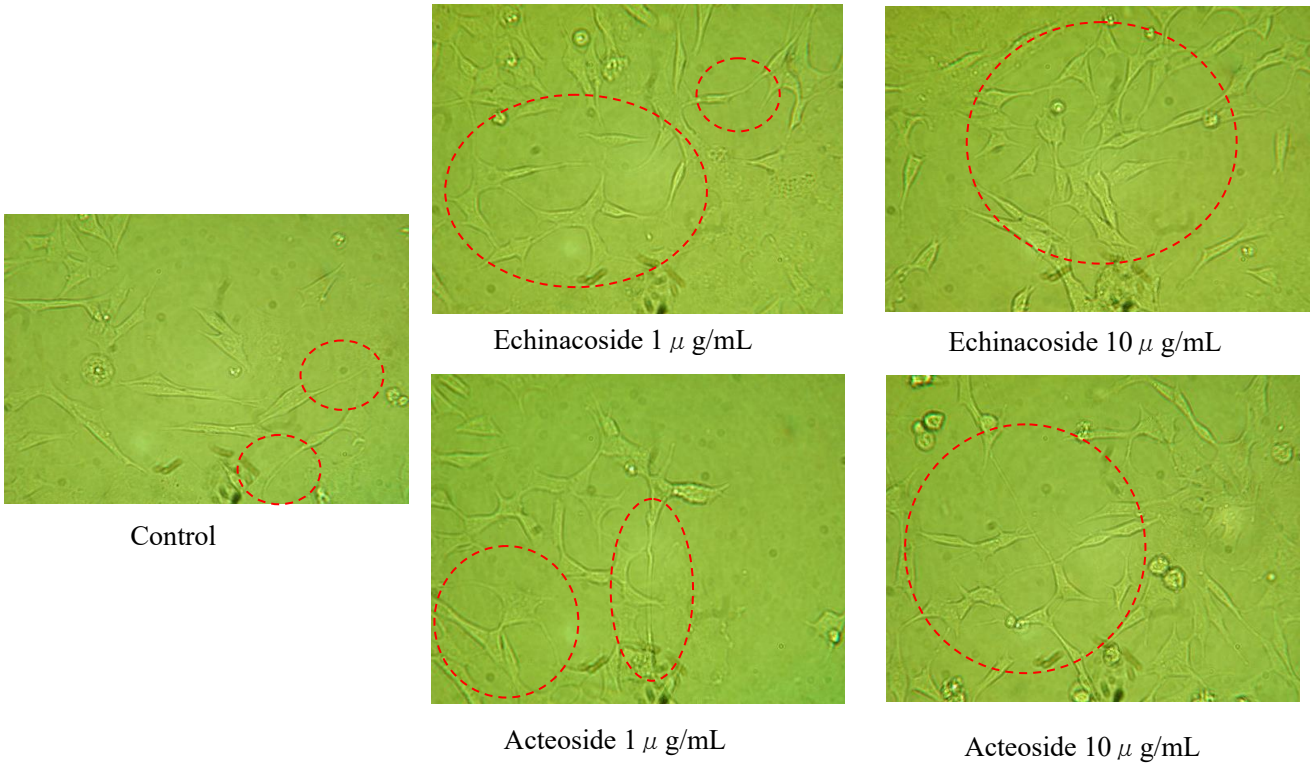


Fig. 17 The Effect of echinacoside and acteoside on neurite outgrowth (microscopic illustrations, ×400)

【Method】

Human fibroblasts (SK-N-SH) were suspended ($1x10^4$ cells/mL) in a MEM medium (10% FCS, 100 units/mL penicillin, 100 μg/mL streptomycin contained) and 5 mL of each was inseminated into a 60 mm petri dish. Cistanche Tubulosa extract prepared in various concentrations was added and changes on cell count were observed using a microscope. Proliferation of the cells was quantified by MTT assay method on the fifth and sixth day after commencement of incubation.

5) Effect on cerebral ischemia-reperfusion (*in vivo*)

Cistanche salsa, a congener of Cistanche Tubulosa, has been reported to prevent cerebral ischemia-reperfusion and protect against apoptosis of CA_1 region of hippocampus (the part in the brain involved in memory and spatial learning ability. Cerebral ischemia is the initial pathological part involved in Alzheimer's disease) [7]. Mice were separated into five groups [sham surgery group, cerebral ischemia group, cerebral ischemia-reperfusion group, cerebral ischemia-reperfusion + positive control (gingko leave extract) group, and cerebral ischemia-reperfusion + Cistanche salsa extract group]. Each test sample was orally administered to mice for eight days. One hour after the final administration, the right carotid artery of the mice was pinched under anesthetic condition by a silken thread for three hours to induce cerebral ischemia. Then reperfusion was carried out 24hours and 48 hours later to create cerebral ischemia-reperfusion models. Cerebral ischemic area was measured by staining brain slices and the apoptosis rate in CA_1 region of hippocampus 24 hours after the cerebral ischemia-reperfusion was examined by the TUNEL method using a kit. As tabulated in Table 2, the area of cerebral ischemia was significantly reduced in group of

cerebral-ischemic mice treated with Cistanche Tubulosa salsa upon compared with control. In addition, Cistanche salsa extract was shown to have similar potency with gingko leaf extract, the positive control, in lowering the apoptosis rate in CA_1 region in hippocampus 24 hours after the cerebral ischemia-reperfusion (Fig. 18). Therefore, it is suggestive that Cistanche salsa protects brain cells and prevents cerebral infarction and Alzheimer's disease by preventing apoptosis in the hippocampus. Cistanche Tubulosa extract is expected to exert similar activity as it belongs to the same family as Cistanche salsa while contains higher amount of bioactive components.

Table 2 The Effect of Cistanche salsa on the area of infarction of ischemia-reperfusion model at 0, 24 and 48h in mice (Mean±S.D., **: p<0.01, n=13-18)

Group	Dose (mg/kg)	0h	24h	48h
		Area of cerebral ischemia (%)		
Sham surgery		0	0	0
Cerebral ischemia		57.47±5.37		
Cerebral ischemia-reperfusion		56.96±6.43	72.98±6.57	60.45±6.06
Gingko leave extract	100	20.32±3.45**	25.67±5.38**	23.83±3.78**
Cistanche salsa extract	62.5	27.23±5.66**	31.13±3.92**	22.27±5.32**
Cistanche salsa extract	125	21.45±4.47**	25.81±6.74**	20.06±4.69**
Cistanche salsa extract	250	22.03±6.22**	25.94±4.07**	22.14±4.75**

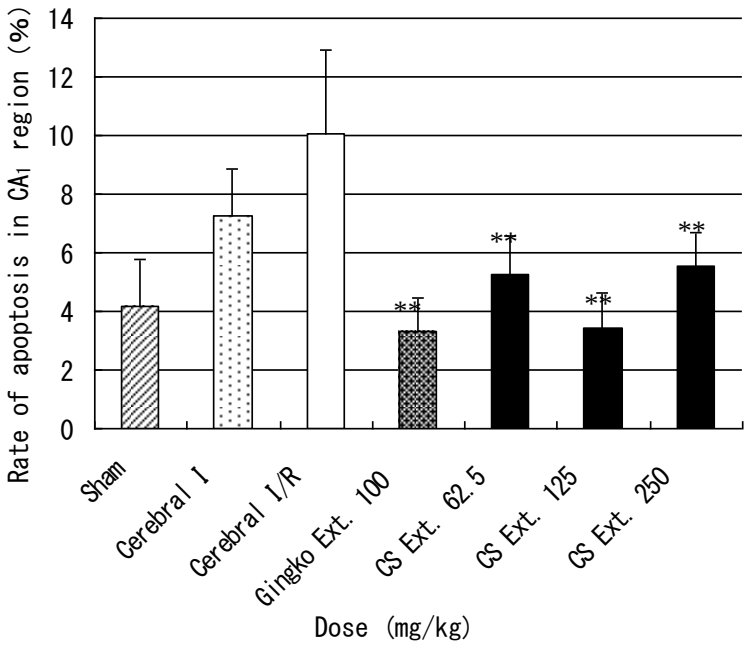


Fig. 18 The Effect of Cistanche salsa extract (CS Ext.) on neuron apoptosis in CA_1 region at ischemia-reperfusion model after 24h (Mean±S.D., **: p<0.01, n=10)

7) Xiao-wen Wang, *et al.*, Protective effects of glycosides of *Cistanche* on cerebral ischemia-reperfusion damage of brain tissue in CA_1 region of hippocampus in awake mice. *Stroke and Nervous Diseases*., **10**(6), 325-328, 2003.

6) Enhance the level of neurotransmitters (*in vivo*)

Cistanche salsa, a congener of Cistanche Tubulosa, has been reported to increase the amount of neurotransmitters in rat brain[8]. Each sample was administered to rats for 40 days consecutively. Rats' brain were removed 12 hours after the final administration of sample and the level of neurotransmitters such as dopamine (DA), noradrenaline (NA), and serotonin (5-HT) was measured according to HPLC-ECD method. Result showed that level of NA and 5-HIAA, a metabolite of serotonin, significantly increased I the hypothalamus. Similarly, level of DA and its metabolite DOPAC significantly increased (Table 4). Results indicated that Cistanche salsa extract enhance brain functions by increasing the amount of neurotransmitters. Similarly, these effect may be extrapolated to Cistanche Tubulosa extract since it belongs to the same family as Cistanche salsa while containing higher level of bioactive components.

Table 3 The effect of Cistanche salsa extract (CS Ext.) on the level of monoamine transmitters in rat's hypothalamus. (μg/g tissue, Mean±S.D., n=6)

(mg/kg)	DA	DOPAC	NA	5-HT	5-HIAA
Cont.	0.42±0.05	0.08±0.02	1.58±1.09	1.84±0.15	0.85±0.15
CS Ext. 200	0.48±0.06	0.08±0.01	1.69±0.18	2.04±0.26	1.02±0.17
CS Ext. 400	0.46±0.04	0.06±0.01	1.98±0.19**	2.27±0.30	1.04±0.13*

*: p<0.05, **: p<0.01

Table 4 Recovery ratios of neurotransmitters/ metabolites in rat. (μg/g tissue, Mean±S.D., n=6)

(mg/kg)	DA/DOPAC	5-HT/5-HIAA
Cont.	5.64±1.41	2.16±0.23
CS Ext. 200	5.81±0.67	2.04±0.35
CS Ext. 400	7.62±1.20*	2.19±0.17

*: p<0.05

8) Influence of *Cistanche* on the amount of monoaminergic neurotransmitters in rat brain. *Chinese Herb*, **24**(8), 417-419, 1993.

7) Prevention of cerebral infarction and myocardial infarction (*in vivo*)

① Inhibition of platelet aggregation (data of Sinphar and Peking University)

The effect of Cistanche Tubulosa extract on platelet aggregation was examined on rats. Aspirin was used as positive control in the experiment and each sample was orally administered to rats for seven days. Blood was collected from a main artery one hour after the final administration followed by addition of adenosine disodium diphosphate (ADP) solution to induce platelet aggregation. Aggregation rate was measured by SPA-4 multi-functional aggregometer. As illustrated in Fig. 19, rate of platelet aggregation was significantly reduced in group treated with Cistanche Tubulosa Extract and its potency is similar to that of aspirin.

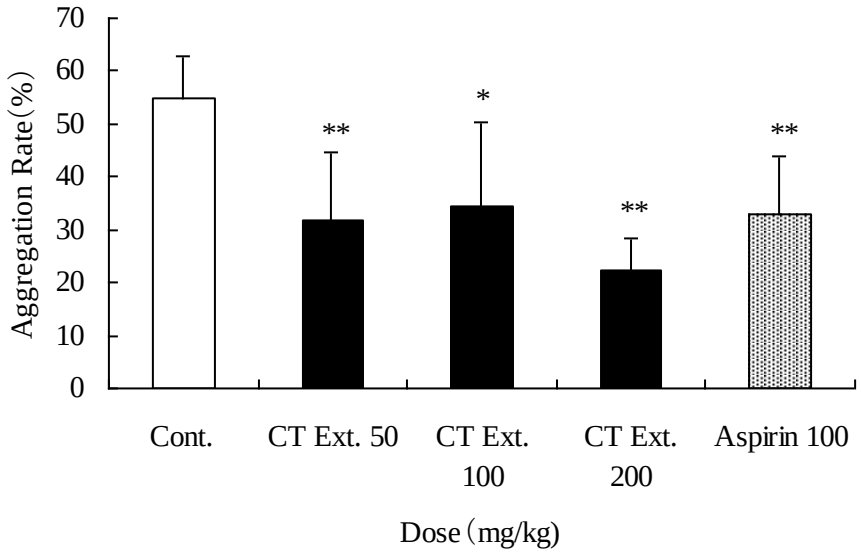


Fig. 19 Effect of Cistanche Tubulosa extract (CT Ext.) on platelet aggregation in rats (Mean±S.D., *: $p<0.05$, **: $p<0.01$, n=10-11)

② Inhibition of formation in bypassed vein (data from Sinphar and Peking University)

The effect of Cistanche Tubulosa extract on clot formation in bypassed vein was examined in rats. Aspirin was used as positive control in the experiment and each sample was orally administered to rats for seven days. Bypassed vein was isolated at the end of the experiment and weight of clot was measured. As illustrated in Fig. 20 below, clot formation in bypassed vein was significantly prevented in group treated with Cistanche Tubulosa Extract and it is suggestive that Cistanche Tubulosa Extract prevents cerebral infarction and myocardial infarction.

