A provisional translation of the publications available in Chinese on research into Pine Pollen.

Research on the Therapeutic Effect of Pine Pollen on Prostatic Hypertrophy (BPH) in Rats (2005,Cong Tao)

In studying the therapeutic effect of Pine Pollen on rat benign prostatic hypertrophy (BPH), also known as enlarged prostate, and the mechanism of disease of BPH, twenty four SD rats were divided into three groups. The three groups were fed with a normal diet. The diets of the second and third groups were supplemented with three starch tablets (as a placebo) and three pine pollen tablets per day, respectively. Additionally, group two and group three were fed with group one. After two weeks of feeding, group two and group three were injected with testosterone propionate (4mg/kg), mixed with olive oil. At the same time, group one was injected with olive oil (1ml/kg) alone. Two weeks later, the sample slices of prostate were observed, and the sex hormones contents, trace elements zinc, copper, and antioxidant enzymes were taken from the blood serum, prostate and liver. The results showed that the prostate hyperplasia symptoms of the groups fed Pine Pollen were significantly better than those of placebo group. The testosterone level of group three was lower than that of group two and no distinct difference from the control group. The estradiol level in the blood serum of rats in group three was the lowest. Therefore, the experiment concludes that consumption of Pine Pollen could improve the symptoms of BPH and the preventive mechanism can regulate sex hormones balance.

At present, the index to measure the degree of benign prostatic hypertrophy (BPH) is via prostate index blood tests and biopsy. The above results shows that prostate weight, prostate index, and prostate cell proliferation of the Pine Pollen group of rats was distinctly lower than the placebo group, clearly showing that Pine Pollen has a preventive and therapeutic effect on BPH (Benign Prostatic Hyperplasia).
It is generally recognized that three biochemical mechanisms trigger BHP: male sex hormones, estrogen hormones, and growth factor. The above experiment results shows that the serum testosterone content of BPH rats increase greatly which is because of the testosterone propionate injection. Testosterone level of Pine Pollen group of rats is obviously lower than the placebo group of rats, and the serum estrogen level is also lower than the other two groups. These differences show that Pine Pollen not only can effectively control rats' serum testosterone level but also can adjust the serum estrogen level as well. These two effects can assist in reducing the hyperplastic prostate and result in a better treatment effect without side effects. Application of this plant male andro-gamete(Pine Pollen) in the treatment of sex organ diseases conforms with the therapeutic principles of Traditional Chinese Medicine.
Additional, in vitro proliferation experiments were conducted on different sources of cells with Pine Pollen. The result show that cells from the prostate can be inhibited by Pine Pollen while the non-prostate sources cells were not. This demonstrates that Pine Pollen has a selective

inhibitory effect on prostatic cell proliferation, and has a better inhibitory effect on hormone insensitive cells than hormone-dependent cells.
Meanwhile, biochemical analysis showed that levels of blood urea, nitrogen, and blood sugar of prostatic hyperplasia rats were lower in the Pine Pollen group than in the control group, and creatinine levels showed a decreasing trend. This means that the liver function of prostatic hyperplasia rats was affected at a certain degree with liver function decreased. Because weight, serum cholesterol, and triglyceride levels of the Pine Pollen group of rats has a distinct decrease over the control group, then we can conclude that Pine Pollen has a positive clinical meaning for middle age men, in addition to the positive effects on BHP.

Influence of Pine Pollen on Immune Modulating Functions. Zhang Tun, Jianping Zhu.

Chinese Journal of Hospital Pharmacy, 2006(5): 638-639

Zhang Tun and Jianping Zhu conducted an observational research project on the influence of Pine Pollen on the immune modulating function in mice.
Method: Random group the mice into negative control group(purified water), low-dosage, medium-dosage and high-dosage groups, and feed each group 0.25g, 0.5g and 1.5g/kg (body weight) with cell-broken Pine Pollen powder which is equivalent to 5 times, 10 times and 30 times of human clinical dosage. 30 days later, serum hemolysin measurement, mice peritoneal macrophages phagocytosis ability test on chicken erythrocyte, dinitrofluorobenzene-induced delayed type hypersensitivity test (DTH), NK cell activity test, mice antibody formation(Hemolytic plaque number), thymus index and spleen index test were conducted. The results are:

