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# DeerVelvet

Technical manual

New Zealand
Deer Products

# 11 HEALTHY JOINT FUNCTION

## 11.1 Health Benefits

Velvet antler has been and still is widely used to assist healthy joint function in China. Lately, arthritis sufferers in Western countries have also begun to take velvet to alleviate their symptoms. Recent *in vitro* and *in vivo* studies support this use, and have suggested that velvet antler may indeed be beneficial to joint function.

## 11.2 Suggested Physiological Rationale

The precise mechanism of any effect of velvet antler on joint function is not known, although immunoregulatory and anti-inflammatory activities and an apparent ability to inhibit connective tissue degradation may play important roles. Deer velvet contains chondroitin sulphate, which has been shown to be clinically effective for treatment of arthritis. However, the effective doses of velvet administered in studies described below did not deliver enough chondroitin for it to solely, or even primarily, be responsible for the observed responses.

## 11.3 Research Support

Yudin and Dobryakov (1974) concluded that Pantocrine showed marked anti-inflammatory activity.

Wang (1996) reported that intra-peritoneal injection of velvet antler polysaccharides (100 mg/kg) inhibited the swelling induced in the forepaws of mice by dextran and egg white injection. In contrast, oral administration had no effect, which suggests the active polysaccharides did not survive the digestion process.

Intravenous administration of 10–50 mg/kg of a heat stable 68-amino acid polypeptide purified from velvet antler also showed a pronounced anti-inflammatory effect in rats (Zhang *et al.* 1992; Zhang *et al.* 1994). This occurred in adrenalectomised rats as well as in normal rats, although the reduction in swelling caused by dextran and cotton pellet granulomas was not as marked in the adrenalectomised animals (Table 10). In these rats, though, the effect of the antler polypeptide was similar to that of the steroid positive control, dexamethazone. The authors inferred from these results that the anti-inflammatory effects of the polypeptide are not completely dependent upon the pituitary-adrenal system.

There is a report that velvet powder had a pronounced analgesic effect when given orally to rats (Shin *et al.* 1989). A contribution to pain relief by velvet would obviously be of benefit to arthritis sufferers. However the doses administered to the rats were very high (500 mg/kg or more), so the relevance of the observation to standard use in humans is impossible to judge.

Table 10. Effect of a polypeptide (PAP) isolated from velvet antler on hind paw swelling of normal and adrenalectomised rats

Treatments were administered by intravenous injection. Data presented are the means of groups of six animals ± standard deviation (Zhang *et al.* 1994). *P<0.05, **P<0.01, ***P<0.001 *vs* control.

| Group | Dose (mg/kg) | Swelling of hind paw | |
|---|---|---|---|
| **Normal rats** | | | |
| Control | 0 | 22.5 ± 2.1 | |
| PAP | 5 | 18.4 ± 3.3 | *** |
| | 10 | 12.3 ± 4.1 | *** |
| | 20 | 8.2 ± 3.2 | *** |
| **Adrenalectomised rats** | | | |
| Control | 0 | 20.7 ± 2.4 | |
| Dexamethazone | 1 | 16.0 ± 3.1 | ** |
| PAP | 10 | 17.2 ± 3.6 | * |
| | 20 | 14.5 ± 4.1 | ** |

## Rheumatoid arthritis

In Korea, hot water extract of deer velvet is a widely used treatment for some immune-related diseases such as rheumatoid arthritis. Studies in immunisation-induced animal models of rheumatoid arthritis have shown that velvet extract was able to inhibit the development of the disease, when given by intra-peritoneal injection (Kim *et al.* 2003; Kang *et al.* 2006; Suh *et al.* 2007; Kim *et al.* 2008a; Kim *et al.* 2008b) or bilateral Shinsu (B23) acupuncture (Kim *et al.* 2004d; Kim *et al.* 2005). Velvet treatment had significant effects on a range of factors associated with the onset of arthritis in the animals, including suppression of the excessive rises in inflammatory mediators (Kim *et al.* 2003; Kang *et al.* 2006; Suh *et al.* 2007), inhibition of abnormally high activities of synovial fluid proteases (Suh *et al.* 2007; Kim *et al.* 2008a), prevention of oxidative damage to synovial fluid proteins by reactive oxygen free radical species (ROS) (Kim *et al.* 2008a), and inhibition of leukocytosis (Kim *et al.* 2008b). In rodents with collagen-induced arthritis it inhibited the formation of anti-collagen antibodies (Kim *et al.* 2003; Kang *et al.* 2006; Suh *et al.* 2007), and in adjuvant-induced arthritic rats velvet extract alleviated reductions in bone minerals, strength and trabecular bone formation and the increase in osteoclast number (Kim *et al.* 2005).