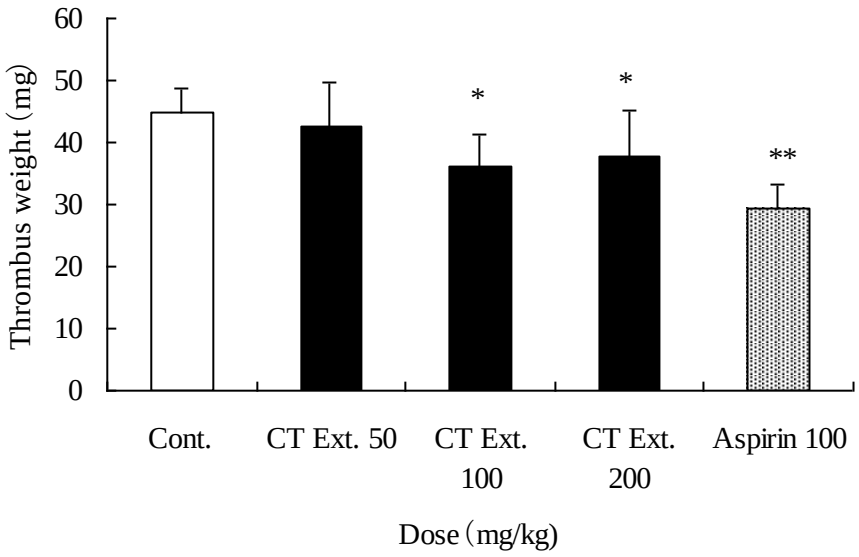


Fig. 20 The Effect of Cistanche Tubulosa extract (CT Ext.) on thrombosis in artery-vein bypass in rat (Mean±S.D., *: $p<0.05$, **: $p<0.01$)

8) Clinical Trial (Phase I-III，Data from Sinphar group)

①Phase I

1.1 Toxicity Test on Single Dose Administration (Oral)

Experimental Group	1	2	3	4	5	6	7
Dosage (mg, single)	150	300	600	900	1500	1800	2400
Increasing times	1	2	4	6	10	12	16
Nos. of subject	2	4	6	6	4	4	4

Nos. of subject：30

Method：Oral (Echinacoside 25%, Acteoside 3%)

Result：

No abnormal changes reported in body temperature, breathing frequency, heart rate, systolic arterial pressure, diastolic pressure, hepatic/renal functions, fasting blood glucose level, blood in general, urine in general, stool in general, or electrocardiogram on any subject people. No side effect reported during the test period. Results confirmed the safety of the extract in single dose administration in normal human being.

1.2 Toxicity Test on Continuous Administration (Oral)

Nos. of subject：12

Research centers：成都漢方医薬大学付属病院

Duration：10days （Start：2001.11）

Group	1	2
Dosage (mg/times)	600	900
Nos. of subject	6	6

Dosage：Oral Administration (three times a day) (Echinacoside 25%, Acteoside 3%)

Result：

No abnormal changes reported in body temperature, breathing frequency, heart rate, systolic arterial pressure, diastolic pressure, hepatic/renal functions, fasting blood glucose level, blood in general, urine in general, stool in general, or electrocardiogram on any subject people. No side effect reported during test period. Results confirmed the safety of the extract in continuous oral administration in normal human being.

1.3 Conclusion

No side effects nor abnormal changes reported on test parameters during single dose and continuous oral administration tests conducted in Phase I of the clinical trial of Cistanche Tubulosa extract. This clarified that Cistanche Tubulosa Extract is safe to be used in Phase II and III of clinical trial. In phase II and III of the clinical trial, Cistanche Tubulosa Extract (containing min. echinacoside 25%, min. acteoside 3%) 600mg was orally given to test subjects three times a day.

②Phase II

2.1 Method : Double blind tests were carried out in 5 research institutes upon approval by the Ethics Committee. Test subjects were divided into two groups: one group consuming Cistanche Tubulosa extract and another group as positive control consuming Hydergine, a pharmaceutical agent[*1]). The trial was conducted over period of 3 months. Test parameters include test subjects' cognitive functions (mini mental state examination: MMSE), social ability (berg balance scale: BBS), and ability of daily living (ADL) and complete physical examination by doctor was carried out before and after the administration, the effect of Cistanche Tubulosa extract on clinical treatment of vascular dementia was evaluated and compared with positive control group. Safety of the extract for clinical treatments was evaluated at the same time.

Research institutions：福建省漢方医薬研究院，戸州医学院付属漢方病院，陝西漢方医薬大学付属病院，西安市漢方病院，成都漢方医薬大学付属病院。
Duration：2002.3～2002.10

2.2 Nos. of subject：Cistanche Tubulosa extract：120
Positive control (pharmaceutical product, Hydergine)：120
Dosing period：Three months
Dosage：Oral Administration (600 mg, three times a day)

2.3 Result
2.3.1 Efficacy result

Efficacy ratio	MMSE	BBS	ADL	Symptoms
Cistanche Tubulosa Extract	75.66%	66.09%	50.43%	84.35%
Positive control	72.32%	54.46%	40.18%	70.54%

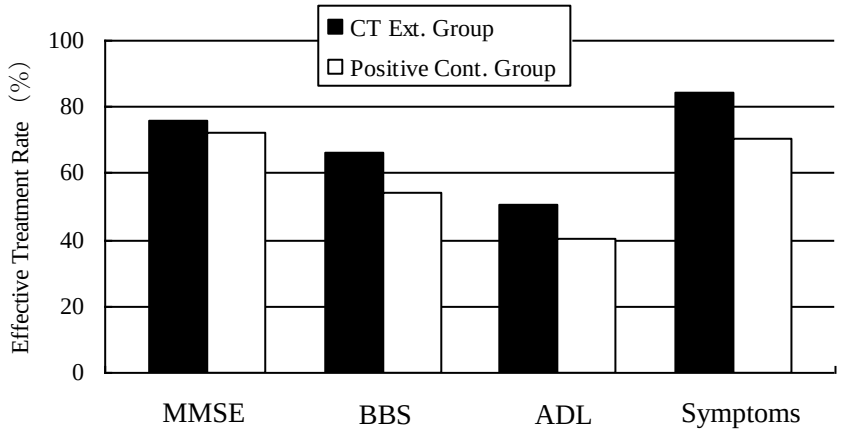


Fig. 21 The Effect of Cistanche Tubulosa Extract (CT Ext.) vs. Positive Control Group (Phase II)

2.3.2 Analysis of the Efficacy & Safety of Cistanche Tubulosa on Vascular Dementia

Cardiovascular Disease / Patient's Review	Treatment Rate	Improved	Unchanged	Worsen
Mild (46 subjects)	63.04%	−	34.78%	2.17%
Moderate (61 subjects)	34.43%	44.26%	21.31%	0.00%
High (9 subjects)	22.22%	55.56%	22.22%	−

1. Cistanche Tubulosa Extract effectively improved the conditions of subjects with moderate symptoms of the disease.
2. Treatment efficacy of Cistanche Tubulosa Extract is similar to that of positive control in subjects with severe symptoms of the disease.

2.3.3 Efficacy result of each facility

In Phase II of the clinical trial of Cistanche Tubulosa Extract, there is no significant difference reported in subjects with vascular dementia among the 5 research institutes, (p>0.05). This indicates that the effectiveness of Cistanche Tubulosa Extract was similar in all research institutes.

2.4 Long-term efficacy

Upon completion of Phase II clinical trial of Cistanche Tubulosa Extract, a survey was conducted to investigate the long term effect of Cistanche Tubulosa Extract on vascular dementia. Examination on cognitive function of test subjects was conducted at home. Result showed that, symptoms of test subjects treated with Cistanche Tubulosa Extract were either remained unchanged or improved 3 months later. The efficacy rate of Cistanche tubulosa Extract was 90.32% while efficacy rate of the positive control was 78.05%. In the response of survey from test subjects treated with Cistanche Tubulosa Extract, the score generated on evaluation of their cognitive function was 7.89±4.40 points higher than the score before the administration and 0.35±2.95 points higher than the score at the completion of the administration. Meanwhile, the score generated on evaluation of cognitive function of test subjects in positive control group was 5.73±3.23 points higher than the score before the administration. However, the score was 0.80±1.58 points lower than the score at the completion of the administration. The results show a significant difference between the two groups (p<0.05) and indicated that long-term effectiveness of Cistanche Tubulosa extract is better than Hydergine, the positive control.

2.5 Safety evaluation

A full analysis on blood, urine, stool, hepatic function (ALT), renal function (BUN, Cr), and electrocardiogram of all test subjects was carried out before and after the trial. There was no negative findings reported with Cistanche Tubulosa Extract. Although there was a significant difference in the effect on electrocardiogram (p<0.05) as compared to the positive control (Hydergine), there was no significant difference in other safety parameters(p>0.05).

2.6 Conclusion

Results from Phase II clinical trial concluded that Cistanche Tubulosa Extract is safe and effective in the treatment of vascular dementia.

③Phase III

3.1 Method Double blind tests were carried out in 4 research institutes upon approval by the Ethics Committee. Test subjects were divided into two groups: one group consuming Cistanche Tubulosa extract and another group as positive control consuming Hydergine, a pharmaceutical agent[*1]). The trial was conducted over period of 3 months. Test parameters include test subjects' cognitive functions (mini mental state examination: MMSE), social ability (berg balance scale: BBS), and ability of daily living (ADL) and complete physical examination by doctor was carried out before and after the administration, the effect of Cistanche Tubulosa extract on clinical treatment of vascular dementia was evaluated and compared with positive control group. Safety of the extract for clinical treatments was evaluated at the same time. Research institutions：成都漢方医薬大学付属病院，西安市漢方病院，戸州医学院付属漢方病院，福建省漢方医薬研究院。

Duration：2002.12～2003.8

3.2 Nos. of subject：Cistanche Tubulosa extract：333

Positive control (pharmaceutical product, Hydergine)：111

Dosing period：Three months

Dosage：Oral Administration (600 mg, three times a day)

3.3 Result

3.3.1 Efficacy result

Efficacy ratio	MMSE	BBS	ADL	Symptoms
Cistanche Tubulosa Extract	77.74%	72.10%	57.37%	91.19%
Positive control	64.15%	62.26%	38.68%	66.98%

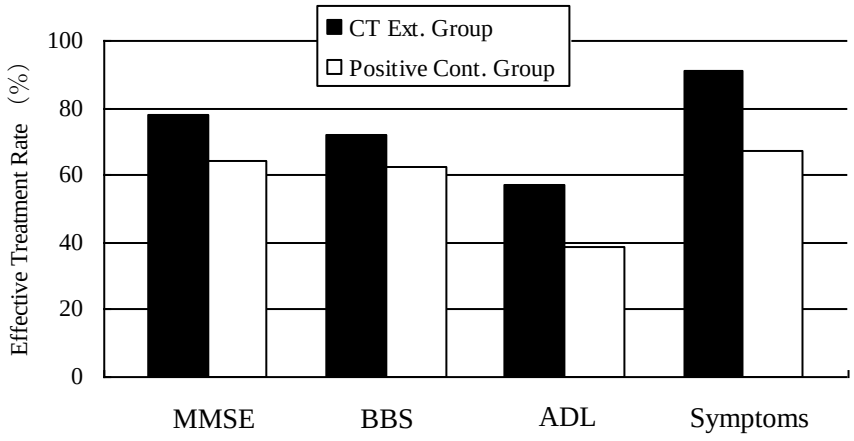


Fig. 22. The Effect of Cistanche Tubulosa Extract (CT Ext.) vs. Positive Control Group (Phase III)

3.3.2 Analysis on the Efficacy & Safety of Cistanche Tubulosa on Vascular Dementia

Cardiovascular Disease / Patient's Review	Treatment Rate	Improve	No changes	Worse
Mild (137 subjects)	83.94%	−	16.06%	0.00%
Moderate (182 subjects)	37.36%	49.45%	13.19%	0.00%
High (3 subjects)	66.67%	33.33%	0.00%	−

1. Cistanche Tubulosa Extract is more effective in subjects with moderate symptoms of the disease compared with positive control group.
2. There is no significant difference observed in subjects with severe symptoms when compared with positive control group.

3.3.3 Efficacy result of each facility

In Phase II of the clinical trial of Cistanche Tubulosa Extract, there is no significant difference reported in subjects with vascular dementia among the 4 research institutes, (p>0.05). This indicates that the effectiveness of Cistanche Tubulosa Extract was similar in all research institutes.

3.4 Long-term efficacy

Upon completion of Phase II clinical trial of Cistanche Tubulosa Extract, a survey was conducted to investigate the long term effect of Cistanche Tubulosa Extract on vascular dementia. Examination on cognitive function of test subjects was conducted at home. Result showed that, symptoms of test subjects treated with Cistanche Tubulosa Extract were either remained unchanged or improved 3 months later. The efficacy rate of Cistanche Tubulosa Extract and positive control group was 64.71% and 69.77% respectively. In the response of survey from test subjects treated with Cistanche Tubulosa Extract, the score generated on evaluation of their cognitive function was 5.39±3.26 points higher than the score before the administration and 1.71±2.43 points higher than the score at the completion of the administration. Meanwhile, the score generated on evaluation of cognitive function of test subjects in positive control group was 4.47±2.70 points higher than the score before the administration. However, the score was 1.47±1.84 points lower than the score at the completion of the administration.

3.5 Safety evaluation

There was no negative findings reported on test subjects treated with Cistanche Tubulosa Extract.

3.6 Conclusion

Results from Phase III clinical trial concluded that Cistanche Tubulosa Extract is safe and effective in the treatment of vascular dementia.

*1: Hydergine (Nonproprietary name：Dihydroergotoxine mesylate)

A drug to improve brain metabolism and peripheral blood circulation. It improves blood flow by releasing vascular tone and dilating blood vessels. It also accelerates oxygen and blood supply to the brain and improves metabolism of brain cells.

(2) Anti-aging effect

1) Free Radical Scavenging Ability (*in vitro*)

Cistanche salsa extract (phenylethanoid glycosides-rich fraction) has been reported to inhibit reactive oxygen species and prevent DNA damage[9]. The effect of Cistanche sals extract on reactive oxygen species (O_2-: Super oxide, OH: Hydroxyl radical, H_2O_2: hydrogen peroxide, $^1O_2$: singlet oxygen) was examined where IC_{50} (concentration of the sample to perform 50% inhibition) was measured by a chemiluminescence method. In addition, using the same method, IC_{50} was measured to evaluate the protective effect of Cistanche salsa Tubulosa Extract against DNA damage caused by hydroxyl radical. Result revealed that Cistanche salsa extract exert potent inhibitory effect on reactive oxygen species and thus prevent DNA damage (IC_{50}: 0.4211 μg/mL). The values of IC_{50} for each reactive oxygen species are tabulated in Table 5. These results indicated the anti-ageing effect of Cistanche salsa extract in the inhibition of reactive oxygen species. Cistanche Tubulosa is believed to function similarly as it belongs to the same family as Cistanche salsa while contains higher concentration of bioactive components.

Table 5. The Free Radical Scavenging Ability of Cistanche salsa extract

Free Radicals	IC_{50} (mg/mL)
O_2^- (superoxide)	0.0731
・OH (hydroxyl radical)	7.031
H_2O_2 (hydrogen peroxide)	0.098
$^1O_2$ (singlet oxygen)	0.1254

9) Xiaowen W., *et al.*, Free radical scavenging ability from *Cistanche* glycosides and its protection ability against DNA damage induce by OH. *Chinese Pharmaceutical Journal*, **36**(1), 29-32, 2001.

