



FERTILITY SUPPORT

GRAMINEX Flower Pollen Extract

Graminex Flower Pollen Extract and its Effect on Fertility

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Effect of Cernitin pollen-extract on the Sex-hormone-induced Nonbacterial Prostatitis in Rats

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Cernitin pollen-extract (Cernilton®, CN-009) is a preparation made from eight kinds of pollen. The active components are water-soluble (T60) and fat-soluble (GBX) fractions. CN-009 has been used for the treatment of chronic prostatitis in Europe and Japan. To study the action of CN-009 on the prostatitis, we examined the effect of CN-009 on the sex-hormone-induced nonbacterial prostatitis in rats.

Aged Wistar rats (10 months old) were castrated and then injected 17β -estradiol (0.25 mg/kg, s.c.) for 30 days. These treatments reduced the weight of prostate and induced the inflammation and epithelial cell dysfunction of the lateral prostate lobe in the rats. Testosterone (2.5 mg/kg, s.c.) injected for the last 14 days of the treatment of 17β -estradiol to the rats restored markedly the estradiol-induced prostatitis. Those changes were similar to the findings reported by others. CN-009 was administered orally for the last 14 days of the treatment of 17β -estradiol to the rats. The administration of 378 mg/kg of CN-009 did not change in the prostatic histopathological findings, while 1260 mg/kg of CN-009 increased the number of intracellular secretory granules of epithelial cells and diminished weakly the invasion of inflammatory cells into the lumen or the stroma in the prostatic gland.

These results suggest that CN-009 may recover the prostatic epithelial cell dysfunction and have the mild anti-inflammatory properties.

KEY WORDS

Cernitin pollen-extract, Cernilton, CN-009, Aged Wistar rat,
Castration, Sex-hormone-induced nonbacterial prostatitis

Efficacy of Cernilton administration for infertile males associated with asymptomatic pyospermia

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Introduction

The cases, that white blood cell is significantly higher in semen, accounts for 16 ~ 17% of male infertility patients. Interestingly, it was common that no bacterial finding is presented in these cases, using standardized bacterial test, PCR methods for Chlamydia trachomatis (*C. trachomatis*), and semi-quantitative analysis for Urea plasma urealyticum (*U. urealyticum*). Although these cases are classified in nonbacterial chronic prostatitis, it has been generally recognized to be associated with male infertility. In present study, we reported that administration of Cernilton reduce PMN-elastase activity and to improve seminal findings in semen for 17 male infertility patients with no bacterial finding in semen.

Material and Methods

17 male infertility patients associated with nonbacterial asymptomatic pyospermia were treated with Cernilton 6 tablets daily over 12 weeks, then sperm density, progressively motile sperm ratio, sperm motility and PMN-elastase activity in semen were measured.

Results

In all patients, progressively motile sperm ratio, sperm motility and PMN-elastase activity in seminal fluid were improved.

Conclusion

Administration of Cernilton is seemed to be effective in the treatment of infertile males associated with nonbacterial asymptomatic pyospermia.

Effects of pollen extract EA-10, P₅ on chronic prostatitis or infertility with chronic prostatitis

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KEY WORDS prostatitis; infertility; free radicals; pollen

ABSTRACT

AIM: To determine the drug action mechanism of pollen extract EA-10, P₅ on the treatment of chronic prostatitis (CP) or infertility with CP. **METHODS:** Malondialdehyde (MDA), super oxide dismutase (SOD), and nitrogen monoxide (NO) were measured by biochemical assay, and zinc content was assayed by atomical spectrophotography in the pre-treatment and post-treatment of CP or infertility with CP.

RESULTS: Compared with control group, leukocytes in expressed prostatic secretion (LEPS), MDA, and NO were increased, and zinc content and SOD were decreased significantly in the pre-treatment of CP. After the treatment, LEPS was improved, and MDA and NO were reduced, while zinc content were increased apparently and the alteration of SOD was not evident ($P>0.05$). In the pre-treatment of infertility with CP, LEPS, MDA, NO, sperm viability, and seminal leukocytes were obviously higher and seminal plasma SOD, zinc content, and sperm motility were obviously lower than those in control group. After the treatment, LEPS, sperm motility, and sperm viability were improved, MDA, NO, and seminal leukocytes were decreased, SOD and zinc content were increased markedly. **CONCLUSION:** There was inter-correlation between oxygen free radicals (OFR) and occurrence, development, and recovery of CP; Change of OFR may be involved in the drug action mechanism of EA-10, P₅ in the treatment of CP or infertility with CP.