A registered medicine (Cervusen®) containing deer velvet (70% by weight) in combination with deer sinews (10%) and Korean ginseng (20%) also provided protection against arthritis when administered orally to rats in a modified adjuvant-induced arthritis model (Ghosh *et al.* 2001). Measures of acute inflammatory activity were decreased in rats treated with 2 or 5 mg/kg Cervusen®, and the destruction of cartilage and bone in knee joints of these animals was also substantially reduced.

The use of deer velvet powder to alleviate symptoms of rheumatoid arthritis has also been investigated in two human clinical studies. The first was an initial phase II trial (Allen *et al.* 2002a;

Allen *et al.* 2004a) that was intended to assess the safety of velvet taken together with standard rheumatoid arthritis medications, and to provide data that could be used to estimate required sample size for a subsequent larger trial. Forty patients with stage II rheumatoid arthritis were randomly assigned to groups of 10 patients each. One group received placebo and the other three groups received 2, 4, or 6 capsules (each containing 215 mg) of velvet powder daily for one month. In addition, all subjects continued to take their conventional arthritis medications (*e.g.* non-steroidal anti-inflammatory drugs, disease-modulating arthritis drugs, and analgesics). At the end of the study, there were no significant differences between groups in number of adverse events or health status. However, the greatest improvement was in the group receiving 6 capsules of velvet, and the least was in the placebo group. It was concluded that elk velvet antler could be taken safely in conjunction with a number of rheumatoid arthritis medications and warranted further study to assess efficacy.

In a follow-up triple-blind placebo-controlled study (Allen *et al.* 2008), 168 patients with stage 2 to 3 rheumatoid arthritis and suffering residual symptoms after standard treatment were randomly assigned to receive either velvet (1000 mg) or a placebo, daily for 6 months. Measures included patient assessment of pain, tender and swollen joint counts, patient and physician assessments of disease activity, patient questionnaires of functional ability and quality of life, and blood tests for C-reactive protein (a marker of acute inflammation). No significant differences between groups were found for any of these parameters in the study, and the pattern of change of the measures across time points was essentially the same for both groups. This resulted in the overall conclusion that velvet antler does not effectively manage residual symptoms in patients with rheumatoid arthritis. However, the authors did note that, because of ethical considerations arising from the severity of the disease in the patients, they were unable to compare velvet to standard medications; all patients needed to continue receiving standard treatments in addition to the experimental treatment. This meant that only small changes due to velvet treatment might be expected. Furthermore, the study was somewhat underpowered to demonstrate statistically significant results, as the investigators only managed to recruit 168 patients instead of the target 220 that were estimated to be needed from the analysis of the results of their initial trial. Of interest, some individuals in the velvet group reported that they felt markedly better following treatment, whereas none in the placebo group described a similar improvement. These factors led the study authors to suggest that deer velvet may have a positive effect in some individuals with rheumatoid arthritis.

## Osteoarthritis

A double-blind, placebo-controlled human study was conducted to determine if the velvet containing product Cervusen® was effective in ameliorating clinical symptoms in osteoarthritis (OA) sufferers (Edelman *et al.* 2000; Edelman *et al.* 2002). Following baseline examination, 54 patients were randomly assigned to receive either Cervusen® (2 x 350mg capsules/day) or placebo (2 x 350 lactose capsules/day) for 6 months. Clinical assessments were conducted at 1 and 3 months post-baseline, and at the end of the study. No adverse side-effects were reported for the duration of the study. Cervusen-treated patients showed improvements relative to baseline in self assessments of pain, and in both self and physician assessments of global

improvement, which were all significant at 3 and 6 months  No significant improvement from baseline was observed for the placebo-treated group for any of the parameters examined.  The authors suggested that the lead time required to reach clinical efficacy, along with their pre-clinical data, were suggestive that Cervusen® provided symptomatic relief in osteoarthritis by disease modification achieved through oral tolerisation to cartilage derived antigens.

