



Review

Bioactive components of velvet antlers and their pharmacological properties



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ABSTRACT

Velvet antler is one of the most important animal medicines, and has been used with a variety of functions, such as anti-fatigue, tissue repair and health promotion. In the past few years, the investigation on chemical compositions, bioactive components, and pharmacological effects has been performed, which demonstrates that velvet antlers could be used as an important health-promoting tonic with great nutritional and medicinal values. This review focuses on the recent advance in studying the bioactive components of velvet antlers.

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1. Introduction

In recent years, there has been an increasing awareness of the benefits of natural products, and many plant-derived medicines have been reported. For examples *Rehmannia glutinosa* is one of the earliest known edible crude herbs used for various Chinese patent medicines, such as liuwei dihuang decoction, and its active principles possess wide pharmacological actions on the blood system, immune system, endocrine system, cardiovascular system and the nervous system [1]; *Panax ginseng* is one of the most valuable herb medicines and has been used in Asia as a tonic to improve stamina and vitality [2]; the lateral roots of *Aconitum carmichaelii* is the main ingredient of the Sini decoction, a well-known formula to treat myocardial infarction [3]. However, the researches on animal medicines or functional foods are still relatively few, although animal medicines have been proven of various important components, such as proteins, peptides, fatty acids, glycosaminoglycans, prostaglandins, vitamins, minerals, dietary fiber, essential oils and carotenoids [4,5], which can be used in the prevention and treatment of various diseases.

As a typical traditional animal medicine, velvet antlers (Fig. 1) have been used for over 2000 years, and have pharmacological effects to improve immune system, physical strength, and sexual function [6,7]. Now, velvet antlers from sika deer and red deer are designated as medicinal antlers in the pharmacopeias of countries, such as China, Japan and Korea. Besides, they are also used as supplement to prevent diseases.

Generally, deer antler is used by oral administration in the formulations of decoction or medicinal liquor. The most important source of velvet antlers is antler removal from farmed deer, which has been permitted in several countries, such as China, New Zealand, Australia and Canada [8–10]. It is currently estimated that the global production of velvet antlers is near to 1300 tons/year, which is still rapidly growing to meet the requirements of medicinal markets [6].

Velvet antlers are the unique organs that display an annual cycle of full regeneration in mammals [11]. In addition, antler growth is a very rapid process, with the maximum rate of elongation recorded for wapiti (*Elaphurus davidianus*) antlers being 2.75 cm per day [12]. During this period, the constitutive tissues, such as cartilage, bone, nerves, skin, and blood vessels also grow at the same rate [13]. Therefore, antlers are considered as valuable models for studying the signaling pathways. Recent evidence suggests that antler regeneration is a stem cell-based process, and some growth factors, such as VEGF, EGF, FGF and NGF, have been proven involved in the exceptional growth [14–17], in which some undiscovered modulating factors with low abundance or short-half-life in the development of normal tissues may be over-expressed and more likely be found. Thus, the investigation on bioactive components in velvet antlers might lead to the discovery of new active factors.

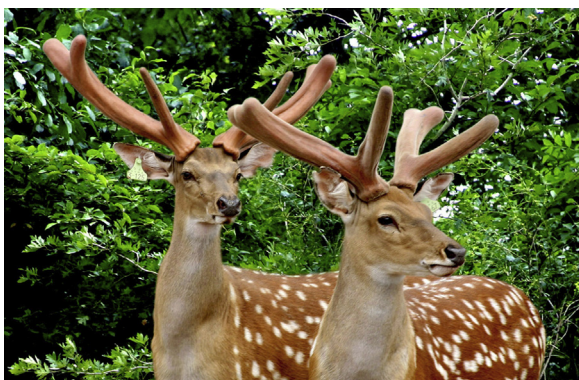


Fig. 1. Velvet antlers of farmed sika deer.