INTRODUCTION

Chronic prostatitis (CP) is one of the most common diseases in andrology. Its therapeutic efficacy is not very satisfactory. Recent studies showed that CP might defect semen quality. Thus, it is significant to make an investigation of pathogenesis and medication of CP. Oxygen free radicals (OFR) which causes tissue damage by lipid peroxidation (LPO)[1], includes mainly super oxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl free radical ($\cdot OH$), and nitrogen monoxide (NO). LPO has yielded several types of secondary free radicals and a large number of reactive compounds (including MDA), resulting in the destruction of cellular portion. Of course, cells are equipped with various antioxidants, such as vitamin E, vitamin C, glutathione (GSH), super oxide dismutase (SOD), catalase (CAT), and so on. These can scavenge supernumerary OFR and protect organism from cytotoxic effect of OFR[2,3]. In addition, there was apparent negative correlation between semen OFR level and semen quality, but with the increasing of semen OFR level and prolonging of contact time between OFR and sperm, sperm vital force would obviously decrease [4,5]. Studies also showed seminal MDA might be increased apparently in chronic bacterial prostatitis, resulting in the influence of sperm vitality and sperm motility [6,7]. These data indicated that OFR played an important role in pathogenesis of CP and infertility. EA-10, P₅ is regarded

as a satisfactory drug in the treatment of CP. At present, it is still unknown that whether OFR, antioxidase, and zinc content in semen will be regulated in the treatment of CP or infertility with CP by EA-10, P5. Therefore, we investigated whether EA-10, P5 could inhibit LPO, and thus to obtain the primary conclusion about drug action mechanism of EA-10, P5 in our treatment.

MATERIALS AND METHODS

Population

All 68 cases of CP (group I) and 63 cases of infertility with CP (group II) were divided into two groups, which were then subdivided into three treatment subgroups respectively (group A: EA-10, P5 + Roxithromycin, group B: EA-10, P5 alone, and group C: Roxithromycin alone). Twenty cases who were normal healthy donors of proven fertility were used as control group. The treatment period was four weeks. Group A received EA-10, P5 (product from Sweden Pharmacia Allergon AB, 375 mg/pill) and roxithromycin (150 mg/pill) twice daily. Group B-C received respectively EA-10, P5 and Roxithromycin twice daily. During the treatment, all 131 cases were treated with sitting bath in hot water and controlled diet (wine and pungent diet prohibited).

Semen samples and treatment Semen samples were obtained from all cases by masturbation after 3 d of abstinence. Samples were incubated for 20 min in 37 °C warm bath box. Firstly, regular semen analysis and seminal MDA content were analyzed after semen has been liquefied completely; Secondly, liquefied semen was centrifuged at 1000×g for 10 min, and seminal plasma was used to determine the content of NO and SOD. Finally, surplus seminal plasma was frozen at -20 °C until further use for zinc content assay.

Determination of seminal MDA content and SOD activity Seminal MDA content was determined by thiobarbituric acid (TBA) method [8]. SOD activity was measured as the inhibition of nitroblue tetrazolium reduction due to superoxide anion generation by xanthine plus xanthine oxidase [9].

Zinc and NO content in seminal plasma assay Zinc content was assayed by a method based on atomical spectrophotography [10]. The NO concentration was estimated by a method based on nitrite salt response with sulfanilamide to form diazole, which could appear purplish red color reacting with naphthalene ethylenediamine in the acid conditions. The absorbance of 530 nm was measured [11].

Semen parameters All semen analysis adopt with color quality analysis system of WLJY-9000, which was devised by skill-trade Company Weili Peking. All parameters were settled down to refer to standard of World Health Organization (WHO) [12].

Statistical Data were expressed as mean ±SD and analyzed with *t*-test. Value of *P*<0.05 was considered to be statistically significant.

RESULTS

Changes in symptom and LEPS in CP or infertility with CP After the treatment by EA-10, P5 +Roxithromycin, EA-10, P5 alone, and roxithromycin alone in CP or infertility with CP, remissive rate of symptom was 92 %, 66.67 %, 68.17 %, and 90 %, 61.91 %, 63.64 %, while effective rate of LEPS was 88 %, 57.14 %, 59.09 %, and 85 %, 52.38 %, 54.55 %, respectively. Therapeutic efficacy in group A was significantly higher than that in group B or C (*P*<0.01) (Tab 1, 2).