2) Enhance SOD activity and prevent lipid peroxidation (*in vivo*)

Cistanche salsa extract (phenylethanoid glycosides-rich fraction) has been reported to enhance SOD activity and prevent lipid peroxidation[10]. Each sample was orally administered to mice for 18 days. Two hours after the final administration, blood was collected from mice and SOD activity in red blood cells and serum MDA (malondialdehyde) content were measured. The effect of Cistanche salsa on DNA and RNA of major organs (heart, brain, liver, and kidneys) was examined. As illustrated in Fig. 23, Cistanche salsa significantly enhanced SOD activity while reducing MDA level in blood. Moreover, as shown in Table 6 & 7, levels of DNA and RNA of the liver and kidney increased in groups treated with Cistanche salsa. Therefore, it is indicative that the anti-ageing effect of Cistanche salsa is contributed by its inhibition on lipid

peroxidation and enhancement of antioxidant activity. Similarly, Cistanche Tubulosa is believed to possess similar activity as it belongs to the same family as Cistanche salsa while contain higher level of bioactive components.

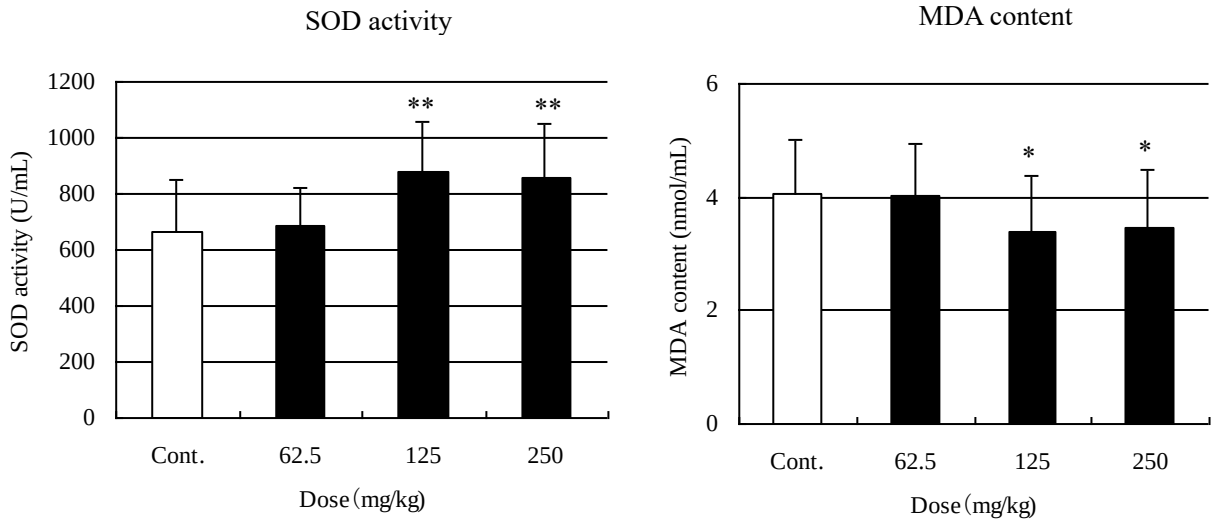


Fig. 23 The Effect of Cistanche salsa extract on red blood cell SOD activity and serum MDA content in normal mice. (Mean±S.D., *: p<0.05, **: p<0.01, n=20-30)

Table 6. The Effect of Cistanche salsa extract (CS Ext.) on DNA of major organs in normal mice. (Mean±S.D., *: p<0.05, **: p<0.01, n=20-30)

Group	Dose(mg/kg)	DNA (μg/100mg)			
		heart	brain	liver	kidneys
Cont.		72.2±13.3	53.7±14.1	96.1±17.0	141.6±20.7
CS Ext.	62.5	73.7±14.2	58.7±20.0	96.4±10.2	146.2±20.9*
	125	72.2±14.6	58.1±16.6	105.3±10.6*	163.4±22.2**
	250	73.4±12.5	63.3±18.6	109.7±17.8**	164.3±19.2**

Table 7. The Effect of Cistanche salsa extract (CS Ext.) on RNA of major organs in normal mice. (Mean±S.D., **: p<0.01, n=20-30)

Group	Dose(mg/kg)	RNA (μg/100mg)			
		heart	brain	liver	kidneys
Cont.		76.1±17.9	81.2±17.3	253.5±56.7	133.4±17.9
CS Ext.	62.5	78.3±18.3	80.7±17.1	252.6±42.9	142.9±28.9
	125	76.4±18.4	81.4±15.3	299.5±52.9**	161.3±27.8**
	250	77.0±13.8	90.6±18.2	319.9±39.5**	167.3±25.6**

10) Linlin L., *et al.*, Effects on *Cistanche* glycosides anti-lipid peroxidation and anti-radiation. *China Journal of Chinese Material Medicine*, **22**(6), 364-367, 1997.

3) Cistanche species on peroxidation (*in vitro, in vivo*)

The Effect of Cistanche Tubulosa (CT Ext.) and Cistanche salsa extracts (CS Ext.) on lipid peroxidation was compared based on following test method[11].

1. The Effect of Cistanche species extract on serum MDA in rabbit (*in vitro*)
   Whole blood of rabbit was incubated with Cistanche Tubulosa extract Cistanche salsa extract separately. The malondialdehyde (MDA) level, a measurable parameter serum lipid peroxidation, was quantified by TBA method. Result showed that both Cistanche Tubulosa and Cistanche salsa extracts significantly lowered serum MDA level (Fig. 24) while Cistanche Tubulosa demonstrated a stronger activity as compared to Cistanche salsa.

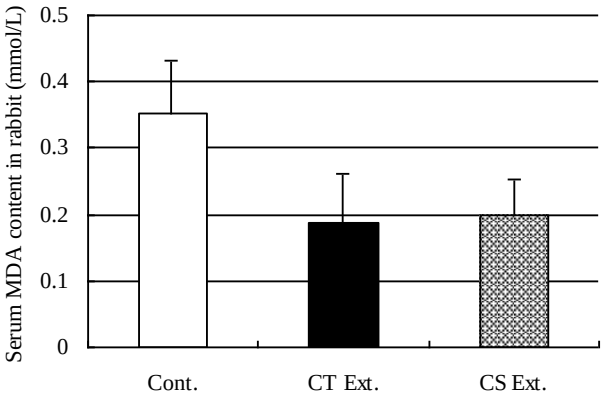


Fig. 24 The Effect of Cistanche species extract on serum MDA level in rabbit (Mean±S.D., *: $p<0.05$, $n=8$)

2. The Effect of Cistanche species extract on liver MDA level in mice (*in vitro*)
   Liver homogenate of mice was incubated with Cistanche Tubulosa extract and Cistanche salsa extract separately. The malondialdehyde (MDA) level, a measurable parameter serum lipid peroxidation, was quantified by TBA method. Result showed that both Cistanche Tubulosa and Cistanche salsa extracts significantly lowered serum MDA level in the supernatant (Fig. 25) while Cistanche Tubulosa demonstrated a stronger activity as compared to Cistanche salsa.

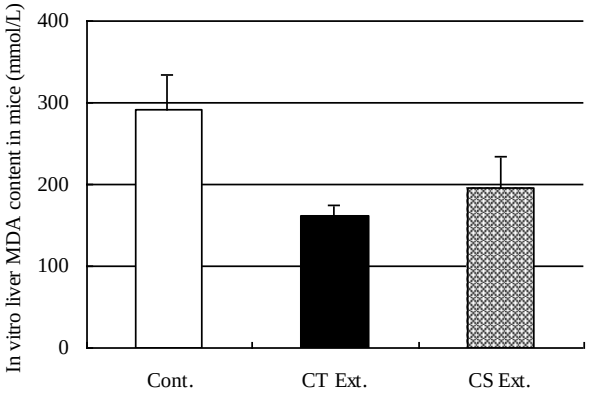


Fig. 25 The Effect of Cistanche species extract on liver MDA content in mice (Mean±S.D., **: $p<0.01$, $n=8$)

3. The Systemic (via oral administration) Effect of Cistanche species extract on liver MDA content in mice (*in vivo*)

   Cistanche Tubulosa extract and Cistanche salsa extract were orally given to mice once a day for ten days consecutively. On day-11th, the MDA level of mice's liver homogenate was quantified by TBA method. As illustrated in Fig. 26, both Cistanche Tubulosa and Cistanche salsa extracts significantly reduced the level of liver MDA. Nevertheless, both Cistanche Tubolosa Extract and Cistanche salsa extract demonstrated similar potency in the inhibition of lipid peroxidation.

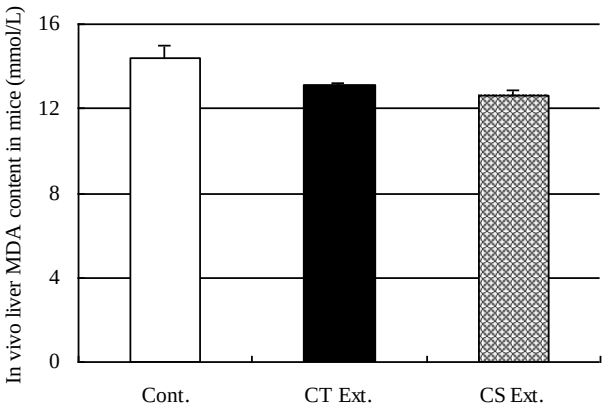


Fig. 26 The Effect of extract of Cistanche species on liver MDA in mice (Mean±S.D., *: $p<0.05$, $n=6$)

11) Dawen S., *et al.*, The effects of traditional Chinese medicine *Cistanche* species on the immune function and lipid peroxidation. *Acta Academiae Medicinae Shanghai*, **22**(4), 306-308, 1995.

4) Anti-aging effect on aging mouse model (*in vivo*)

Echinacoside (ECH), an important bioactive constituent of Cistanche Tubulosa extract, has been shown to have anti-aging activity on aged mice model[12]. D-galactose was administered to mice subcutaneously once a day for six weeks consecutively to create aged mice model. Each test sample was orally administered during the same period and vitamin E (VE) was given as positive control in one of the groups. At the end of the experiment, blood sample of mice was collected and major organs (heart, liver, kidney, and brain) were removed. The level of reactive oxygen species (ROS) in tissues of heart, liver, kidney, and brain was measured respectively by EPR (electron paramagnetic resonance) method. In addition, blood GSH-Px level was quantified by DTNB method [5,5'-dithiobis(2-nitrobenzoic acid) method] while serum SOD level was quantified by EPR method to study the effect of extracts physiological antioxidation system. As conducted previously, liver MDA level was measured by TBA method but level MAO (monoamine oxydase) was measured using a MAO activity measurement kit. As shown in Fig. 27, level of ROS in tissues of heart, liver, kidney and brain was significantly reduced in group receiving echinacoside upon comparison with aged mice

model. Meanwhile, groups receiving echinacoside (ECH) and vitamin E (VE) had a significantly increased in blood GSH-Px level and serum SOD level respectively. In addition, brain MAO level and liver MDA level had significantly reduced (Table 8). The lowering of liver MDA level and brain MAO level has proven the anti-oxidative effect of Cistanche Tubulosa Extract and suggesting the anti-ageing effect of Cistanche Tubulosa Extract.

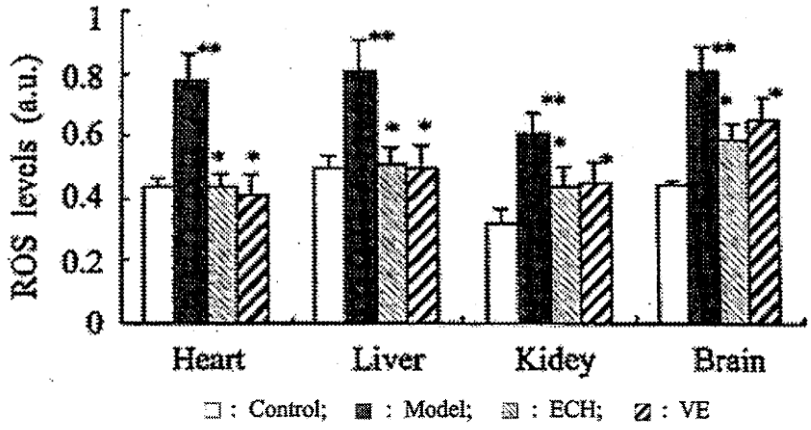


Fig. 27 The level of ROS in heart, liver, kidney and brain tissue among different groups (Mean ±S.D., **: p<0.01 vs control group *: p<0.01 vs model group, ECH: 50 mg/kg, VE: 50 mg/kg, n=10)

Table 8 The level of whole blood serum GSH-Px, SOD, brain MAO and liver MDA among different groups of mice (Mean±S.D., n=10)

	GSH-Px (U/mgprot)	SOD (U_{ESR}/ml)	MDA (nmol/mgprot)	MAO (U/h/mgprot)
Control	60.63±7.80	350.5±11.3	1.52±0.20	24.76±1.19
Model	32.99±10.75 **	300.0±14.2**	2.46±0.32**	28.09±3.76**
ECH (50 mg/kg)	54.27±7.97*	338.0±18.5*	1.95±0.21*	24.71±0.88*
VE (50 mg/kg)	45.87±7.42*	343.0±18.4*	1.95±0.37*	22.59±2.52*

**P<0.01 vs control group; *P<0.01 vs model group.

12) Gulinuer M., *et al.*, Anti-aging function study on echinacoside. *Acta Biochimica et Biophysica Sinica*, **20**(3), 183-187, 2004.

(3) Skin beautifying effect

1) Inhibition of hyaluronidase (*in vitro*, Data from Oryza)

Hyaluronidase, a hydrolase of hyaluronic acid, is widely distributed in tissues of animal including the skin. Hyaluronic acid, the substrate of the enzyme, is a type of mucopolysaccharide present in the skin, ligaments, joint fluids, and vitreous bodies in huge quantity. In the skin, hyaluronic acid is involved in protecting cells, distributing nutrients, and maintaining cells' moisture content and elasticity. It also performs crucial role as lubricant between joints to maintain tissue structure/functions and lubricity. The content of hyaluronic acid decrease with age and diseases which leads to dry, rough skin texture with lack of suppleness, sometimes appearance of dark spot and wrinkle. Besides, poorly lubricated joints may cause arthritic pain. As shown in Fig. 28, Cistanche Tubulosa Extract inhibited hyaluronidase (type 1) activity suggesting the anti-ageing potential of Cistanche Tubulosa Extract. Besides, hyaluronidase (type 1) activity was similarly inhibited by echinacoside and acteoside respectively (Fig. 29). Therefore, it is suggestive that echinacoside and acteoside are contributory to the inhibition of hyaluronidase activity by Cistanche Tubulosa Extract.