Up to now, many investigations have been undertaken to determine the chemical constituents as well as the pharmacological effects of velvet antlers. Herein, this review attempts to summarize the recent advance in studying the bioactive components of velvet antlers, which might be beneficial to encourage the in-depth study on their pharmacological effects, and provide further insight into the mechanisms associated with therapeutic effects.

2. Bioactive components

Through the in-depth study on white-tailed deer (*Odocoileus leucurus*), red deer (*Cervus elaphus*), elk (*E. davidianus*) and sika deer (*Cervus nippon*) have been studied, mineral elements [18,19] amino acids [20–24], polypeptides [31,32], proteins [17,20,23,27,28], polysaccharides [33–35], fatty acids [36], phospholipids [37] and biological base [40,56] have been proven as the bioactive components (shown in Table 1), and their contents change obviously with the growth of velvet antlers [20,26].

2.1. Amino acids, polypeptides and proteins

Velvet antlers are rich in amino acids, polypeptides and proteins, which are considered as the most prominent bioactive components.

Up to now, 19 kinds of amino acids have been isolated and identified from antlers. Jeon et al. [20] processed velvet antlers (*C. nippon*) by hydrolysis with 6 M HCl, and by using an amino-acid analyzer, they found 16 amino acids. Among them, aspartic acid, glutamic acid, proline, glycine and arginine accounted for approximately 32.5–37.2% of the total amino acids. Similarly, Wang et al. [21,22] not only extracted free amino acids, but also processed hydrolytic amino acids from velvet antlers (*C. nippon*). They found 17 free amino acids, and seven of them accounted for almost 30% of the total mass. In the case of hydrolytic products, 16 amino acids were detected and the proportions of them are similar with that of free amino acids. Li et al. [23] compared the amino acid content in different sections from sika deer (*C. nippon*), and found that the total mass percentage of 16 kinds of amino acids in velvet antlers was more than 44%.

In addition, Sunwoo et al. [24] reported that the amino acid content decreased downward from the tip to the base section of velvet antlers of wapiti (*E. davidianus*) in accordance with the theory of traditional Chinese medicine that the tip section is considered as the most valuable part of velvet antler. Therefore, the composition and content of amino acid may be one of the factors indicating the quality of velvet antlers.

Besides amino acids, Jeon et al. [20] also analyzed the crude proteins of velvet antlers of sika deer (*C. nippon*), stags on 40 days (FDG) and 60 days (SDG) after antler regeneration. They found that the crude protein was the highest in the top section, decreased markedly in other sections further down the antler. Besides, FDG had a higher content of crude protein than SDG. In the report of Je [27], antlers were harvested from elk (*E. davidianus*) stags on 65 days (VA65), 80 days (VA80) and 95 days (VA95) after regeneration, and the chemical composition of each antler was determined in five sections (top, upper, middle, base, and bottom). The crude protein content was the highest in the top section, and decreased markedly in the other sections further down the antler. In another study [28], velvet antlers of sika deer (*C. nippon*) were harvested on 40, 50 and 60 days after casting, and the analysis of crude proteins showed similar results.

Recently, many studies on the velvet antlers were performed in terms of molecular biology. These studies reveal that various growth factors and proto-oncogenes are expressed in the growing tip of antler. Lai et al. [16] detected the distribution of the growth factors, FGF-2 and VEGF, and their receptors FGFR1, FGFR2

Table 1
Summary of the chemical constituents present in deer antlers.