Tab 1. Changes in symptom and LEPS in different treated groups of CP. bP<0.05 vs EA-10, P5+Roxithromycin groups.

Treatment	Case	Symptom improved		LEPS improved.	
		Efficiency	Percent/%	Efficiency	Percent/ %
EA-10, P5+Roxithromycin	25	23	92	22	88
EA-10, P5	21	14	66.67b	12	57.14b
Roxithromycin	22	15	68.17b	13	59.09b

Tab 2. Changes in the symptom and LEPS in different treated groups of infertility with CP. bP<0.05 vs EA-10, P5 +roxithromycin groups.

Treatment	Case	Symptom improved		LEPS improved.	
		Efficiency	Percent/%	Efficiency	Percent/%
EA-10, P5+roxithromycin	20	18	90	17	85
EA-10,P5	21	13	61.91b	11	52.38b
Roxithromycin	22	14	63.64b	12	54.55b

Changes in LEPS, MDA, SOD, Zinc content, and NO in CP Compared with control group, LEPS, MDA, and NO were increased, while zinc content and SOD were decreased significantly in the pre-treatment ($P<0.01$). After the treatment, LEPS and zinc content were improved, while MDA and NO were decreased apparently vs pre-treatment ($P<0.01$), but there was no obvious alteration of SOD ($P>0.05$) (Tab 3).

Tab 3. Changes in LEPS, MDA, SOD, Zn 2+ content, and NO in different treated groups of CP. Mean \pm SD. bP<0.05, cP<0.01 vs control. dP>0.05, fP<0.01 vs pre-treatment at the same group. hP<0.05 vs EA-10, P5+Roxithromycin group.

	EA-10,P5+Roxithromycin							
	Control (n=20)	(n=25).		EA-10, P5 (n=21)		Roxithromycin (n=22)		
		pre-treat	post-treat	pre-treat	post-treat	pre-treat	post-treat	
LEPS /Hp	3.4 \pm 2.1	25 \pm 16b	5.0 \pm 2.8f	23 \pm 13b	7 \pm 4f	25 \pm 14b	7 \pm 4f	
MDA/ μ mol-L-1	4.1 \pm 1.1	8.3 \pm 1.9c	4.3 \pm 1.4f	8.3 \pm 1.7c	5.4 \pm 1.6bf h	8.4 \pm 1.8c	5.2 \pm 1.2bf h	
Zn2+/ μ mol-L-1	2.3 \pm 0.6	1.2 \pm 0.4b	1.8 \pm 0.5f	1.2 \pm 0.5b	1.6 \pm 0.5f	1.2 \pm 0.4b	1.6 \pm 0.5f	
SOD/kU-L-1	20 \pm 119	850 \pm 118b	851 \pm 122d	838 \pm 110b	840 \pm 113d	829 \pm 120b	831 \pm 123d	
NO/ μ mol-L-1	4.6 \pm 1.6	63 \pm 20c	39 \pm 16bf	63 \pm 20c	45 \pm 18bf	63 \pm 21c	47 \pm 18bf	

Changes in LEPS, MDA, SOD, Zinc content, NO, and semen parameters in infertility with CP In the pre-treatment, LEPS, MDA, NO, sperm viability, and seminal leukocytes were obviously higher and SOD, zinc content, and sperm motility were obviously lower than those in controlled group ($P<0.01$). After the treatment, LEPS, SOD, zinc content, sperm motility, and sperm viability were improved and MDA, NO, and seminal leukocytes were decreased significantly ($P<0.01$). Compared with the pre-treatment, MDA levels and seminal leukocytes were reduced significantly in group A than these in group B or C in the post-treatment ($P<0.01$) (Tab 4).

Tab 4. Changes in LEPS, MDA, SOD, Zinc content, NO, and Semen parameters in different treated groups of infertility with CP. Mean \pm SD. aP>0.05, bP<0.05, cP<0.01 vs control. dP>0.05, eP<0.05, fP<0.01 vs pre-treatment at the same group. hP<0.05 vs EA-10, P5+Roxithromycin groups.