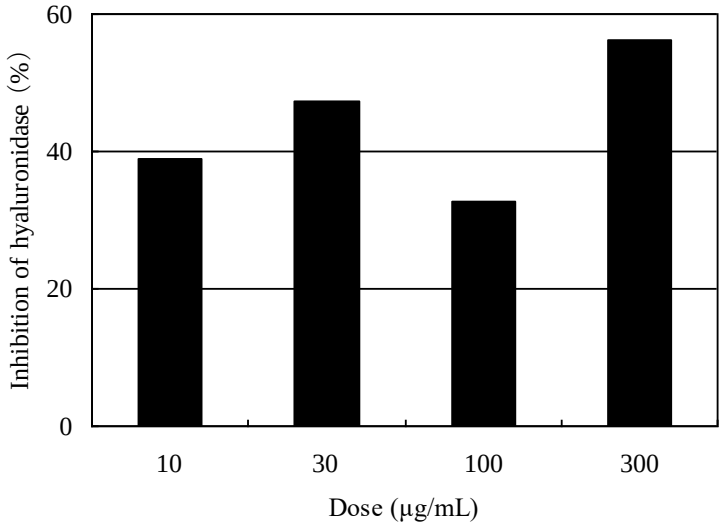


Fig. 28 The Effect of Cistanche Tubulosa Extract on hyaluronidase (type I)

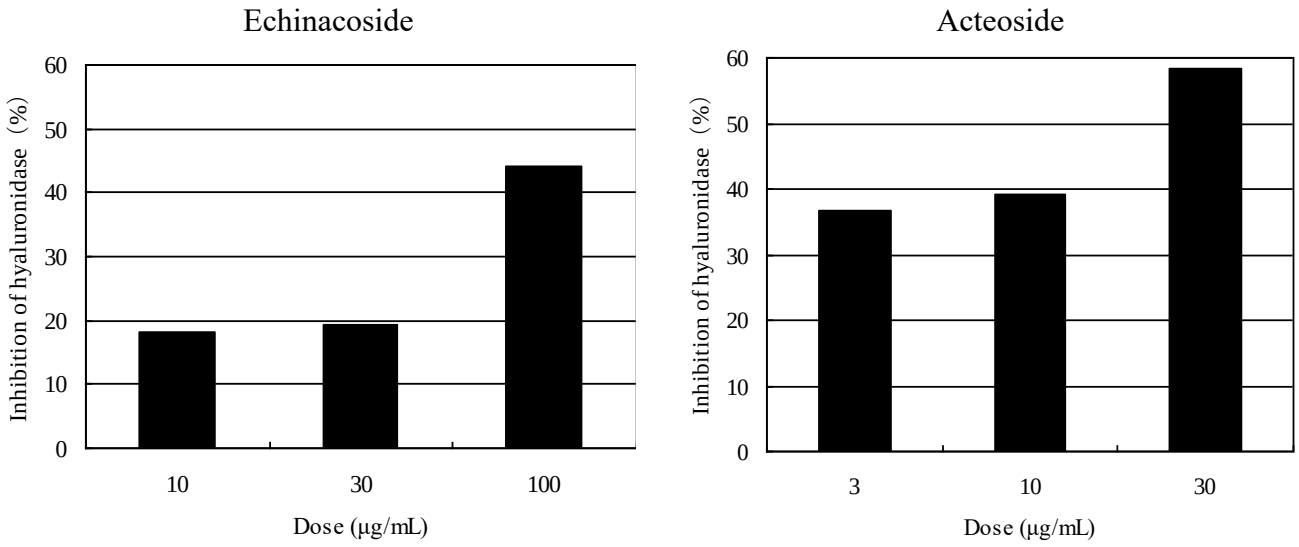


Fig. 29 The Effect of echinacoside and acteoside on hyaluronidase (type I)

【Method】Each samples were dissolved in DMSO, and hyaluronic acid was hydrolized by hyaluronidase. After reaction with *p*-dimethylamino- benzaldehyde, absorbance was measured.

2）Inhibition of Elastase (*in vitro*, Data from Oryza)

Elastin is a protein in the connective tissue. Elastin is responsible for the tissue to resume its original shape after being stretched. The amount of elastin decreases with age after early adulthood due to reduced capacity in elastin production and concomitant degradation of elastin by elastase leading to sagging and wrinkling skin. The effect of Cistanche Tubulosa extract, acteoside and echinacoside on elastase activity was examined using Elastase Assay Kit. Results revealed a concentration dependent elastase inhibitory effect demonstrated by Cistanche Tubulosa Extract, Acteoside and Echinacoside respectively (Fig. 30). It is suggestive that echinacoside and acteoside are contributory in the inhibition of elastase by Cistanche Tubulosa Extract.

[Method]
50μL of each sample (concentration 10, 30, 100, 300, 1000μg/mL) were dissolved in 10% DMSO in a 96-well-plate. Then, 50μL of DQ elastin(100μg/mL) was added followed by 100μL of elastase (0.2U/mL). Upon completion of reaction, absorbance was measured at wavelength 485nm & 530nm respectively.

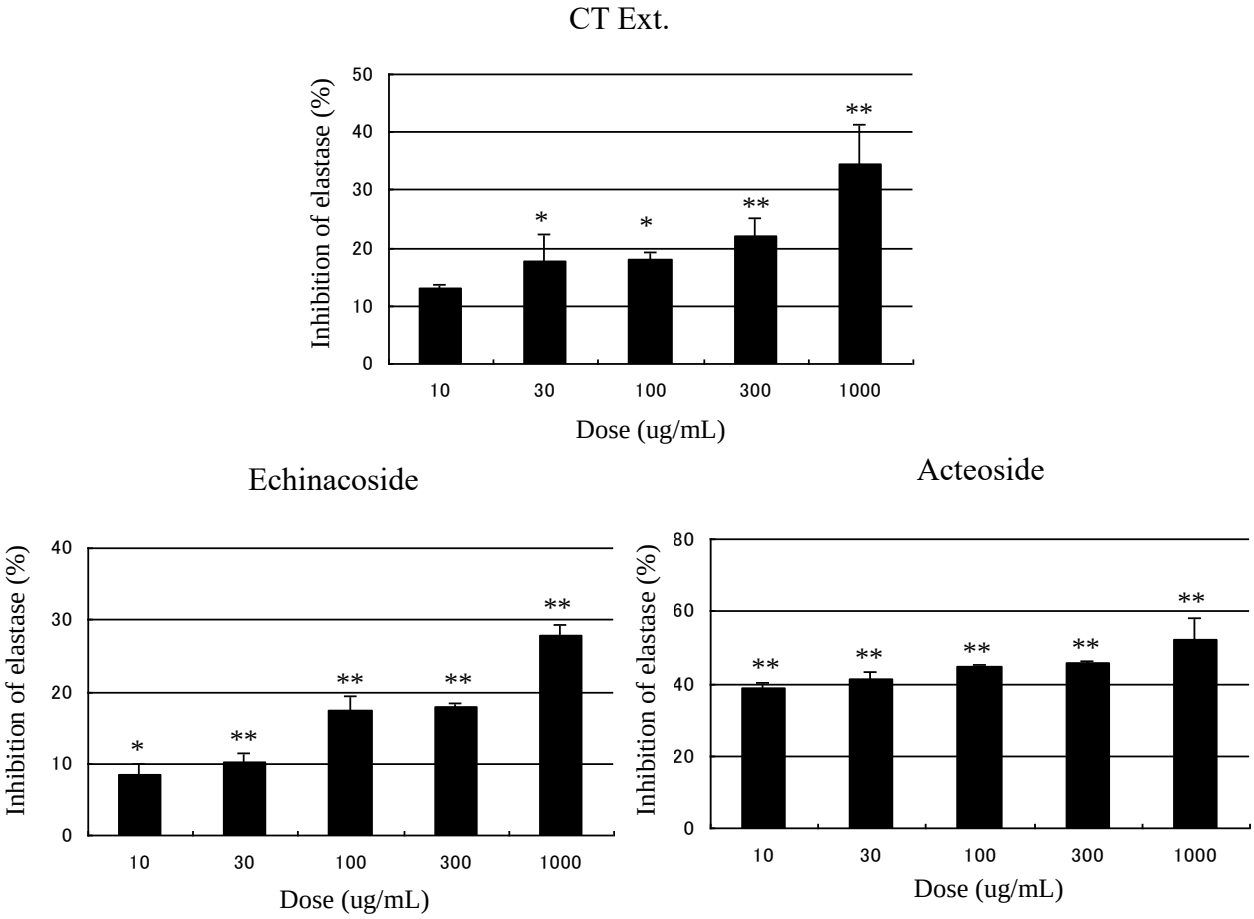


Fig. 30 The Effect of Cistanche Tubulosa Extract (CT Ext.), echinacoside, and acteoside on elastase (Mean±S.E., *n*=3, *: $p<0.05$, **: $p<0.01$)

3) Inhibition of Tyrosinase (*in vitro*, Data from Oryza)

Melanin is the primary determinant of human skin colour. The biosynthetic pathway of melanin is catalysed by tyrosinase:
Tyrosine → Dopa → Dopaquinone
Ageing and over exposure to UVB radiation may increase the production of melanin which result in skin pigmentation and dark spots. The tyrosinase inhibitory effect by Cistanche Tubulosa extract and Acteoside has been reported while involvement of echinacoside is unknown. Experiment was promted to evaluate the effect of echinacoside on tyrosinase activity. As illustrated in Fig. 31, echinacoside demonstrated a dose-dependent inhibitory effect on tyrosinase. Clearly, both echinacoside and acteoside are contributory in the inhibition of tyrosinase by Cistanche Tubulosa Extract.

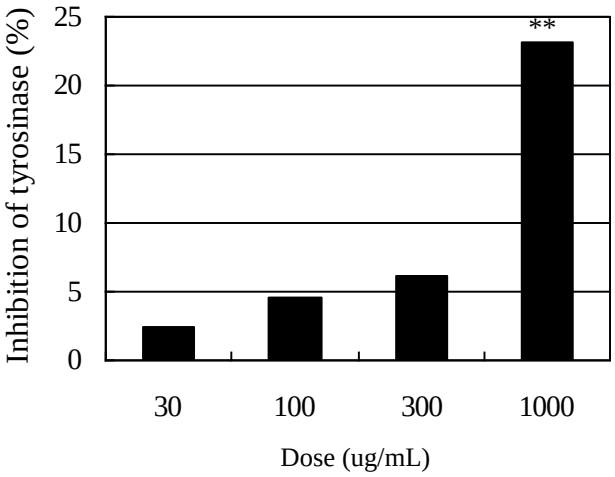


Fig. 31 The Effect of echinacoside on tyrosinase (n=4, **: p<0.01)

[Method]
0.3% L-DOPA (70μL/well) was added to various concentration of sample solution followed by pre-heating at 37°C for 5min. Then, tyrosinase solution (mushroom-derived, 1.6units/mL) was added and allow for reaction at 37°C for 5min. Microplate absorbance was measured at wavelength 492nm upon completion of reaction.

4) Prevention of Photo-ageing of Skin (*in vivo*, Data from Oryza)

Reactive oxygen species generated from UV-rays is one of the major risk factors contributing to ageing skin. Photo-induced aged mice (by radiating UV ray to hairless mice) were used in an experiment to study the effect of Cistanche Tubulosa Extract on skin ageing. The effect of Cistanche Tubulosa Extract on wrinkle formation and skin ageing-related genes (hyaluronidase: Hyal1, collagenase: MMP-1) was evaluated. As illustrated in Fig. 32, Cistanche Tubulosa Extract at concentration of 200mg/kg reduced the total wrinkling area due to UV exposure. Besides, quantification by RT-PCR revealed that the genes expression of hyaluronidase and collagenase in response to UV ray exposure was similarly reduced in mice treated with Cistanche Tubulosa extract (200 mg/kg) (Fig. 33). The results were similar with the results on evaluation on wrinkle formation shown in Fig. 29. Based on above findings, Cistanche Tubulosa Extract is proven to prevent UV-induced photo ageing of skin, thus reduce wrinkling formation.

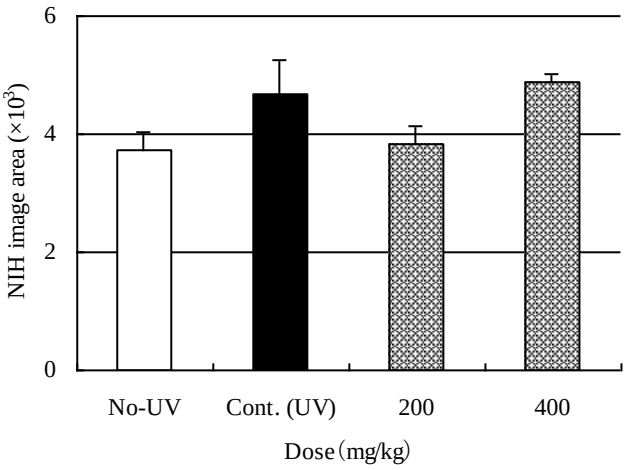


Fig. 32 Anti-wrinkle effect of Cistanche Tubulosa Extract (Mean±S.E., *n*=4)

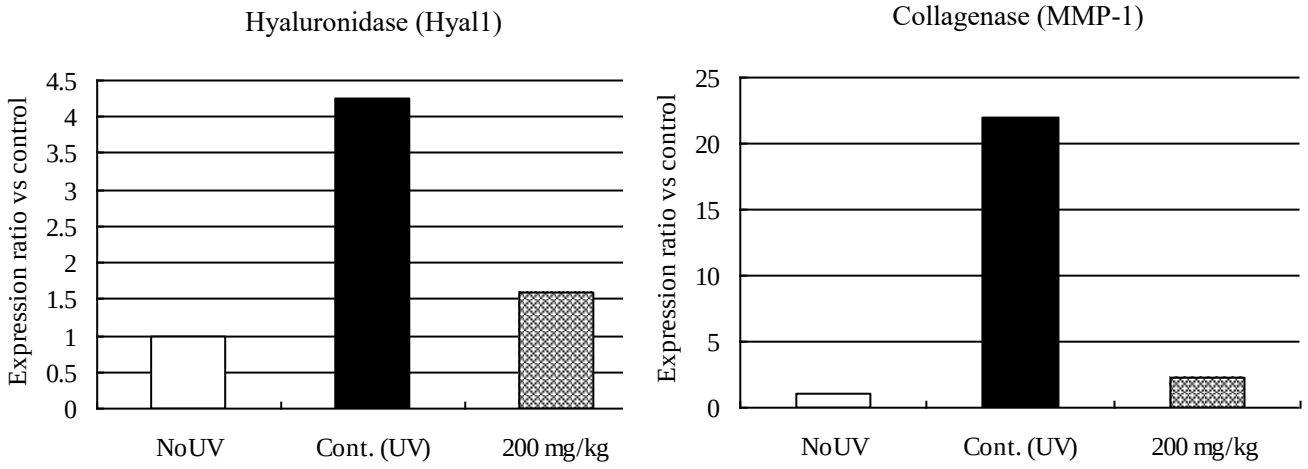


Fig. 33 The Effect of Cistanche Tubulosa Extract on Genetic Expression of Hyaluronidase and Collagenase (There is no RT-PCR analysis in group C.T. Ext 400mg/kg as RNA extraction was unsuccessful.)