Moreau *et al.* (2004) have provided evidence that velvet may also be effective for treatment of OA in dogs.  Canadian elk velvet antler powder was evaluated on client-owned dogs with OA in a double-blind, and placebo-controlled study.  Thirteen dogs received a placebo for 30 days and then velvet for 60 days.  Twenty-five other dogs received velvet for 60 days.  Gait analysis measured with a force plate, clinical signs assessed by an orthopaedic surgeon, performances in daily life activities and vitality assessed by the owners, and complete blood analyses were obtained at days 0, after 30 days of placebo and/or 60 days of velvet treatment.  On placebo, the 13 dogs did not show significant improvement; however, their gait, their performances in daily life activities, and their vitality were significantly improved on velvet supplementation, based on changes in values exceeding those observed when placebo was administered.  The 25 dogs on velvet for 60 days showed similar improvements.  No clinical changes were revealed by blood analyses.  Administration of velvet powder was considered to be effective in alleviating the condition in arthritic dogs.  Based on this study, Sanderson et al. (2009) concluded in a recent review of treatments for the management of canine OA that there is a "moderate level of comfort" for the efficacy of velvet in modifying the structures involved in the disease, but cautioned that further high quality studies need to be conducted.

# 12 STRONG BONES

## 12.1 Health Benefits

Osteoporosis is a metabolic disorder characterised by loss of bone density and associated increased risk of fracture. It is a significant public health problem, affecting 10 million women in the US alone with a further 34 million at risk of developing the disease. A range of drugs are available for treatment, but most have quite serious side effects. In China and Korea, deer velvet has long been highly valued for its bone strengthening effect. Given this background, and the low toxicity of the product in its various forms, it is no wonder that Western consumers are also beginning to consider the use of velvet for treatment of osteoporosis and other bone-related problems.

## 12.2 Suggested Physiological Rationale

Healthy and strong bones require a carefully regulated balance between bone resorption and bone formation. These processes are primarily facilitated by two cell types, osteoclasts and osteoblasts, respectively. Chondrocytes are also important bone-related cells, which produce cartilage. Deer velvet might be expected to have an effect on osteoporosis, and bone in general, by directly or indirectly affecting the proliferation or maturation of any or all of these cell types. Strong evidence is being accumulated that shows deer velvet does indeed exert such influences on all three cell types.

## 12.3 Research Support

### *In vitro* studies

Multiple studies have demonstrated that deer velvet contains factors that have direct effects on bone-related cells *in vitro*.

Total polypeptides isolated from Chinese sika deer (*Cervus nippon* Temminck) velvet by Wang Ben Xiang and co-workers were found to promote the proliferation of chondrocytes derived from rabbit ribs and embryonic human joints, as well as osteoblast precursor cells of embryonic chick calvaria (Guo *et al.* 1998; Zhou *et al.* 1999). A polypeptide consisting of 59 amino acids and having a molecular weight of 7,262 Dalton was purified from the mixture and was found to strongly stimulate the proliferation of all three cell types. In addition, a smaller polypeptide (molecular weight ~3,600 Dalton, as determined by SDS-polyacrylamide gel electrophoresis) was isolated that had weaker stimulatory activity with the two types of chondrocytes, and which did not affect the proliferation of the osteoblast precursor cells (Zhou *et al.* 1999). Comparable polypeptide fractions isolated from sika deer velvet and from red deer velvet were equally effective at stimulating the proliferation of rabbit costal chondrocytes, although there were differences in their compositions and their activities with epidermal cells (Zhou *et al.* 2001).

Chen *et al.* (2008) demonstrated that addition of velvet polypeptides to cultured rat chondrocytes affected the expression of proteins that control the cell life cycle, and resulted in postponing of senescence of the chondrocytes.

Other Chinese researchers separated a water-based velvet extract into five fractions based on their molecular weights, using gel filtration chromatography (Chen *et al.* 2004). The two fractions that contained the largest proteins were found to promote the proliferation of a rat osteoblast-like cell line (UMR 106), while the other three lower molecular weight fractions were inhibitory. After further purification the most active of the growth promoting fractions was identified as containing deer serum albumin (Lin *et al.* 2005). In addition to stimulating the proliferation of UMR 106 cells, the deer serum albumin isolated from velvet was shown to increase the secretion of IGF-I by the cells by about 50%. These activities were affected by the processing of the deer velvet, with greater activity displayed by extract from freeze-dried velvet than from velvet that was exposed to heat during traditional processing (Ke *et al.* 2008).