	Control (n=20)	EA-10,P5+Roxithromycin					
		(n=25)		EA-10,P5 (n=21)		Roxithromycin	
	pre-treat	post-treat	pre-treat	post-treat	pre-treat	post-treat	
LEPS/Hp	3.4 \pm 2.1	23 \pm 13c	6 \pm 4f	23 \pm 12c	7 \pm 5f	23 \pm 12c	6 \pm 4f
MDA/ μ mol-L-1	4.1 \pm 1.1	9.2 \pm 1.6c	5.5 \pm 2.1f	9.1 \pm 1.9c	7.5 \pm 2.4beh	9.1 \pm 1.7c	7.2 \pm 2.5 bch
Zn2+/ μ mol-L-1	2.3 \pm 0.6	1.1 \pm 0.4c	1.6 \pm 0.4bf	1.1 \pm 0.4c	1.5 \pm 0.4bf	1.1 \pm 0.3c	1.4 \pm 0.4bf
SOD/kU-L-1	920 \pm 119	653 \pm 115c	736 \pm 125bf	663 \pm 91c	727 \pm 104bf	660 \pm 97c	722 \pm 109b f
NO/ μ mol-L-1	4.6 \pm 1.6	78 \pm 20c	55 \pm 18bf	76 \pm 27c	63 \pm 27bf	77 \pm 25c	61 \pm 21bf
10-9 x Sperm density/L-1	76 \pm 24	82 \pm 49a	79 \pm 46ad	79 \pm 42a	77 \pm 41ad	80 \pm 41a	79 \pm 40ad
Sperm motility/%	75 \pm 12	37 \pm 14c	46 \pm 14bf	38 \pm 17c	43 \pm 19bf	37 \pm 16c	43 \pm 18bf
Sperm viability/%	14 \pm 8	36 \pm 14c	24 \pm 10bf	34 \pm 14c	28 \pm 11bf	34 \pm 13c	28 \pm 11bf
10-9x Seminal leukocytes/L-1	0.5 \pm 0.3	1.6 \pm 0.9c	0.7 \pm 0.4af	1.6 \pm 0.8c	0.9 \pm 0.4bf	1.6 \pm 0.8c	0.9 \pm 0.5bf

DISCUSSION

In this test, we have used EA-10, P5 and roxithromycin to treat CP and infertility with CP. Roxithromycin has a good effect to chlamydia besides much of Gram-negative bacteria [13]. Therapeutic efficacy was lower in our works than that in literature. But our therapeutic efficacy was still satisfactory. We considered that the reason may be as follows: (1) Chronic bacterial prostatitis may be selected in all the chosen cases, which might influence therapeutic efficacy of EA-10, P5. (2) The treatment period was shorter compared with that illustrated in literature. In addition, we have found that therapeutic efficacy in group A was better than in group B or C. This indicated that EA-10, P5 should be used together with effective antibiotic in the treatment of CP.

Some studies have proved that OF was related to occurrence and development of CP[3- 4,14]. In our studies, MDA was higher and SOD was lower significantly in the pre-treatment of CP than those in the control group, which suggested that there be an increase of OFR, a decrease of antioxidation, and reinforce a of LPO. But MDA was decreased after the treatment, indicated that OFR was scavenged massively and LPO was obviously inhibited. Similarly, MDA was higher and SOD was lower significantly in pre-treatment of infertility with CP than those in the control group, which suggested that oxidation be increased and antioxidation be decreased in semen. At the same time, we discovered that sperm motility was declined and sperm viability was raised significantly. But after the treatment, MDA was decreased and SOD was increased significantly than those in the pre-treatment ($P<0.01$), accompanying with improvement of sperm motility and sperm viability apparently. This indicated that LPO was inhibited and antioxidation was reinforced. From the result above, we believed that EA-10, P5 could reduce LPO

and enhance antioxidation in the treatment of CP or infertility with CP. In our treatment, antibiotic and EA-10, P5 were used not only to cure CP but also to improve semen quality. We found that EA-10, P5 had an effect on weakening oxidative stress and increasing antioxidation in prostatic secret ion and semen. This suggested that change of OFR may be involved in the drug action mechanism of EA-10, P5 in the treatment of CP or infertility with CP. At present, it is known that ferulic acid was an antioxidant containing phenolic hydroxyl [15]; and P5, one of valid portion in pollen extract EA-10, P5, may have anti-oxidative effect owing to providing phenolic hydroxyl too. Nevertheless this view still needs to be confirmed by more investigation. It was reported that zinc content in prostatic secretion and semen was higher than in other organ and body fluid, which showed that zinc played an important role in keeping function of prostate and other accessory sex glands. Our studies showed that zinc content was increased accompanying with improvement of an illness state. EA-10, P5 can enhance zinc content in seminal plasma, which may be related to improve local circumstance. In summary, all these results could provide us with a possible therapeutics approach to treat infertility with CP. In order to improve therapeutic efficacy, anti-infection and anti-oxidation should be adopted in the treatment of CP or infertility with CP.