【Method】Hairless mice were separated into four groups (No UV group, UV + solvent group, UV + 200 mg/kg of Cistanche Tubulosa extract group, and UV + 400 mg/kg of Cistanche Tubulosa). Each test sample was orally administered to mice for six consecutive weeks. Meanwhile, mice were exposed to UV ray three times a week for six weeks (1st week: 50 mJ/cm^2, 2nd week: 70 mJ/cm^{2,} 3rd and 4th weeks: 80 mJ/cm^2, 5th and 6th weeks: 200 mJ/cm^2). At the end of the experiment, replicas of the back of the hairless mice were taken using Skin Cast. NIH Image analysis of skin replicas was carried out to evaluate the effect of test samples on wrinkle formation. Last, skin of the hairless mice was removed, RNA of the skin was extracted for reverse transcription reaction, and gene expression was analyzed and compared by RT-PCR.

(4) Anti-fatigue

Anti-fatigue of mice（*in vivo*）

Cistanche Tubulosa extract has been reported to have anti-fatigue activity with prolonged survival time of oxygen deficient mice, prolonged survival time in sodium nitrite (toxic agent), and prolonged tolerance time of increased weight bearing swimming [13].

1. Survival under anaerobic condition

Each sample was 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. As shown in Fig. 34, the survival time of group oxygen deficient mice receiving Cistanche Tubulosa Extract was significantly longer. The prolonged survival time is concentration dependent.

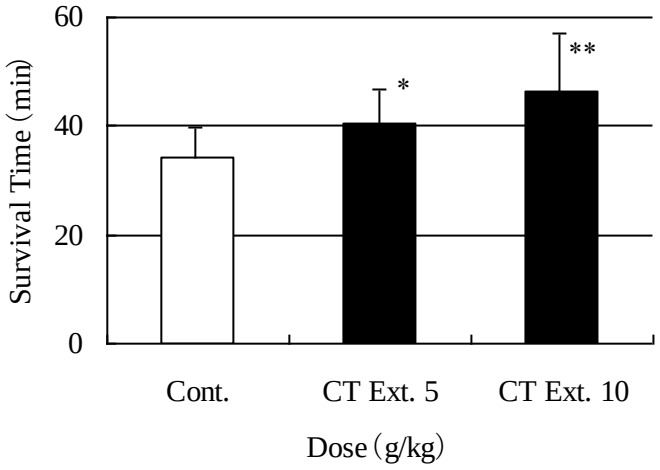


Fig. 34 The Effect of Cistanche Tubulosa extract (CT Ext.) on survival time of oxygen deficient mice. (Mean±S.D., *: p<0.05, **: p<0.01, n=10-12)

2. Survival post administration of Sodium Nitrite (toxic agent)

Each test sample was administered to mice intraperitoneally followed by administration of sodium nitrite (250 mg/kg) 30 minutes later. The survival time of mice in sodium nitrite was measured. Result showed that the survival time in sodium nitrite in mice receiving Cistanche Tubulosa Extract was significantly longer (Fig. 35). The effect was concentration-dependent.

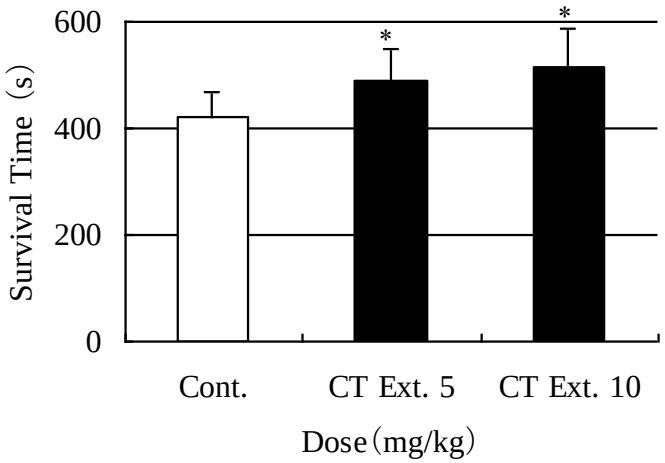


Fig. 35 The Effect of Cistanche Tubulosa Extract on survival time of mice in sodium nitrite. (Mean±S.D., *: $p<0.05$, $n=10$)

3. Endurance on forced swimming test

Each test sample 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. As shown in Fig. 36, mice receiving Cistanche Tubulosa Extract demonstrated a prolonged tolerance time in forced swimming test. The effect was concentration-dependent. Summarizing the above findings, Cistanche Tubulosa Extract exhibited anti-fatigue effect with increased tolerance on ischemic condition and resistance to toxic substance, sodium nitrite.

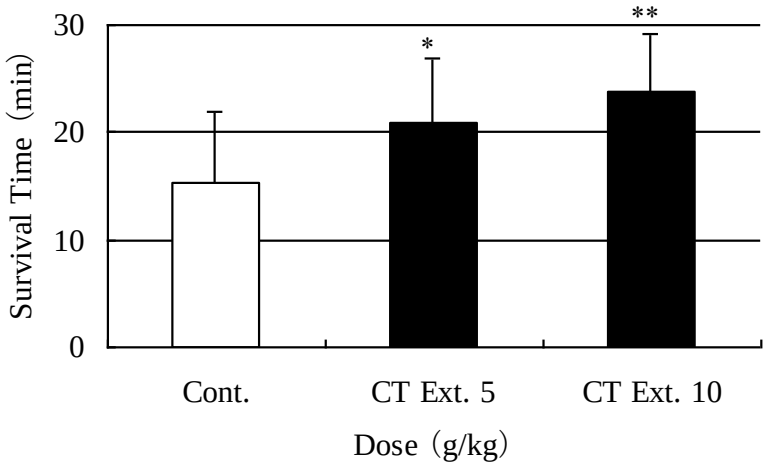


Fig. 36 The Effect of Cistanche Tubulosa Extract on forced (increased weight bearing) swimming test. (Mean±S.D., *: $p<0.05$, **: $p<0.01$, $n=12$)

13) Zhiqiang W., *et al.*, Effects of CTG on oxygen insufficiency tolerance and fatigue tolerance. *Chinese Traditional Herbal Drugs*, **27**(supplementary issue), 137-138, 1996.

(5) Aphrodisiac effect

1) Effects of the constituents of Cistanchis herba on sex behavior in stressed mice (*in vivo*)

Phenylethanoid glycosides-rich fraction of Cistanche salsa extract and its bioactive components, echinacoside and acteoside have been reported to improve sexual ability[14]. The effect of Cistanche salsa extract and its bioactive components, echinacoside and acteoside on stress-induced reduced libido was evaluated for 15 consecutive days. Ten female mice and one male mouse were placed together for ten minutes and the number of mice that experienced mounting or intromission, the number of such behavior, and also the time spent for mounting were measured. Groups and the number of days with significant difference observed were described below. In group of mice receiving Cistanche salsa extract, mounting and intromission observed on day-3 and day-5 respectively while the time spent for mounting and intromission observed on day-4. In group of mice receiving echinacoside and acteoside, sexually active mice were observed on day-4 and day-5 respectively while mounting and intromission observed on day-3 to day-6. Also, time spent for mounting and intromission was observed on day-4 to day-6 respectively. Results indicated that Cistanche salsa extract (phenylethanoid glycosides-rich fraction), achinacoside and acteoside improved condition of stress-induced reduced libido in mice. Similarly, belonging to the same family as Cistanche sals, Cistanche Tubulosa with higher content of bioactive components is believed to be aphrodisiac on stressed mice.

14) Sato T., *et al.*, Pharmacological studies on *Cistanchis Herba*. I. Effects of the constituents of *Cistanchis Herba* on sex and learning behavior in chronic stressed mice (1), *Yakugaku Zasshi*, **105**(12), 1131-1144, 1985.

2) Enhance male hormone production (*in vitro, in vivo*, Data from Oryza)

1. The effect of Cistanche Tubulosa Extract on the genetic expression of enzymes of male hormone production in the liver

The effect of Cistanche Tubulosa extract (CT Ext.) on the gene expression of **3β-hydroxysteroid dehydrogenase** (3β-HSD), an enzyme that is responsible for the synthesis of testosterone, and **5α-reductase-2 and aldo-keto reductase**, enzymes that are responsible for the synthesis of dihydrotestosterone was examined. Cistanche Tubulosa extract (400 mg/kg) was administered to mice once daily for two weeks and liver RNA was extracted. DNA micro array analysis was conducted on one specimen in each group of mice. In the specimen of mice receiving Cistanche Tubulosa extract, the genetic expression of 3β-hydroxysteroid dehydrogenase (3β-HSD) was enhanced by 1.5 times. Meanwhile, the genetic expression of 5α-reductase-2 and aldo-keto reductase, was doubled (Fig. 37). Meanwhile, gene expression not being analyzed by micro array was analyzed by RT-PCR with exception on C17-20 lyase. As illustrated in Fig. 38, genetic expression of P450 SCC, 17α-hydroxylase, 17β-hydroxysteroid dehydrogenase, and 5α-reductase-2 was enhanced. In particular, gene expression of 5α-reductase-2 was 15 times higher as compared to the control. The enhancement of genetic expression by Cistanche Tubulosa extract (400 mg/kg) on enzymes responsible for the production of male hormone suggested the positive effect of Cistanche Tubulosa Extract on male hormone production.

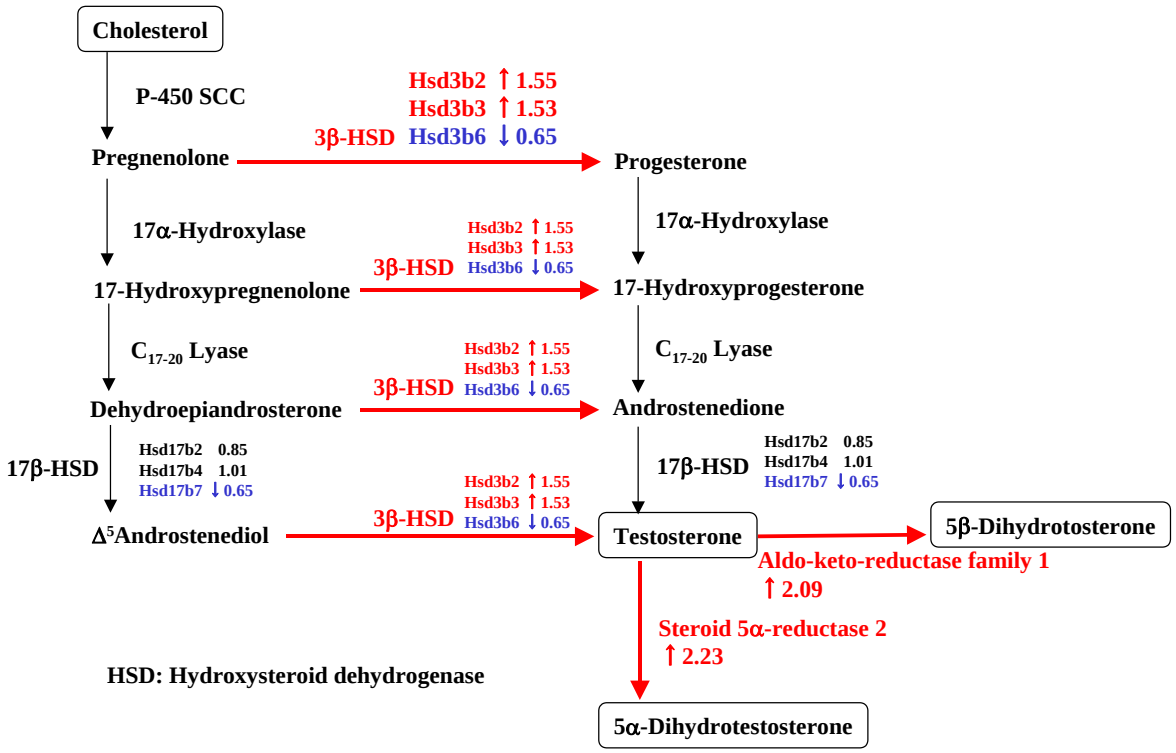


Fig. 37 DNA micro array analysis of the liver [↑ (Increase), ↓ (Decrease) and fold change rate is relative to control=1]

