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DeerVelvet

Technical manual

New Zealand
Deer Products

10 IMMUNE FUNCTION

10.1 Health Benefits

Velvet antler is often used as a tonic by Koreans at the beginning of and during winter. In Traditional Chinese Medicine (TCM), velvet antler is frequently prescribed as a tonic in situations of stress and fatigue. The TCM Materia Medica indicates that velvet antler administration increases the number of red and white cells. Recent research has some apparently immuno-supportive effects demonstrated in laboratory animals, but the practical implications of these results for humans are not yet known.

10.2 Suggested Immunological Rationale

It may be that velvet antler supports the humoral immune system through its white blood cells and macrophages. A variety of molecules in velvet (lipids, polypeptides, proteoglycans) that span a broad molecular weight range have been identified as having immunomodulatory activity, and it is likely that multiple mechanisms are involved. The precise nature of these, and their interplay, has not been established though.

10.3 Research Support

A study was conducted in Korea by Shin *et al.* (2001), in which the immunostimulating activity of antler extracts were evaluated by the carbon clearance test method established by Wagner *et al.* (1985). ICR mice weighing 25-30 g were dosed orally for 4 consecutive days with velvet antler extracts dissolved in phosphate-buffered saline (PBS) solution. Forty eight hours after the dose, each mouse was intravenously injected with carbon suspension at a dose of 10 µl/g bodyweight. Blood was withdrawn from the orbital vein, and the carbon concentration in the blood was estimated by use of a spectrophotometer. From the optical density at 660 nm, the linear regression coefficient (RC) was calculated by plotting -logE against time. The immunostimulating activity was expressed as the ratio of the regression coefficient of the treated animals (RCtr) to that of the control group (RCc). Zymosan given by intra-peritoneal injection (50 mg/kg of bodyweight) was used as a positive control substance.

As shown in Table 9, the ethanol extracts of antler exhibited a significant enhancement of carbon clearance activity in a dose-responsive manner. The regression coefficient ratio of the group treated with 5 mg/kg/day was 1.18, which represents moderate immunostimulating activity. The regression coefficient ratio of the group treated with 10 mg/kg/day, however, was 1.58, which represents very strong activity. This activity was almost equipotent to Zymosan, a known phagocytosis enhancer. The water extract of antler showed a moderate amount of enhancement of carbon clearance when given as an oral dose of 10 mg/kg each day

The results of the above study backed up those of an earlier experiment by the same group (Shin *et al.* 1989), which showed a weak immunopotentiating effect of velvet powder in the carbon clearance test when orally administered at a dose 500 mg/kg to mice.

Table 9. Effect of ethanol and water extracts of velvet on carbon clearance in mice

Regression coefficients are means ± SEM of five mice (Shin *et al.* 1989). RCtr/RCc (the ratio of RC for treated mice to that of control animals) provides an assessment of immunostimulating potency: values less than 1.0 = not active; 1-1.5 = active; over 1.5 = very active.

Treatment	Dose	Regression Coefficient [a]	RCtr / RCc
Experiment 1 (Ethanol Extract)			
Control		0.0216 ± 0.0017	–
Antler	5 mg/kg/day, p.o.	0.0256 ± 0.0023	1.18
	10 mg/kg/day, p.o.	0.0342 ± 0.0028	1.58
Zymosan	50 mg/kg, i.p.	0.0350 ± 0.030	1.62
Experiment 2 (Water extract)			
Control		0.0208 ± 0.0013	–
Antler	10 mg/kg/day, p.o.	0.0256 ± 0.0010	1.23
Zymosan	50 mg/kg, i.p.	0.0304 ± 0.0016	1.46

Also in Korea, Suh *et al.* (1999; 2000) isolated and characterised monoacetyldigycerides from chloroform fractions of antlers from sika deer. They synthesised one of the monoacetyldiglycerides, *rac*-MADG, and two of its enantiomers (D-MADG and L-MADG), and investigated the effects of these on phagocytosis by mouse peritoneal macrophages. Compared with control macrophages, macrophages treated with *rac*-MADG and in particular with L-MADG showed enhanced phagocytosis. The results suggested that L-MADG enhances the phagocytosis of peritoneal macrophages *via* the secretion of interferon-α.