Kim *et al.* (2006) conducted an experiment to determine whether traditional Korean velvet water extract may have effects on bone remodelling by inducing the differentiation of resting zone chondrocytes (RC). Confluent chondrocyte cell cultures were pre-treated with velvet extract and then the media were replaced with new media containing $10^{-10}$–$10^{-8}$M 1,25-$(OH)_2D_3$ (a vitamin D3 metabolite) and the cells incubated for an additional 24 hours. This second treatment was chosen because prior studies had shown that only the more mature growth zone chondrocytes (GC) respond to the vitamin D3 metabolite. Following pre-treatment for 120 hours with velvet extract, treatment of RC with 1,25-$(OH)_2D_3$ caused a dose-dependent increase in alkaline phosphatase-specific activity and collagen synthesis, but did not affect proteoglycan production. These increases were not observed in RC that were not pre-treated, or were instead pre-treated with 1,25-$(OH)_2D_3$. The results demonstrated that velvet extract was able to directly regulate the maturation of RC chondrocytes into GC chondrocytes, and may therefore play a role in regulating chondrocyte maturation during bone formation.

In order to investigate the putative anti-bone resorptive activity of deer velvet, Li *et al.* (2007b) investigated the effect of a chloroform (*i.e.* lipid) extract of velvet on osteoclast differentiation in mouse bone marrow cultures. The chloroform extract inhibited osteoclast differentiation in mouse bone marrow cultures stimulated by receptor activator of NF-κB ligand (RANKL) and macrophage-colony stimulating factor (M-CSF). The activation of a number of signalling pathways important in osteoclast differentiation was inhibited by the extract. It also inhibited the bone resorptive activity of differentiated osteoclasts that was accompanied by disruption of actin rings and induction of cell apoptosis. These results support the notion that deer velvet extract may be a useful remedy for the treatment of diseases such as osteoporosis by influencing bone-resorption processes.

Mundy and co-workers (1995; 2001) purified a number of novel bone growth factors from deer velvet and from conditioned media of antler cells in culture, based on their abilities to stimulate the proliferation of either MG-63, MC3T3 and/or C433 cells. MG-63 is a human osteosarcoma cell line, while MC3T3 cells are mouse osteoblasts and C433 is a stromal cell line derived from a human giant cell tumour of bone. One of the isolated peptides ("OT-2") had about 70% homology with IGF-I, while another ("OT-4") was similar to IGF-II. A further peptide ("OT-5") was a novel form of basic fibroblast growth factor (bFGF), which did not bind to antibodies to bFGF on affinity columns and showed some differences to bFGF in cell proliferation assays. The results established

that deer velvet contains a number of novel peptides in addition to known growth factors, which are able to stimulate the proliferation of bone-related cells.

## *In vivo* studies

Wang Ben Xiang's group showed that as well as stimulating the *in vitro* proliferation of chondrocytes and osteoblast precursor cells (see above), the mixture of velvet polypeptides isolated from sika deer velvet hastened the repair of experimental bone fracture repair in rats when injected at the site of injury (Zhou *et al.* 1999). After seven weeks of treatment a significantly higher proportion of fractures had healed in rats injected with 20 mg/kg velvet antler polypeptides as compared to control rats (75% *vs* 25%, respectively). The strengths of the repairs were also enhanced by treatment, as assessed by removing the previously fractured long bones and determining the maximum weights they could support before breaking again. The untreated bones broke again with a loading of 802 g, but the bones of the rats injected with 20 mg/kg velvet antler polypeptides did not break until the load reached 1548 g. The hydroxyproline and calcium contents in the callus were also significantly higher in the velvet treated rats than in control animals, showing that the velvet polypeptides had enhanced collagen accumulation and calcium deposition at the fracture sites.

Mundy *et al.* (1995) injected proteinaceous material extracted from deer velvet above the calvaria of 5 week old mice once a day for 3 days. Four days later a substantial amount of new woven bone was observed on the outer surface of the calvarial bone, and this attained the appearance of lameliar bone over the following 1 to 2 weeks. The velvet extracts so tested were deduced to have therapeutic potential for the enhancement of bone growth and fracture repair in animals and in humans.

In rats with adjuvant-induced arthritis, velvet extract administered by traditional Korean acupuncture alleviated reductions in bone minerals, strength and trabecular bone formation and the increase in osteoclast number associated with the disorder (Kim *et al.* 2005).