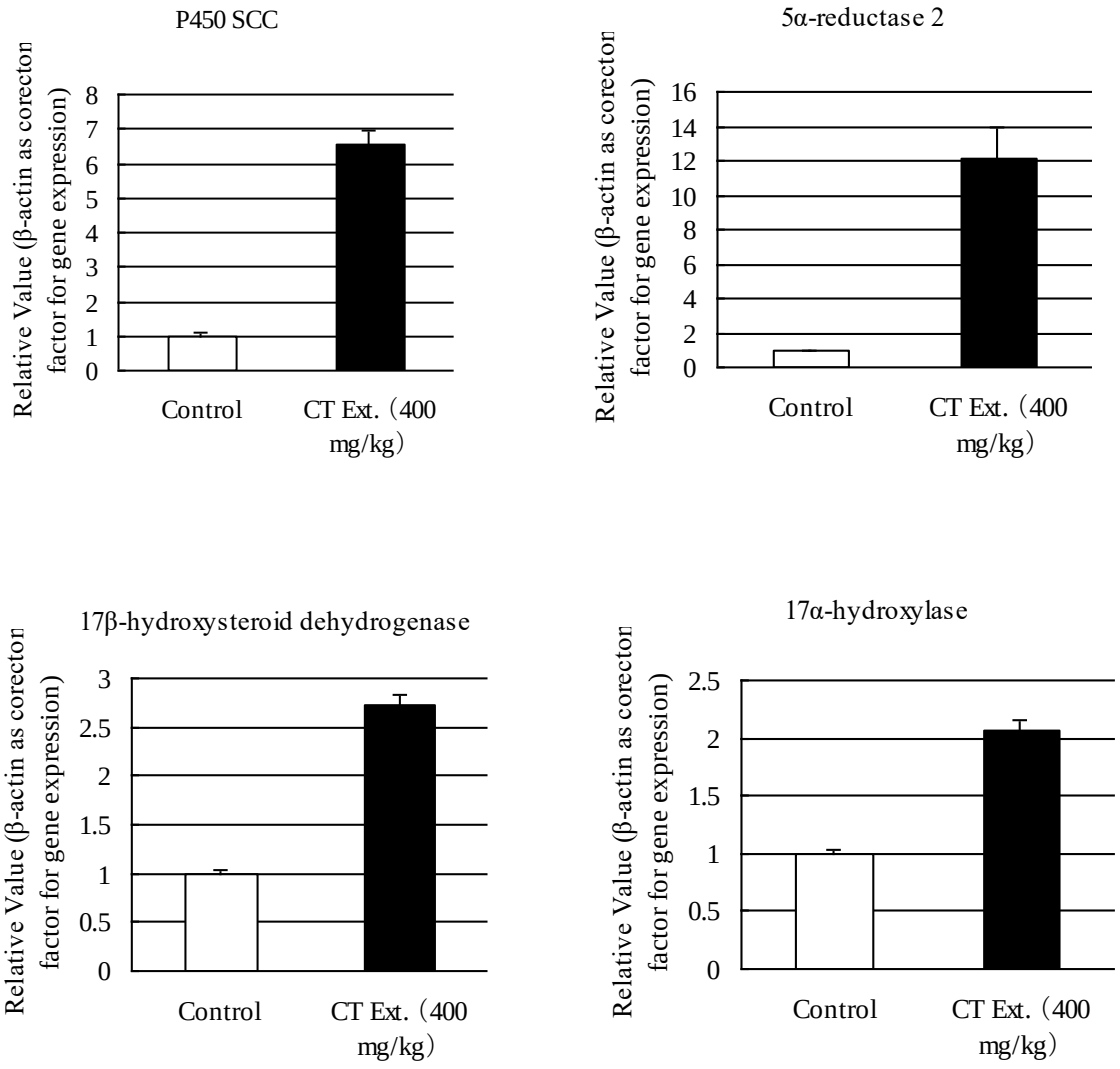


Fig. 38 RT-PCR analysis of genetic expression in the liver (Mean±S.E., *n*=3-7)

2. The effect of Cistanche Tubulosa Extract on gene expression of male hormone production in the testis

Based on above findings, further experiment was prompted to study the effect of Cistanche Tubulosa Extract (CT Ext.) on the genetic expression of 5α-reductase-1 and -2 in the testis. However, there is no enhancement of gene expression in the testis (Fig. 39).

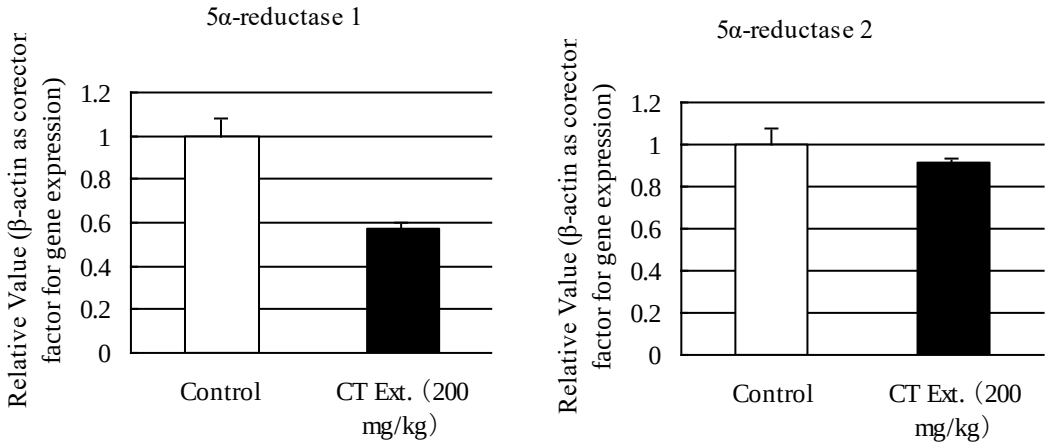


Fig. 39 RT-PCR analysis in testis (*n*=5-7)

【Method】Cistanche Tubulosa extract (200 mg/kg) was orally administered to male ddY mice (5 months old) for two weeks. After stabilizing the testis by "RNA later," RNA was extracted using a kit manufactured by Quiagen. c-DNA was produced by the reverse transcription reaction in the conventional manner and the gene expression was examined by RT-PCR.

3. The effect of Cistanche Tubulosa Extract on mouse testosterone level

Based on findings of 1 and 2 above, the effect of Cistanche Tubulosa extract (CT Ext.) on blood testosterone concentration was evaluated. However, no significant difference observed though there was tendency of increased concentration of testosterone (Table 9).

Table 9. The effect of Cistanche Tubulosa Extract on mouse testosterone level

	Dose (mg/kg)	*n*	Testosterone（ng/mL）
Mice (5 month)	-	6	29.53±10.4
CT Ext.	200	7	40.41±29.64
	400	7	79.11±44.66

Mean±S.E.

【Method】Cistanche Tubulosa extract was orally administered to male ddY mice (5 months old) for two weeks and whole blood sample was collected. After separating the serum, level of testosterone was measured using the Testosterone EIA kit (Cayman Chemical Corporate).

4. The Effect Cistanche Tubulosa Extract on testosterone production in Leydig cells

Leydig cell exists in the testis and produces testosterone. Using Leydig cell line (R2C), the effect of Cistanche Tubulosa Extract on testosterone production was evaluated. As shown in Fig. 40 below, Cistanche Tubulosa Extract and its bioactive components, echinacoside and acteoside, increased the production of testosterone in Leydig cells.

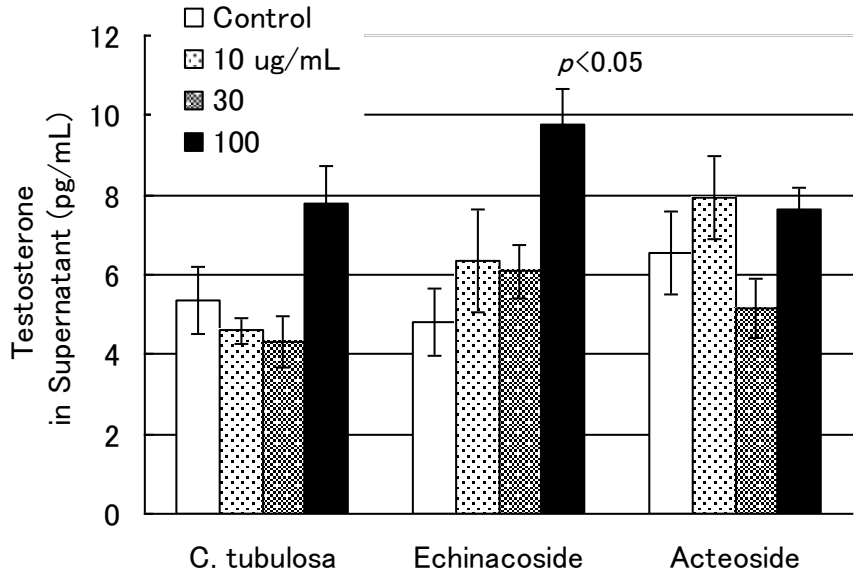


Fig. 40 The Effect of Cistanche Tubulosa extract, echinacoside and acteoside on testosterone production in Leydig cells (n=4, Mean±S.E)

【Method】Leydig cells (R2C) derived from testicular cancer of rat were cultured in a 24-hole plate (2.5×10^5 cells/500 uL) and incubated for 24 hours. After addition of test samples, the cells continue to be incubated for four hours, and the supernatant was separated to measure the level of testosterone.

(6) Immune boosting effect

The effect of Cistanche Tubulosa extract on mouse lymphatic cells (*in vivo*)

Cistanche salsa extract (phenylethanoid glycosides-rich fraction) has been reported to enhance immune function[15]. ^{60}Co (cobalt 60) was radiated on mice to lower the immune function. Each test sample was orally administered to the mice for 15 days consecutively. At the end of the experiment, blood was collected from mice and specimen slides were prepared. The diameter of lymphocytes (cells that defend the host from attack from bacteria and viruses), was measured under microscope to evaluate the effect of Cistanche salsa extract. As shown in Fig. 41, size of lymphocytes was significantly enlarged in group of mice receiving Cistanche salsa extract as result of antigen stimulation (cobalt 60) and it is suggestive that Cistanche Tubulosa Extract may exert similar effect as it belongs to the same family as Cistanche salsa while containing higher level of bioactive components.

In addition, Cistanche Tubulosa extract has been reported to activate lymphoid cells and increase the killing rate on cancer cells[16]. Therefore it is further convinced that Cistanche Tubulosa extract enhances immune function.

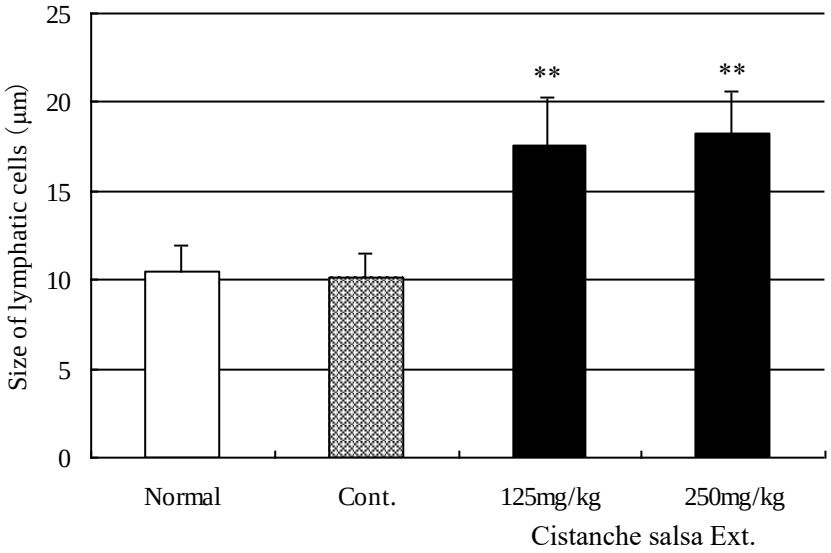


Fig. 41 The effect of Cistanche salsa Extract on size of lymphocytes
(Mean±S.D., **: p<0.01, n=15)

15) Xiaowen W., *et al.*, Morphological changes of peripheral blood corpuscles of radiated mice feeded with *Cistanche*. *ACTA Academiae Medicinae Xinjiang*, **18**(2), 83-86, 1995.

16) Dawen S., *et al.*, The effects of traditional Chinese medicine *Cistanche* species on the immune function and lipid peroxidation. *Acta Academiae Medicinae Shanghai*, **22**(4), 306-308, 1995.

(7) Metabolism enhancing effect

1) The effect on cholesterol metabolism (*in vivo*, Data from Oryza)

The effect of Cistanche Tubulosa extract (CT Ext.) on the gene expression of enzymes responsible for lipid metabolism in the liver was examined. Cistanche Tubulosa extract (400 mg/kg) was administered to mice for two weeks and liver RNA was obtained for DNA micro array analysis.

DNA micro array analysis was conducted on one specimen of mice of each group. As shown in Fig. 42, the gene expression of HMG CoA reductase, a rate-limiting enzyme responsible for cholesterol synthesis converting 3-hydroxy-methyl-3-methylglutaryl CoA (HMG CoA) to mevalonic acid is down-regulated. Down-regulating the genetic expression of HMG COA reductase suggested the potential of Cistanche Tubulosa Extract in preventing hypercholesterolemia.

Furthermore, genetic expression of apo-lipoprotein B, VLDL receptor, and lipoprotein lipase was enhanced in group of mice receiving Cistanche Tubulosa extract (Table 10). Genetic expression of apo-lipoprotein B and VLDL receptor of remaining mice was analyzed by RT-PCR. Results revealed an enhanced genetic expression of both apo-lipoprotein B and VLDL receptor (Fig. 43). Apo-lipoprotein B and VLDL receptors are responsible for the transfer and cellular uptake of blood cholesterol, the up-regulation of genetic expression indicated the potential of Cistanche Tubulosa Extract in lowering blood cholesterol level.

Besides, serum cholesterol and liver cholesterol in group of the mice receiving Cistanche Tubulosa extract was measured. As tabulated in Table 11, there is a slight reduction in the ratio of LDL-cholesterol/pre β+β lipoprotein (fraction containing VLDL and LDL-cholesterol) while ratio of HDL-cholesterol/α-lipoprotein (fraction containing HDL-cholesterol) was slightly increased. Minimal changes observed as normal mice were used in the experiment. Further experiment is prompted in the future using hypercholesrolaemic mice (pathological condition) to obtain a more comprehensible result.

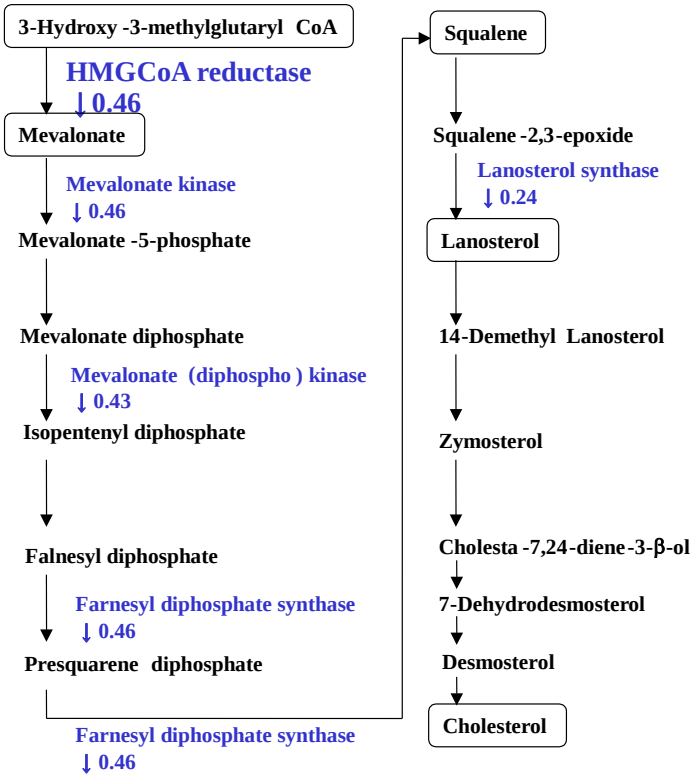


Fig. 42 DNA micro array analysis on enzymes in the Liver [↓ (Decrease) and fold change rate is relative to control=1]

Table 10 Up-regulation of cholesterol transporter gene DNA Micro Array analysis

	Fold Change	Function
Apolipoprotein B	↑2.87	Delivery VLDL cholesterol
VLDL receptor	↑9.00	Uptake of VLDL into cells
Lipoprotein lipase	↑2.08	Degradation and uptake of VLDL into cells

Fold change rate is relative to control=1

Table 11 Blood Lipid Profile of Liver Homogenate of mouse serum

	Total Cho. (mg/dL)	LDL-Cho. (mg/dL)	Ratio of Preβ+βlipo protein (%)	HDL-Cho. (mg/dL)	Ratio of α-lipoprotein (%)	Liver Cho. (mg/g)
Control	129.8±12.8	10.0±1.4	17.0±1.5	119.8±11.3	76.1±1.9	3.1±0.1
CT Ext.400mg/kg	132.0±8.7	9.5±0.5	16.4±0.9	123.8±7.7	78.3±1.2	3.2±0.2

Mean±S.E., *n*=5-7.