Influence of Pine Pollen on the Immune Modulation Function in Mice

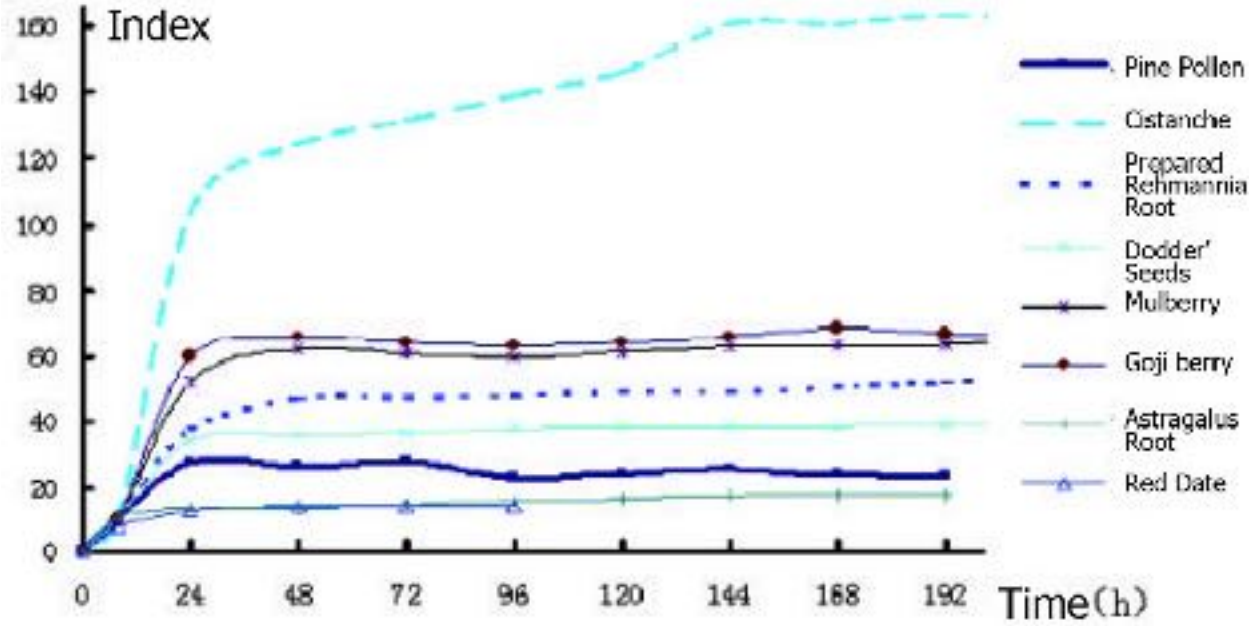
Group	Antibody titre Level	Phagocytic Rate (%)	Phagocytic Index	Swelling Level (Mg)	N K Cell activity (%)	Thymus Index (%)	Spleen index (%)	Hemolytic Plaque Number(*1000/total splenectomy)	OD Value plus Control A	OD value without Control A	Difference value
Negative control group	66.0±18.5	11±2.0	0.17±0.03	19.0±3.1	27.4±5.9	0.29±0.04	0.64±0.07	9.7±2.2	1.388±0.027	1.364±0.029	0.024±0.009
Low dosage group	68.6±34.2	11±1.6	0.17±0.03	17.6±5.4	20.7±1.8	0.27±0.05	0.66±0.13	9.7±2.9	1.405±0.033	1.314±0.038	0.091±0.014
Medium dosage group	101.2±35.1a	14±1.9a	0.23±0.04a	22.4±3.5	33.0±4.9a	0.26±0.04	0.60±0.10	14.4±3.2a	1.403±0.032a	1.312±0.039	0.121±0.015a

The Antibody titre level, Phagocytic rate, Phagocytic index, Swelling level, Hemolytic plaque number, N K cell activity and the left and mice right ear difference value of

dinitrofluorobenzene-induced delayed type hypersensitivity shows a significant increase for the medium-dosage and high-dosage groups compared with the negative control group; while Thymus index and Spleen index shows no great change.
The above experiment shows that cell cracked Pine Pollen can greatly increase the cellular immune function and cytophagy function, and that this change may be attributed to the nutrition contained in Pine Pollen.