Japanese scientists reported that a high molecular weight fraction of a hot-water extract of velvet antler had anti-complementary activity (Zhao *et al.* 1992), and thus had effects on the innate immune system. Chondroitin sulphate moieties were shown to be important for the activity of the complement-activating proteoglycans.

In China, Wang (1996) reported that intra-peritoneal injections of Pantocrine (0.5-2 mg/kg) stimulated the phagocytic function of macrophages in both normal and immune-deficient mice. Similarly, Li *et al.* (2004) showed that alcohol velvet extracts of either Chinese wapiti or New Zealand red deer velvet were active stimulators of macrophage phagocytosis, when given to mice by oral administration.

Pan *et al.* (2007) isolated polypeptides from velvet and administered these to mice by daily intra-peritoneal injections (400 mg/kg) for 20 days. The velvet polypeptides promoted the proliferation of T and B lymphocytes, and also enhanced the secretion of IL-12 (a T cell stimulating factor) by activated peritoneal macrophages isolated from the mice. The isolated velvet polypeptides were thus concluded to possess immunopotentiation activity.

Kim *et al.* (1995) conducted a number of experiments to investigate the effects of velvet extract on the following indices of immune function: lymphocyte production and maturation (blastogenesis) in spleen, thymus, lymph node, and bone marrow cells of Balb/C mice, the haemagglutination reaction against sheep red blood cell (SRBC), the plaque forming cell (PFC) assay against SRBC, and on IL-2 production. Velvet extract demonstrated a potent mitogenic activity on spleen and lymph node cells, but had only mild activity on the thymus and born marrow cells. The active mitogenic component of the velvet extract was shown by use of ultrafiltration to consist of materials with molecular weights over 5,000 Dalton. Velvet extract was shown to significantly increase the mitogenic effect on the lipopolysaccharide (LPS)-stimulated spleen cells, but decrease the mitogenic effect on the concanavalin A (Con A) stimulated spleen cells at concentration of 0.3%, 1% and 3%. Velvet extract did not show a positive haemagglutination reaction and was found to inhibit the Con A-induced haemagglutination reaction against SRBC. It significantly increased the number of PFC at concentrations of 0.1% and 1%. When IL-2 or IL-4 production was determined after the proliferation of CTLL-2 cells, velvet extract was not shown to stimulate the production of IL-2. From these results, it was concluded that velvet was capable of increasing antibody production by B cells, but not IL-2 production by helper T cells.

This conclusion is not supported, however, by the results of Sim and Sunwoo (2001) in an experiment in which rats were immunised against ovalbumin (see page 41 for details of the protocol). Titres of specific anti-ovalbumin antibodies were lower in velvet-treated animals, as were total levels of immunoglobulins at the highest dose of velvet. More research is thus required on the effect of velvet on the humoral immune system.

Other Korean researchers (Kim *et al.* 2004c) used bioassay-guided fractionation after silica gel column chromatography to identify an immunomodulator in velvet. Structural analysis was performed with one- and two-dimensional nuclear magnetic resonance techniques and tandem mass spectrometry coupled with fast atom bombardment. The phosphatidylcholines sub-fraction, isolated from a 70% ethanol extract of velvet, induced the proliferation of spleen cells in synergy with Con A. According to the structural analysis, the phosphatidylcholines were classified as a family (1,2-alkyl-sn-glycerol-3-phosphocholines) containing arachidonyl (C20:4), stearoyl (C18:0), oleoyl (C18:1), linoleoyl (C18:2), palmitoyl (C16:0), and myristoyl (C14:0) chains in their fatty acyl chains. Because the unsaturated fatty acids showed an inhibitory effect on the immune system, dialkyl phosphatidylcholines with different chain lengths from C10:0 to C20:0 that stimulate the proliferation of spleen cells were examined extensively. Among other saturated phosphatidylcholines used, dimyristoyl phosphatidylcholine (C14:0) induced the proliferation of spleen cells more efficiently, whereas dimyristoleoyl phosphatidylcholine (C14:1) effected little change in the proliferation of spleen cells. Collectively the results suggested that phosphatidylcholines with saturated fatty acyl chains are immunostimulating factors and that they may modify the proliferation activity of known mitogens. Further, the chain length and saturation of the fatty acids may play important roles in the proliferation of spleen cells.