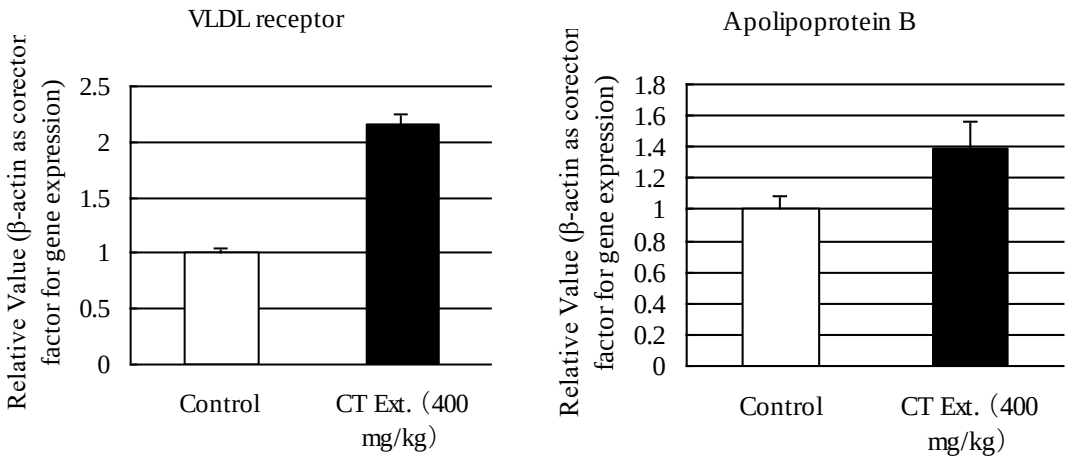


Fig. 43 RT-PCR analysis of Liver (enhancement of apolipoprotein B and VLDL receptor, Mean±S.E.)

2) The Effect of Cistanche Tubulosa Extract on Fatty Acid Metabolism (*in vivo,* Data from Oryza)

Genetic expression responsible for lipid metabolism in the liver were analyzed. As tabulated in Table 12, gene expression of Lipin 1, PPARα, Acetyl-CoA acyl transferase 1A, Acetyl-CoA acyl transferaase 1B and Carnitine Palmitoyl Transferase (CPT) 1 were up-regulated in specimen of mice receiving Cistanche Tubulosa Extract. Lipin 1 and PPARα regulate lipid metabolism. Recently, Lipin 1 was reported to accelerate lipid metabolism. Acetyl-CoA acyl transferase and CPT are enzymes involved in beta-oxidation in which the former is involved in fatty acid degradation and the latter is responsible for the uptake of fatty acid into mitochondria. Up-regulation of these gene expression indicated that Cistanche Tubulosa extract (CT Ext.) accelerates the metabolism of fatty acid in the liver. Genetic expression of Lipin 1 and CPT 1 of remaining mice was analyzed by RT-PCR. Similarly, as shown in Fig. 44, genetic expression of Lipin 1 and CPT 1 was up-regulated. Based on above findings, Cistanche Tubulosa Extract accelerate lipid metabolism in the liver.

Table 12 Genetic Expression of enzymes of Fatty Acid Metabolism

	Fold Change	Function
Lipin 1	↑ 5.11	Regulate lipid metabolism
PPARα	↑ 2.14	Regulate lipid metabolism (β -oxidation)
Acetyl-CoA acyl transferase 1A	↑ 2.78	Regulation of β -oxidation
Acetyl-CoA acyl transferase 1B	↑ 2.07	Regulation of β -oxidation
Carnitine palmitoyl transferase (CPT) 1	↑ 2.67	Uptake of fatty acids into Mitochondria (Rate limiting enzyme of β -oxidation)

Fold change rate is relative to control=1

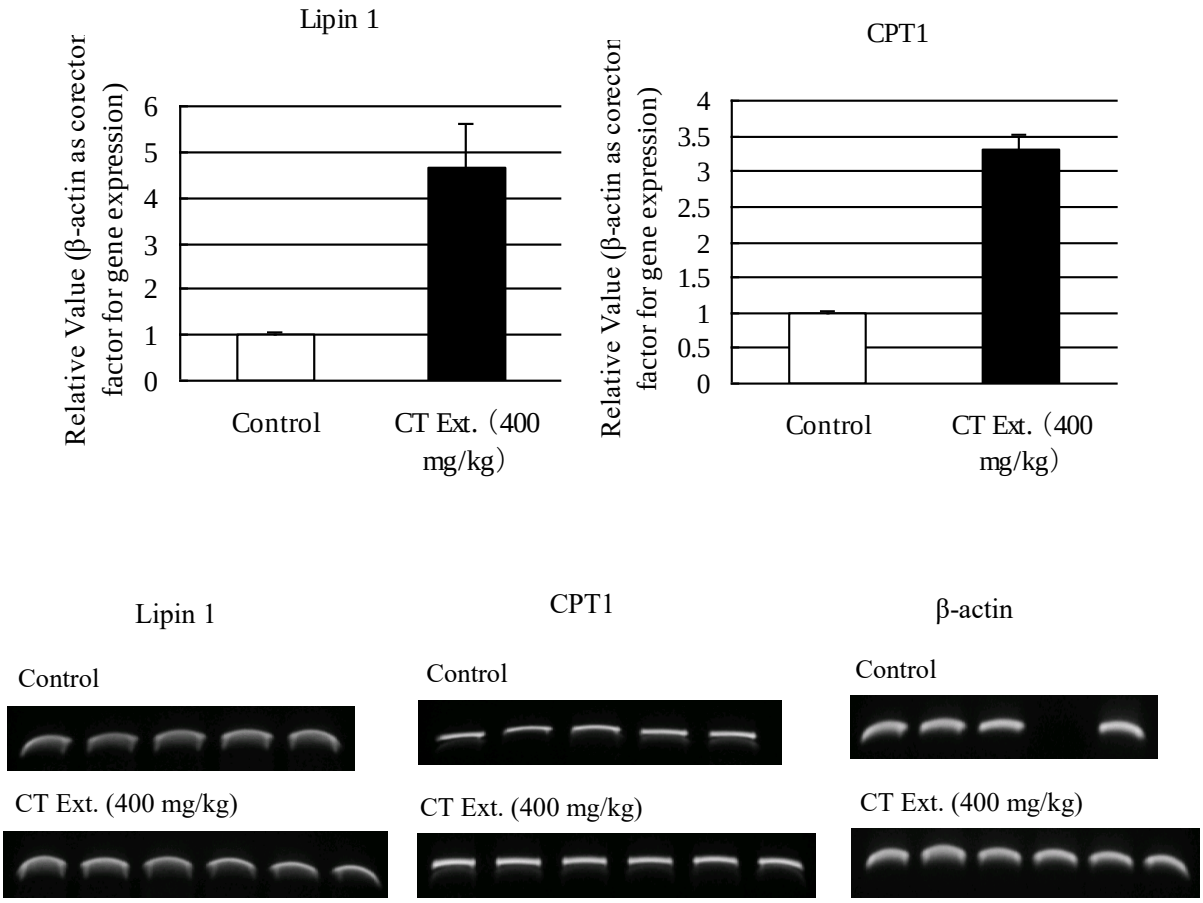


Fig. 44 RT-PCR analysis in Liver (enhanced of lipin 1 and CPT1, Mean±S.E., *n*=5-7)

【Method】Cistanche Tubulosa extract (400 mg/kg) was orally administered to male ddY mice (5 months old) for two weeks. After stabilizing their liver tissue by "RNA later," RNA was obtained using a kit manufactured by Quiagen. DNA microarray analysis was conducted on one specimen each experimental group. Gene expression of remaining mice was evaluated by RT-PCR.

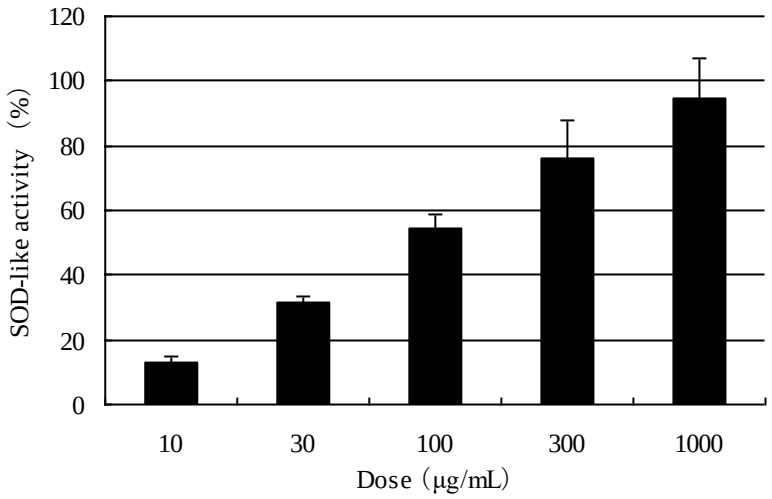
【Reference】Shimoda H., Tanaka J., Takahara Y., Takemoto K., Shan S. J., Su M. H. The hypocholesterolemic effects of *Cistanche tubulosa* extract, a Chinese traditional crude medicine, in mice. *Am. J. Chin. Med.*, **37**(6), 1125-38, 2009.

(8) Antioxidant Activities

SOD-like Activity and DPPH Radical Scavenger Activity（*in vitro*, ORYZA Data）

Endogenous metabolic processes and stress produce free radicals in the body. Free radicals such as reactive oxygen species (ROS) activate series of cells oxidation process resulting in cell death, ageing & degenerative diseases. Studies reported that ageing is accelerated by with the increase in endogenous free radicals. Experiment was prompted to study the effect of Cistanche Tubulosa Extract on super oxide dismutase (SOD) model and 1,1-diphenyl 2-picryl-hyrazil (DPPH) radical scavenging model. As illustrated in Fig. 45, Cistanche Tubulosa Extract loaded with plant polyphenols exhibited a dose-dependent antioxidative effect on SOD and DPPH radical scavenging models respectively.

①SOD-like Activity



②DPPH Radical Scavenger Activity

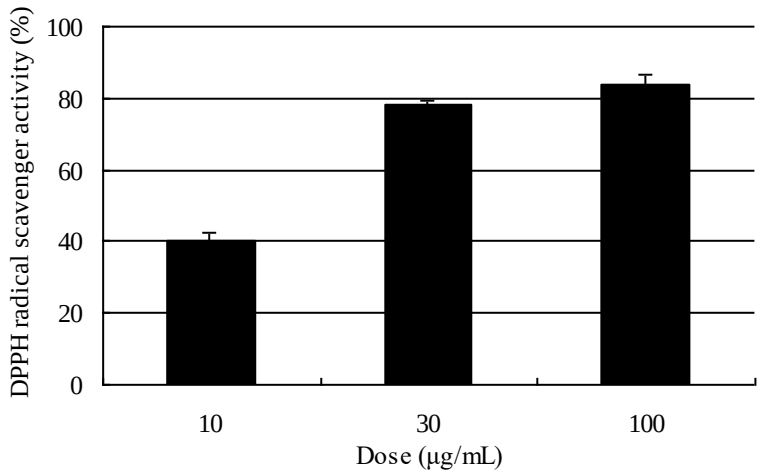


Fig. 45 Antioxidative Activity of Cistanche Tubulosa Extract (Mean±S.D., *n*=5)

4. Stability of Cistanche Tubulosa Extract

(1) Thermo stability

As illustrated in Fig. 46, echinacoside, acteoside and phenylethanoid glycosides content of Cistanche Tubulosa Extract is highly stable at 100°C and 120°C for 1 hour. It is stable at normal food processing temperature.