Class	Chemical constituents	Reference		
Mineral elements	Calcium (Ca)	[18,19]		
	Phosphorus (P)			
	Sodium (Na)			
	Potassium (K)			
	Magnesium (Mg)			
	Iron (Fe)			
	Zinc (Zn)			
	Copper (Cu)			
	Chromium (Cr)			
	Strontium (Sr)			
	Nickel (Ni)			
	Cobalt (Co)			
	Manganese (Mn)			
	Vanadium (V)			
	Tin (Sn)			
Amino acids	Arginine (Arg)	[20–24]		
	Histidine (His)			
	Glutamic acid (Glu)			
	Proline (Pro)			
	Aspartic acid (Asp)			
	Serine (Ser)			
	Threonine (Thr)			
	Glycine (Gly)			
	Alanine (Ala)			
	Isoleucine (Ile)			
	Leucine (Leu)			
	Phenylalanine (Phe)			
	Methionine (Met)			
	Lysine (Lys)			
	Tyrosine (Tyr)			
	Valine (Val)			
	Cysteine (Cys)			
Hydroxyproline (Hyp)				
Proteins and peptides	Tryptophan (Trp)	[16]		
	FGF-2, VEGF, FGFR1, FGFR2, FGFR3 and VEGFR-2			
	Soluble proteins		[17]	
	Crude proteins		[20,27,28]	
	Collagen content		[28,23]	
	Tripeptide with molecular weight of 395.1 kDa polypeptide		[31]	
	Decorin		[32]	
	Sulfated-GAGs and GAGs		[55]	
	Uronic acid		[25,28]	
	Chondroitin sulfate A, B and C		[23,34,35]	
Saccharides	Chondroitin sulfate	[33]		
	Phospholipids	[35,55]		
	Sialic acid	[36]		
	Keratan sulfate	[42]		
	Sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, lysophosphatidylcholine and lysophosphatidylethanolamine	[55]		
	Polyunsaturated fatty acids	[59]		
	Lipids and polyamines	Prostaglandins of A, B, E and F	[36]	
		Total saturated fatty acid, total monounsaturated fatty acid, conjugated linoleic acid, polyunsaturated fatty acid and ω -3 fatty acid		
		17 α -Hydroxyprogesterone, progesterone and testosterone		[37]
				[38,39]
		[37]		
Biological bases	Uracil, hypoxanthine and undine	[40,56,59]		

and FGFR3, and VEGFR-2, by means of immunohistochemistry and light microscopy in sections of growing antlers of red deer (*C. elaphus*). Their findings suggested that FGF-2 induced the expression of VEGF, stimulating and maintaining high rates of neovascularization and angiogenesis, and thereby provided nutrients to both velvet antlers and bones during the rapid growth and development. Nieto Diaz et al. [17] reported that several soluble proteins secreted by velvet antlers (*C. elaphus*) could strongly promote neurite outgrowth. By means of specific blocking antibodies, they

demonstrated that nerve growth factor was partially responsible for these effects although other yet unidentified proteins seemed also to be involved.

The collagen content in velvet antlers of sika deer (*C. nippon*) and elk (*E. davidianus*) was also studied by Jeon [28]. They calculated the collagen content by multiplying the hydroxyproline content by 7.25 [29], and hydroxyproline content was determined by the method of Bergman and Loxley [30]. The results showed that collagen content in velvet antlers was the lowest in the top section, and increased downward from the top to the bottom section. Similarly, Sunwoo et al. [24] reported that the proportion of collagen in velvet antler of elk (*E. davidianus*) increased downward from the upper sections to the base sections (approximately 1.4, 2.5 and 3.2 times higher in the upper, middle and base section, respectively, than in the tip section).

Multifunctional peptides have attracted increasing attention in the food science community because of their therapeutic potential, low toxicity and rapid intestinal absorption. Wang et al. [31] isolated a tripeptide with molecular weight of 395.1 Da from velvet antlers of sika deer (*C. nippon*), which showed antibacterial activity (against *Escherichia coli*, *Staphylococcus aureus* and *Hemolytic streptococcus*), anti-fatigue and anti-swelling of auricle effects in vivo. Weng et al. [32] reported a novel 3.2 kDa polypeptide from velvet antler of red deer (*C. elaphus*), which stimulated the growth of rat epidermal cells and rabbit costal chondrocytes in a dose-dependent manner.