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Findings on ED through the "Pollen Extract G63" of Graminex Company

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Pollen Extract, containing a rich source of nutrition (amino acids, minerals, and vitamins), represents the emergence of the next generation of plant substances that should not be overlooked and is a substance with hidden action not yet fully understood. The Clinic established the first Prostrate Center in Japan and focuses treatment fields such as urology. Naturally, on many occasions we receive requests to provide consultation with regard to ED since most of our patients are male. The main causes of impairment of Hepatic function are mostly nervous stress and hyperlipidaemia. The causes of these problems can be improved; it can be conjectured, through better blood circulation by an antioxidant effect and by the structural repair effect of amino acids. Our report this time studied the effect of Pollen extract as a supplement to improve ED.

[Objective and Method]

At the Clinic, 15 subjects which visited the Clinic from 1 month to 3 months for ED therapy were administered pollen extract G63. The IIEF5 (International Erectile Function Score) was recorded and the before and after therapy effectiveness was determined. However, the 2 subjects which stopped coming to the Clinic after 1 month were eliminated from the effectiveness determination.

The pollen extract G63 used in the trial was produced by Graminex Company in Ohio, USA from the pollen of raw materials such as rye, corn, and timothy hay (referred to as Phlegm pratense in Japan) which were cultivated without using agrochemicals or genetically modified varieties. (However, a slight amount of pollen as weeds from timothy (referred to as Phleum pratense in Japan) was also included.) The pollen which has a double hull is not digested or absorbed even when ingested since it has strong resistance to acid and heat (cannot be destroyed even at 300 deg C). The Graminex Company using a special technology is able to separately extract G60 (water soluble nutrition component) and GFX (lipid soluble component) and we received the product G63 which is a 20:1 combination of G60 and GFX.

The dosage was 6 tablets per day; three tablets each after breakfast and dinner. One 250 mg tablet contains 62.5 mg of pollen extract. (The daily quantity 375mg as pollen extract)

[Results]

Name	Age	Interview Day	IIEF-5	complications	Patient Conversation
1. I_K	60	Before	2	D.M	This never occurred before, but morning ED occurred 1 time. Stool became soft
		After 1 month	2		
T_Y	47	Before	12		The hardness and angle of morning erection was clearly different. There was an effect after administration for 1 week.
		After 1 month	25		
S_A	73	Before	6		Hardness was increased. Erection caused by sexual arousal.
		After 1 month	10		
O_N	74	Before	9	By-pass Surgery	Erection achieved only 1 time in 7 prior to administration, became 4 times in 5 after administration.
		After 1 month	20		
I_T	62	Before	8		Morning erections increased. Improving somewhat. Morning erections increased.
		After 1 month	11		
		After 5 months	13		
		After 5 months	15		

S_I	68	Before	5	Diabetes Concomitant with RACHIKAGORUDO (Sea snake extract) Symptoms improved
		After 1 month	21	
S_A	60	Before	5	Improving a little.
		After 1 month	7	
I_T 65		Before	12	Morning erections increased. Have an impression that it is somewhat improved. Question 2) 3 2 5) 3 1
		After 1 month	9	
T_T	50	Before	13	Stool became soft. Bowl movement 2~3 times on some days.
		After 1 month	13	
MH	74	Before	15	Morning erections started after 10 ~14 days Score unchanged since there was no intercourse.
		After 1 month	15	
_K_A	47	4.25	9	Improved greatly but not quite there yet.
		5.25	15	
_N_S	66	Before	2	Urination also improved.
		After 1 month	4	
_T_M	63	Before	15	
		After 1 month	19	

[Results]

The score improved for 9 patients (69.2%), no change for 3 patients (23.1%), and 1 patient (7.7%) worsened. The score dropped for the 1 medical case that worsened, while morning erections increased.

[Discussion]

Over 90% of ED is caused by nervous stress, hyperlipidaemia, impairment of liver function, and physical fatigue. It can be considered that the strength of erections increased from the effect of antioxidant substances, amino acids, and micro quantities of metal atoms contained in pollen extract which promote improved blood flow, adjustment of the autonomic nerves, recovery from fatigue, repair of damaged hepatocytes, and breakdown of female hormones in the liver.

[Safety]

During the Study, one subject developed diarrhea and there was one that developed soft stool, but administration did not have to be stopped for any of the patients in particular for complaints of worsening physical condition.

10/14/2005