- **Research into the Oxidation Resistance (Antioxidant Properties) of Pine Pollen. Agricultural engineering technology: the agricultural product processing industry in 2008; (Yutian Liu) Yantai University.**

Researcher Yutian Liu, from the Yantai University of China, has conducted a series of research experiments on oxidation resistance (the antioxidant potential) of Pine Pollen, which sheds light on the overall antioxidant properties of the herb. Liu ranked the level of antioxidants in different herbs and foods by measuring the potassium permanganate index value. The result shows that although the antioxidant substance of Pine Pollen, Astragalus Root (*Astragalus membranaceus*), Dodder Seeds (*Cuscuta chinensis*), and Red Date (*Ziziphus jujuba*)is less than other foods and herbs, but that their power to eliminate the hydroxyl free radical is stronger, resulting is a greater therapeutic antioxidant effect.
Furthermore, the experiment concludes that the antioxidant substances in Pine Pollen have a synergistic effect to eliminate hydroxyl free radicals. This conclusion provided the basis for further researches on pine pollen antioxidant application in food industry.

- 

- **Anti-fatigue Function of Pine Pollen. Liu Xie (2004) Chinese Journal of Biochemical Pharmaceutics, CDC, Jiangsu Province, China.**

This research was done to evaluate the influence of Pine Pollen on the swimming time of mice, a common method of accessing fatigue and stress in the laboratory. Researcher Liu fed Pine Pollen to the mice in dosages of 100, 500, and 1000mg/kg, and then tested the loaded swimming time of each group as well as the blood content of lactic acid, hepatic glycogen, and urea nitrogen, after 30 days feeding of each group.

- Impact on Loaded Swimming Time and Biochemical Criterion Post Swimming of Mice (n=8, $\bar{x} \pm s$)

Group	Dosage / (mg/kg)	Swimming Time / Minutes	Urea Nitrogen / (mmol/L)	Blood Lactic Acid / (mg/gl)	Hepatic Glycogen / (mg/100g)
Negative Control Group	0	16.12 ± 6.02	9.58 ± 0.85	85.01 ± 1.76	627.07 ± 317.06
Pine Pollen Low Dosage Group	100	18.98 ± 10.45	9.73 ± 1.33	79.19 ± 6.25	985.34 ± 303.79
Pine Pollen Medium Dosage Group	500	29.00 ± 10.70	8.14 ± 1.37	76.47 ± 6.58	1,484.20 ± 339.43
Pine Pollen High Dosage Group	1,000	31.60 ± 10.20	7.57 ± 1.35	67.94 ± 6.39	2,018.19 ± 412.05

The result demonstrate that Pine Pollen greatly extended the swimming time of the weight loaded mice ($P < 0.01$) and greatly decreased the post exercise content of blood lactic acid and urea nitrogen ($P<0.05$,$P<0.01$). Furthermore, Pine Pollen showed to positively influence the content of hepatic glycogen ($P<0.01$). These tests conclude the anti-fatiguing effect of Pine Pollen.

Research has shown that an increase in skeletal muscle free radicals and other forms of reactive oxygen caused by exercises is one of the important factors resulting in skeletal muscle damage and fatigue.

Antioxidant supplements can effectively inhibit this change and improve exercise performance, which has propelled the significanceof antioxidants in exercise nutrition. Pine Pollen is rich in large amounts of the antioxidants vitamin E, beta carotene, the microelement selenium, and other various flavonoids which play an important role to eliminate the free radical caused by exercises. In particular, selenium helping the synthesis of glutathione peroxidase(GSH-Px) (an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage). Vitamin E and selenium have a synergetic antioxidant effect to protect the body from free radical damage, reduce lipid peroxidation and maintain the structure and function of skeletal muscle, myocardium and other organs.

Beta carotene is currently known as the strongest single oxygen scavenger, which could alleviate the degree of lipid peroxide reaction. Pine Pollen contains rich antioxidant ingredients that can decrease the concentration of free radical after exercises and then alleviate the damage from free radicals, thus slowing down fatigue post exercise.