2.2. Saccharides

Natural polysaccharides are a class of nontoxic macromolecules with physiological activities, which are receiving more and more attention in the development of health products. Wang et al. [33] studied the contents of polysaccharide in different slices prepared from different parts (tip, upper, mid and base) of northeast sika deer (*C. nippon*) velvet antlers, namely, wax slice (WS), powder slice (PS), blood slice (BLS), and bone slice (BNS). They found that the contents of polysaccharide decreased gradually from the tip to the base, and the differences in the polysaccharide contents among various parts of velvet antlers were significant. The changing regularities of the contents of polysaccharide can be used to evaluate the quality of sika deer (*C. nippon*) velvet antlers. Jeon et al. [34] reported that the total uronic acid content reached 118.5 mg/g for upper and middle sections of velvet antlers, and reached 106.1 mg/g for base section when the antlers of elk (*E. davidianus*) were sequentially extracted with hot water at different temperatures, in accordance with the report made by Sunwoo et al. [24]. Meanwhile, Jae et al. also reported that the total uronic acid content reached 15.4 mg/g for upper and middle sections of velvet antlers of wapiti (*E. davidianus*), and reached 16.86 mg/g for base section. Scott and Hughes [35] found that the uronic acid content of deer antler was 300 μ g/g, much lower than Jae's results. The difference might be due to the fact that one analyzed uronic acid content of the extracts, while the other examined the antler itself. Furthermore, Jeon et al. [26,28] reported the contents of sulfated-GAGs and GAGs decreased from the upper sections to the base sections of velvet antlers of sika deer (*C. nippon*). Meanwhile, the GAGs content of antler was higher in the velvet antler cut in 65 days than that cut in 95 days.

2.3. Lipids and polyamines

Ivankina et al. [36] found that the alteration of lipid composition took place at different stages of antler growth, and the bioregulators of lipid origin which were prostaglandins of A, B, E and F groups were found at the same stage. Meanwhile, the greatest amounts of phospholipids and polyunsaturated fatty acids were observed during the most intense soft growth period of velvet antlers of

sika deer (*C. nippon*). It is reported that the ether extract content (crude fat) was the highest in the top section of antlers of sika deer (*C. nippon*), and decreased from the top to the bottom section [28]. Meanwhile, this study found that the proportion of linoleic acid, 11,14,17-eicosatrienoic acid, total ω -3 and ω -6 fatty acids and polyunsaturated fatty acids (PUFAs) for all sections in the VA65 group was higher than in the VA95 group, suggesting that the quality of velvet antler was strongly influenced by the development stages. Jeon et al. [26] investigated the differences in chemical composition of antlers according to growing days (80 d and 90 d after casting) and sections (upper, middle, base) in elk (*E. davidianus*). The contents of ether extract (crude fat) were higher in antlers cut on day 80 than in those cut on day 90. For total fatty acids, unsaturated fatty acids, and essential fatty acids, the contents were higher on day 80 than on day 90. Lee et al. [37] investigated the changes in fatty acid contents in different development stages of antlers in spotted deer (*C. nippon*). Their results showed that total saturated fatty acid was lower in velvet antler harvested 40 days (FDG) than 60 days (SDG). For total monounsaturated fatty acid, conjugated linoleic acid, polyunsaturated fatty acid, ω -3 fatty acid, the contents were higher in FDG than SDG. However the difference between uronic acid in FDG than SDG was not significant. These results indicated that in the longer stage of antler development, the content of such active components was decreased.

SPE was coupled with HPLC–MS/MS for the simultaneous determination of eighteen sex hormones from thirteen velvet antler (*C. nippon* and *C. elaphus*) by Lu et al. [38]. The results showed that testosterone was detectable in almost all samples, 17α -hydroxyprogesterone was observed only in one sample, and progesterone was found in three samples. Lu et al. [39] also reported a method for the simultaneous determination of 11 sex hormones in antler velvet (*C. nippon* and *C. elaphus*) by gas chromatography–tandem mass spectrometry (GC–MS/MS). And an HPLC method for the simultaneous and rapid determination of three main biological base components including uracil, hypoxanthine and undine in antler velvets of sika deer (*C. nippon*) was developed by Zhou et al. [40].