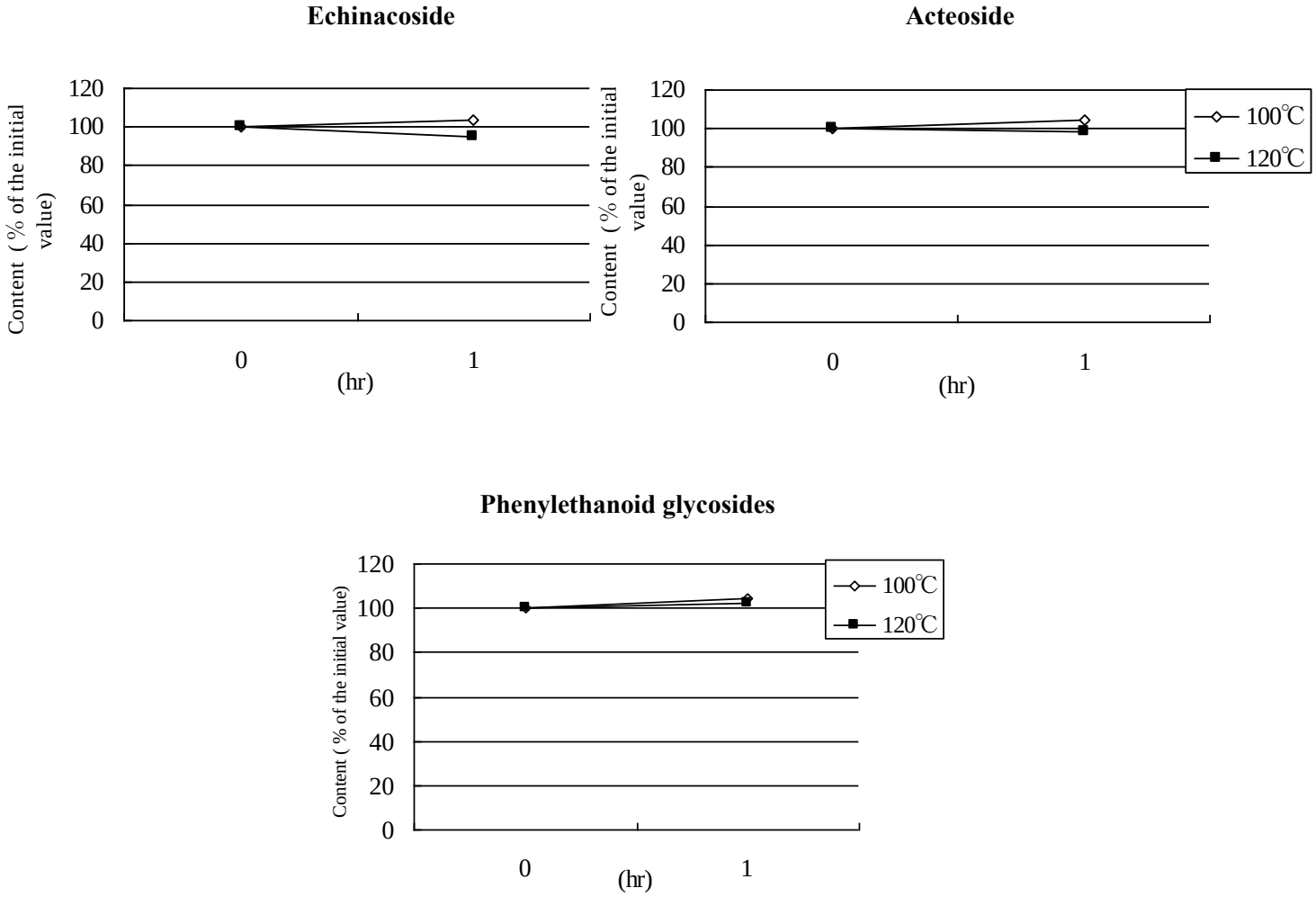
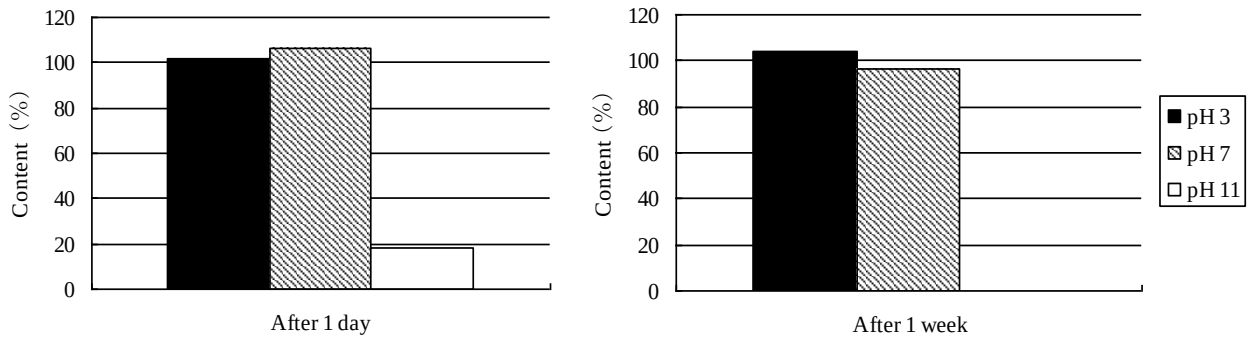


Fig. 46 Thermo stability of Cistanche Tubulosa Extract

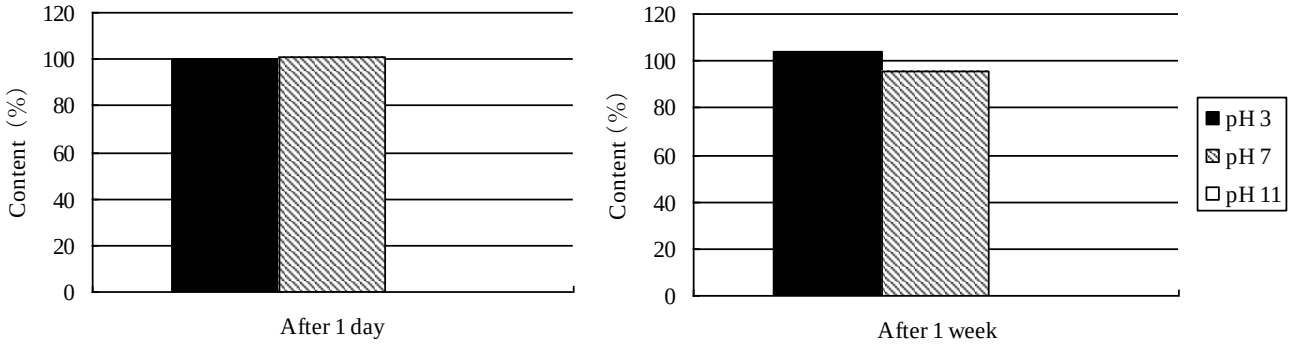
(2) pH stability

Cistanche Tubulosa Extract was dissolved in 30% ethanol, adjusted to its pH and stored at room temperature for 1 day and 1 week respectively. The content of echinacoside, acteoside and phenylethanoid glycosides of Cistanche Tubulosa Extract was measured and results showed (Fig. 47) that echinacoside, acteoside and phenylethanoid glycosides content remained stable at acidic condition.

Echinacoside



Acteoside



Phenylethanoid glycosides

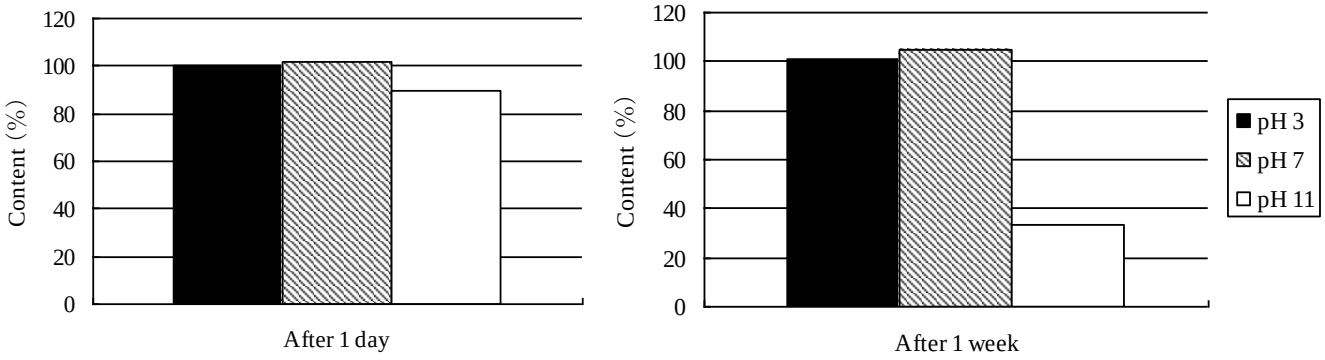


Fig. 47 pH stability of Cistanche Tubulosa Extract（100% as initial value）

(3) Stability in Aqueous Solution

Aqueous solution of Cistanche Tubulosa Extract-P25 0.4% at pH3.5 was prepared and stored at room temperature (under light and dark condition), 40°C (dark condition) & 5°C (dark condition) for 2 weeks. Upon visual observation, no precipitation, turbidity and color change was detected. As tabulated below, Cistanche Tubulosa Extract-P25 is highly in aqueous condition.

	Liquid stability（0.4% solution、pH 3.5）			
	Room temperature (light shielding)	25℃ (without light shielding)	40℃ (without light shielding)	5℃ (without light shielding)
Precipitation, turbidity	Negative	Negative	Negative	Negative
Color changes	Negative	Negative	Negative	Negative

5. Nutrition Information (Cistanche Tubulosa Extract-P25)

Description	Amount	Note	Analytical Method
Moisture	3.2g/100g		Heat-drying at atmospheric pressure
Protein	1.9g/100g	1	Kjeldahl Method
Fat	1.0g/100g		Acid degradation
Ash	2.9g/100g		Direct Incineration
Carbohydrate	91.0g/100g	2	
Energy	381kcal/100g	3	Atwater Method (Revised)
Dietary fiber	0.0g/100g		Prosky Method
Sodium	250mg/100g		Atomic absorption spectrophotometory
Sodium	0.6g/100g		Sodium Equiv. value

1. Nitrogen, protein conversion factor: 6.25
2. Carbohydrate expression standard (Ministry of Health and Welfare's announcement No. 176)
   Calculation: 50 – (water + protein + fat + ash)
3. Energy expression standard (Ministry of Health and Welfare's announcement No. 176)
   Conversion factor: Protein 4, fat 9, sugar 4; dietary fiber 2

Test trustee: SRL, Inc
Date of analysis: December 13, 2006
Test No.: 200611300029

6. Cistanche Tubulosa Extract – Product Safety Profile

(1) Residual Agricultural Chemicals

Cistanche Tubulosa Extract is conformed to regulation stipulated for 447 residual agricultural chemical compounds. No residual agricultural chemicals were detected as confirmed by test trustee.

Test trustee：Masis Co. Ltd.

Data： January 16, 2007

Report No.：9444

(2) Organic Certification

Cistanche Tubulosa Extract is Organic Certified. (Ref: COFCC-R-0704-0096)

CERTIFICATE

(Conversion)

China Organic Food Certification Center

This is to certify that

The Products in the Annex

produced by

Hetian Tianli Pharmaceutical Development Co.,Ltd.

conform to

GB/T 19630.1-.4

"Organic Products"

(Production, Processing, Labeling and Marketing, Management System)

and are certified to be organic products

Category of certification: Production, Processing and Marketing

Production base: Kazinake Development Zone(3000mu), Yutian County

Certificate serial number: COFCC-R-0704-0096

Issue date: 2007.4.26(Valid until 2008.4.25)

(3) Acute toxicity test（LD_{50}）

Acute Toxicity test was conducted according to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products. Cistanche Tubulosa Extract was orally administered to male and female mice at 26.4 g/kg and kept for 8 days. No abnormalities and fatal event observed at 26.4 g/kg. Upon autopsy, no abnormalities were observed. Thus, LD_{50} of Cistanche Tubulosa Extract is deduced to be >26.4 g/kg in both male and female mice.

Furthermore, LD_{50} of Cistanche Tubulosa Extract is deduced to be >17.6 g/kg in both male and female rats.

(4) Genotoxicity

① Ames test

Ames test showed no difference of the colony counting in TA97, TA98, TA100 and TA102 strains with or without Cistanche Tubulosa Extract (8-5000 μg/plate).

② Micronucleus test

Micronucleus test of polychromatic erythrocyte in mice marrow showed that Cistanche Tubulosa Extract (2.5-10 g/kg) has no damage effects to bone marrow cells.

③ Teratogenicity test

Teratogenic test showed that Cistanche Tubulosa Extract (2.5-10 g/kg) has no teratogenesis to mice spermatozoon.

(5) Sub-acute Toxicity

Cistanche Tubulosa Extract was orally administered to male and female rats at 0.65-1.30 g/kg and kept for 30 days. No abnormalities and fatal event were observed at 0.65-1.30 g/kg. Upon autopsy no abnormalities were observed.

(6) 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 were observed at 1.65 g/kg. Upon autopsy, no abnormalities were observed.

Furthermore, Cistanche Tubulosa Extract was orally administered to male and female beagle dogs at 1.50 g/kg and kept for 180 days. No abnormalities and fatal event were observed at 1.50 g/kg. Upon autopsy, no abnormalities observed.

7. Recommended Daily Dosage

The recommended daily dosage for Cistanche Tubulosa Extract–P25 is 100-400 mg/day.

8. Applications

	Applications	Claims	Examples
Foods	Brain function improving food Tonic food Beauty food	1) Improve of brain function 2) Tonic 3) Anti-aging 4) Anti- fatigue 5) Aphrodisiac 6) Beauty	Beverages, hard & soft capsules, tablets, candies, chewing gums, chocolates, wafers, jellies etc
Cosmetics	Beauty cosmetic		Body lotions, body gel etc.

9. Packaging

CISTANCHE TUBULOSA EXTRACT-P25（Water-soluble, for food）
CISTANCHE TUBULOSA EXTRACT-PC25（Water-soluble, for cosmetic）
5kg Interior packaging：Aluminum bag
Exterior packaging：Cardboard

10. Storage

Store in a cool, dry and dark place.

11. Expression

＜Food＞
CISTANCHE TUBULOSA EXTRACT-P25
Expression: CISTANCHE TUBULOSA EXTRACT

＜Cosmetic＞
CISTANCHE TUBULOSA EXTRACT-PC25
INCI; Cistanche Tubulosa Root Extract

PRODUCT STANDARD
PRODUCT NAME

CISTANCHE TUBULOSA EXTRACT-P25

FOOD

This product is extracted from Cistanche tubulosa with aqueous ethanol. It guarantees minimum of 25.0 % echinacoside, 9.0 % acteoside and 50.0 % phenylethanoid glycosides. This product is water-soluble.

Appearance	Brown-light brown powder with light unique smell.	
Echinacoside	Min. 25.0 %	(HPLC)
Acteoside	Min. 9.0 %	(HPLC)
Phenylethanoid glycosides	Min. 50.0 %	(UV)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105℃, 2 h)
Purity Test		
(1)Heavy Metals	Max. 10 ppm	(The Japanese Standards for Food Additives)
(2)Arsenic	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation)
Standard Plate Counts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Cistanche Tubulosa Extract	100 %

PRODUCT STANDARD
PRODUCT NAME

CISTANCHE TUBULOSA EXTRACT-PC25

COSMETIC

This product is extracted from Cistanche tubulosa with aqueous ethanol. It guarantees minimum of 25.0 % echinacoside, 9.0 % acteoside and 50.0 % phenylethanoid glycosides. This product is water-soluble.

Appearance	Brown-light brown powder with light unique smell.	
Echinacoside	Min. 25.0 %	(HPLC)
Acteoside	Min. 9.0 %	(HPLC)
Phenylethanoid glycosides	Min. 50.0 %	(UV)
Loss on Drying	Max. 10.0 %	(1 g, 105℃, 2 h)
Purity Test		
(1)Heavy Metals	Max. 10 ppm	(The Second Method)
(2)Arsenic	Max. 1 ppm	(The Third Method)
Standard Plate Counts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Cistanche Tubulosa Root Extract	100 %

Ref: The Japanese Standards of Quasi-Drug Ingredients.

ORYZA OIL & FAT CHEMICAL CO., LTD. striving for the development of the new functional food materials to promote health and general well-being.

From product planning to OEM - For any additional information or assistance, please contact：

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ORYZA OIL & FAT CHEMICAL CO., LTD.