- **Experimental Study On the Anti-Aging Effects of Pine Pollen. Lixin Zhao (2004). Modern Medical Journal.**

The object of this research is to evaluate the anti-aging effects of Pine Pollen. After mice were fed with animal feeds containing Pine Pollen for 30 days, the activities of serum superoxide dismutase(SOD), catalase(CAT), glutatbione peroxidase(GSH.Per)and the content of malondialdehyde(MDA) in the brain were analyised. Furthermore, levels of lipofuscin (Lf) in brain and liver were assessed, the weight of thymus gland and spleen were taken, and the immune-macrophage function was detected.
Results show that Pine Pollen obviously increases the activities of serum SOD, CAT and GSH-Per, and it decreased the content of malondialdehyde (MDA) in brain and lipofuscin (Lf) in the brain and liver. In addition, Pine Pollen significantly increased the weight of the thymus gland and the spleen and increased the immune-macrophage function. The above results further show the anti-aging effects of Pine Pollen.

- **Impact of Pine Pollen on Serum SOD, CAT, GSH-Per Active in Mice (n=10, $\bar{x} \pm s$)**

Group	SOD / (mU*l-1)	CAT / (u*ml-1)	GSH-Per / u*ml-1)
Young Control Group	39.58 ± 4.87	8.2 ± 2.0	240.6 ± 29.4
Aging Control Group	15.27 ± 3.11	4.4 ± 1.7	134.7 ± 21.7
Pine Pollen 2% Fed Group	37.98 ± 5.38	7.6 ± 1.8	228.4 ± 24.6
Pine Pollen 5% Fed Group	44.35 ± 6.08	8.0 ± 2.7	250.5 ± 27.8

- **Impact of Pine Pollen on Mice Brain and Liver Content of MDA and Lf (n=10, $\bar{x} \pm s$)**

Group	Brain Tissue		Liver Tissue
	MDA / (nmol*g)	Lf / (μ*g)	Lf / (μ*g)
Young Control Group	2.47 ± 0.55	2.61 ± 0.61	9.57 ± 1.74
Aging Control Group	3.24 ± 0.62	4.24 ± 0.87	16.87 ± 2.90
Pine Pollen 2% Fed Group	2.08 ± 0.40	2.91 ± 0.68	11.26 ± 1.69
Pine Pollen 5% Fed Group	1.67 ± 0.38	2.57 ± 0.53	8.40 ± 1.32

Impact of Pine Pollen on Immune Organ Mass (n=10, $\bar{x} \pm s$)		
Group	Thymus(mg/100mg)	Spleen (mg/100mg)
Young Control Group	26.34 ± 6.34	59.01 ± 11.54
Aging Control Group	17.80 ± 3.61	40.82 ± 7.34
Pine Pollen 2% Fed Group	24.06 ± 4.97	61.22 ± 12.67
Pine Pollen 5% Fed Group	30.91 ± 5.77	72.16 ± 13.76

Impact of Pine Pollen on Reticuloendothelial System (n=10, $\bar{x} \pm s$)		
Group	Phagocytic Index	Phagocytic Activity
Young Control Group	0.0346 ± 0.0147	8.9617 ± 2.7043
Aging Control Group	0.0102 ± 0.0087	5.2456 ± 1.3405
Pine Pollen 2% Fed Group	0.0429 ± 0.0210	10.5129 ± 1.8512
Pine Pollen 5% Fed Group	0.0573 ± 0.0255	13.6187 ± 2.7136

The mechanism and reason of aging in the human body is not thoroughly understood, but one of the prominent theories is the Free Radical Theory of aging. This theory suggests that free radicals produced from normal body metabolism each day can be eliminated in normal situations but that the antioxidant activity of the body's natural antioxidant substances like CAT, SOD, GSH-Per decrease with age. The decrease in the body's own production of antioxidants is combined with an overall increased rate of the free radical metabolite MDA. MDA then converts into inactive Lf with phosphatidyl ethanolamine and protein in human body, accumulating in tissues and cells, reducing the RNA, damaging the cell structure and resulting in the aging cell death because cells can no longer mitigate their own metabolism and the free radicals that normal cell metabolism produces. Therefore, improvement of the activity of antioxidents and prevention of damage from free radical has a positive effect in delaying aging in the human body. Pharmacological experiments have proven that Pine Pollen contains various anti-oxidant substances, as mentioned previously, including vitamin E, beta carotene, and the microelement selenium, all of which could inhibit the oxidizing reaction of fat and protein in body and producing an anti-aging effect in the body. After consumption of Pine Pollen, it can promote the activity, count, and the capacity to clear free radicals. At same time, Pine Pollen can clear age related pigment accumulated on the skin, brain, heart, liver, and other organs, inhibit aging processes, and improve immunocompetence. Pine pollen can greatly improve the T-cell changes of D - galactose aging in mice models, enhancing the immunity and prolong the life time of aging mice.