3. Preparation of bioactive components

The extraction and isolation of active components in velvet antlers is critical for studying their bioactivities. Classical single-solvent extraction method is popular in this field, by using water, salt water, acid water, basic water, ethanol or butanol. Recently, multiple and novel technologies have been used to extract chemical components from velvet antlers.

3.1. Extraction and isolation of amino acids, polypeptides and proteins

In traditional medicine, decoction and medicinal liquor are the main formulations of velvet antlers, which have been used for a long time, and many reports have shown that the extracts of velvet antler using hot water and 70% ethanol exhibited multiple activities [41,42]. However, these extraction methods could denature proteins and impair their activities.

Recently, people developed some new methods with good bio-potential for bioactive substances extraction from velvet antlers. Sui et al. [43] developed a sequential extraction strategy to keep the activities of extracted proteins from velvet antlers of sika deer (*C. nippon*), by saline solvent (0.15 M sodium chloride, pH 7.0), mild acid buffer (0.15 M acetate buffer, pH 4.0) and mild alkaline buffer (0.15 M glycine–sodium hydroxide buffer, pH 10.0), by which various components in one sample can be sequentially extracted according to their physical and chemical properties. Hao et al. [44]

extracted the polypeptides of different segments (top, middle and base) of velvet antlers of red deer (*C. elaphus*) with acetic acid solution (pH 3.5) and alcohol, and IGF-1, EGF and NGF were successfully extracted, which were proved different significantly in various parts, and the highest concentration was obtained in the top of velvet antlers.

Yan et al. [45] reported that a velvet antler polypeptide of sika deer (*C. nippon*) was extracted and purified by gel filtration, ion exchange chromatography and RP C18, which showed a single peak in HPLC chromatography and a single band in SDS–PAGE. The amino acid sequence of the polypeptide, EPTVLDEVCLAHGP, was detected with ESI–MS/MS. Wang et al. [31] used 80% methanol to extract polypeptides from sika deer (*C. nippon*) antlers, and separated peptides by an aluminum oxide column. One peptide with molecular weight of 395.14 Da was eluted using 35–50% of ethanol.

Weng et al. [46] isolated and purified a velvet antler polypeptide from red deer (*C. elaphus*) by ion exchange chromatography, gel filtration and RP–HPLC. The polypeptide showed a single peak in HPLC chromatography and a single band in SDS–PAGE. The analysis of amino acid sequence showed that this polypeptide was VLSATDKTNV LAAWG KVGGN APAFG AEALE RM, which could stimulate the growth of rat epidermal cells and rabbit costal chondrocytes in a dose-dependent manner. The homologous polypeptide of velvet antlers of sika deer (*C. nippon*) was also purified using this strategy and also had similar activities [47,48]. Zha et al. [49] synthesized and purified a 3.2 kDa recombinant polypeptide (rPP) according to the amino acid sequence of natural polypeptide (nPP), isolated from velvet antlers, and compared the burn wound healing activity of these two polypeptides. The results revealed that the wound-healing efficacy of rPP was always slightly weaker than that of nPP. However, the yield of rPP was 40-fold higher than that of native one. Taken into account of other factors, such as season restrictions, operation cost and product storage, rPP has decisive advantages over conventional polypeptides for industrial production.