Another study by Korean researchers (Lee *et al.* 2005; Jang *et al.* 2006) examined the efficacy of deer antler extract (DA), medicinal herbs extract (MH), and their mixture (DAMH), on bone growth and serum IGF-I *in vivo* in growing rats. Three-week-old female rats (Sprague-Dawley) were divided into four groups and then fed standard (control) diet or experimental diets containing DA, MH, or the DAMH mixture, for 7 weeks. After that time, the wet weights, breaking forces and bone minerals (Ca, Mg and Zn) of the rats' fibia and tibia were found to be significantly higher in the DA-fed group than in the other groups. All three treatments significantly reduced serum ALP and bone-specific alkaline phosphatase (BALP) activities as compared to the control group. Also, serum IGF-I concentrations were significantly higher in DA-fed group compared to the other groups. The deer velvet extract was thus shown to have promoted bone growth, and it was suggested that this might have been as a result of the increase in IGF-I, a major bone growth factor.

A number of studies have examined the effect of deer velvet on bone in a variety of animal models of osteoporosis.

Duan *et al.* (2007) investigated the effect of total velvet antler polypeptides (Zhou *et al.* 1999) on rats with retinoic acid-induced osteoporosis. Male Wistar rats were given retinoic acid (70 mg/kg/day) intragastrically for 14 days before being sacrificed on day 30. Subcutaneous injection of velvet antler polypeptides at doses of 20, 40 or 60 mg/kg/day over the same period as the retinoic acid treatment significantly increased femur bone mineral density in comparison to control osteoporotic rats. At the highest dose of velvet polypeptides, bone calcium content and bone weight coefficient were also significantly elevated back to the levels of non-osteoporotic control animals. In addition, a number of static histomorphometric indices of the trabecular in the tibia of the rats were significantly improved by all doses of the velvet polypeptides, showing that the velvet treatment was able to improve the structure of cancellous bone as well as increase bone mass in the osteoporotic rats.

In a different model of osteoporosis, groups of ovariectomised rats fed a calcium- and phosphorus-deficient diet were treated with hot water velvet extract (625 or 1,250 mg/kg) orally for 5 weeks (Kim *et al.* 2001). Over that time, serum oestradiol levels significantly decreased by 50% in the ovariectomised control group, but were unchanged in the groups given velvet extract as well as in sham-operated and un-manipulated control groups. Serum osteocalcin and calcitonin levels were significantly raised in the velvet treated groups as compared to the ovariectomised control group, and were restored to the levels of the un-manipulated control group. Control ovariectomised rats had significantly raised serum alkaline phosphatase (ALP) activities and calcium levels, and slightly higher serum phosphorus, but velvet treatment normalised these values back to those of the un-manipulated control animals. These findings suggest possible protective and therapeutic effects of velvet extract against bone loss associated with a significant decrease of serum oestradiol level in ovariectomised rats.

Lee *et al.* (2007b) briefly reported on a study in which osteoporosis was similarly induced by feeding a low calcium diet to female rats for 4 weeks after ovariectomisation. Groups of osteoporotic rats were supplemented with velvet extract (2.5% or 5% of diet) for 6 weeks, were treated with 17β-oestradiol (10 μg/kg, positive control) or were untreated (control). Weight gain was not affected by velvet supplementation, but serum oestradiol levels were significantly elevated in velvet extract-fed groups compared to the control group. Femur weight and mineral (calcium, magnesium) contents were significantly increased both by velvet and by oestrogen treatment compared to control, while urinary hydroxyproline and deoxypyridinoline excretions were significantly decreased. Also serum interleukin-6 (IL-6) was decreased both by velvet supplementation and by oestrogen treatment, suggesting that the bone-sparing effect could be partly attributed to the modulation of osteoclastogenesis induced by IL-6. Like the experiment of Kim *et al.* (2001) that is described above, these results support the conclusion that deer velvet has beneficial effects on oestrogen-dependent bone loss and may be useful for treating postmenopausal osteoporosis.

The ovariectomised rat model was also used by Meng *et al.* (2009) investigate the effects of velvet antler powder on osteoporosis. The results were consistent with those already mentioned. After 13 weeks, supplementation with velvet (0.18 or 0.54 g/kg/day) had improved bone mineral density, bone mineral content and serum osteocalcin of the ovariectomised rats, and reduced serum ALP activity. There were significant differences between the high dose velvet group and

the ovariectomised controls for all measures, and for some measures in the case of the low dose velvet treatment. Both doses of velvet significantly increased the width of trabecula bone and bone trabecula area percentage.