This experiment shows that Pine Pollen increases the activities of serum SOD, CAT and GSH-Per, which means that Pine Pollen can improve the antioxidant ability of the body, eliminating the free radicals produced in the process of aging, and increase the weight of immune organs, thus demonstrating an obvious anti-aging effect.

- **Research on the Protective Effect of Pine Pollen on Injury Caused by Alcohol (2008,Xie Huiping)**

The objective of this experiment was to study the protective effect of Pine Pollen on rat models of liver injure caused by alcohol. Rat models of alcoholic liver were established and each group was fed Pine Pollen at different dosage for 30 days. The contents of malondialdehyde(MDA), triglycerides(TG), and reduced glutathione(GSH) in liver tissue were measured, and the pathological and histological changes of the liver were observed. Results showed that the MDA and TG contents in the liver tissue were markedly lower in the Pine Pollen fed group than those rats in the model control group. The average values of pathological changes in rat liver were markedly lower in the Pine Pollen group than in the model control group. This experiment showed that Pine Pollen has a significantly protective effect on liver injure caused by alcohol.

Impact of Pine Pollen on Weight, Liver Weight, and Hepatosomatic Ratio (R±s)

Group	Dosage group (mg/kg.bw)	Number of rats	Original weight(g)	Medium-term weight(g)	Final weight(g)	Liver weight(g)	Hepatosomatic ratio(%)
Negative control group	0	10	205.60±12.15	282.00±27.79	337.30±19.55	10.96±0.99	3.25±0.26
Model control group	0	10	200.80±14.67	273.30±10.35	333.90±19.40	10.37±0.75	3.11±0.28
Low-dosage group	250	10	202.20±10.80	270.30±14.24	335.40±20.58	10.10±1.06	3.01±0.27
Medium-dosage group	500	10	199.20±13.40	268.20±21.67	334.20±28.25	10.48±1.25	3.14±0.36
High dosage group	1500	10	201.00±14.72	270.60±15.62	342.30±15.64	10.33±0.74	3.19±0.17

- **Impact of Pine Pollen Liver Tissue Content of MDA, GSH, and TG (x̄±s)**

Group	Dosage group (mg/kg.bw)	Number of rats	MDA (mmol/g)	GSH (μ mol/g)	TG (mmol/g)
Negative control group	0	10	1.79±0.68	30.97±4.16	1.37±0.25
Model control group	0	10	5.16±1.31	25.55±6.69	2.70±0.84
Low-dosage group	250	10	3.85±1.29	27.94±5.57	2.49±0.98
Medium-dosage group	500	10	3.75±1.21	31.97±3.29	1.79±0.73
High-dosage group	1500	10	3.36±0.71	33.85±5.69	1.72±0.67

Pathological Examination of Liver Tissue (x̄±s)

Group	Dosage group (mg/kg.bw)	Number of rats	Hepatic fat droplets grade
Negative control group	0	10	0.10±0.32
Model control group	0	10	3.30±0.48
Low dosage group	250	10	2.30±0.82
Medium-dosage group	500	10	2.20±1.14
High-dosage group	1500	10	1.90±0.74

Because of advancements in standard of living, the scope of disease has changed, with an increase of alcoholic liver disease. Alcohol is metabolized by the body through various liver cell enzymes, a process that results in a large number of reactive oxygen molecules (free radicals), producing oxidative damage in the liver. This damages the structure and function of various organelles and enzymes within liver cells, inhibiting biosynthesis of GSH, reducing the antioxidant function of SOD, and causing lipid peroxidation.

Results from the above experiment show that dose-depended intake of Pine Pollen can reduce the content of MDA and TG in the liver tissue of alcoholic liver injured rats, improves the content of reduced glutathione in liver tissue, and reduces the fatty degeneration level of the liver. In a conclusion, Pine Pollen has markedly protective function on alcoholic liver damage.