Recently, an enzymatic hydrolysis method was developed for preparation of velvet antler polypeptides from sika deer (*C. nippon*) by Xu et al. [50], and the optimum hydrolytic condition was as follows. The reaction temperature was 48 °C; pH was 7.2, substrate concentration was 7.5%; enzyme activity was 6000 U/g substrate; the proportion of the enzymes was 1:1 (trypsin:protamex), and reaction time was 4 h. Under this condition, the degree of hydrolysis was 32.5%, and the yield of polypeptide was 72.8%. Wang et al. [51] also optimized the hydrolysis conditions of velvet antler of sika deer (*C. nippon*) by the orthogonal tests. The optimized parameters were as follows: substrate concentration 0.08 g/ml, enzymatic hydrolysis temperature 65 °C, pH 9.0 and hydrolysis time 6.0 h. Under such conditions, the degradation yield of fresh antler velvet was up to 92.6%, and the yield of the amino acids product reached 12.1%.

3.2. Extraction and isolation of saccharides

Ultrasonic wave was used to assist the extraction of polysaccharide from velvet antlers by Zhao et al. [52]. In their study, velvet antler of sika deer (*C. nippon*) were defatted by ethylether, lyophilized and pulverized into powder, and extracted by phosphate buffer. After that, the extract was dialyzed and lyophilized after deproteinization and decolorization. Box–Behnken experiment was designed to optimize extraction conditions for polysaccharides at different growth stages of velvet antlers. The results showed that the polysaccharide content in two branched antlers was the highest. Xiong et al. [53] extracted glycosaminoglycans (GAGs) from velvet antlers of sika deer (*C. nippon*) using Tris–HCl buffer, and the total proteoglycans content was 6.69 mg/g. Ion exchange chromatography (DEAE Sepharose) was also used to isolate proteoglycans, and in three collected fractions, the proteoglycan content was 45.25%, 25.15%, 18.26% respectively. Je et al. [42]

investigated the sialic acid, sulfated-GAGs and uronic acid content in two parts (mixture of upper and middle section and base section) of antlers. Each section was extracted firstly with hot water at 100 °C for 1 h by autoclaving. After filtration, the residual was re-extracted at 110 °C for 1 h, and then the remaining residue was further extracted for the third time at 120 °C for 2 h. All extract solution was collected by filtering. Meanwhile, antlers were also subjected to extraction using 70% ethanol solution for 2 h. The results showed that the uronic acid content of upper section was higher than that in base section, and uronic acid could be effectively extracted at 100 °C. Long et al. [54] reported the isolation of acidic mucopolysaccharide from velvet antlers by hydrolysis. Velvet antlers of sika deer (*C. nippon*) were firstly extracted by ethanol, and the residual was dehydrated by acetone before dried in vacuum. Then the dried sample was hydrolyzed, and the hydrolysate was adjusted to pH 6.0 followed by filtration and precipitation at low temperature for 12 h. After centrifugation, the precipitate was redissolved in salt solution, dialyzed and lyophilized to produce polysaccharide.

Sunwoo et al. [55] extracted proteoglycans by 4M guanidine-HCl from the maturing chondrocyte zone of velvet antlers harvested from wapiti (*E. davidianus*). Proteoglycans were isolated by DEAE-Sephacel chromatography, and separated by Sepharose CL-4B chromatography into three fractions. Among them, fraction I contained a high molecular mass (>1000 kDa) chondroitin sulfate proteoglycan, capable of interacting with hyaluronic acid. Its amino acid composition resembled that was of cartilage proteoglycan, aggrecan. Fraction II contained proteoglycans with intermediate molecular weight, which were recognized by monoclonal antibodies specific to chondroitin sulfate and keratan sulfate. Fraction III contained a low molecular mass (<160 kDa) proteoglycan, decorin, with a glucuronate-rich glycosaminoglycan chain.

3.3. Extraction and isolation of lipid and polyamines

Jeon et al. [26] analyzed the contents of lipids in velvet antler and found that, for total lipid, neutral lipid, and phospholipid, their contents were higher in antlers cut on day 80 than in those cut on day 90. Furthermore, sphingo-phospholipid content was higher than glycerophospholipid content.

Zhou et al. [56] extracted bioactive components from velvet antlers of sika deer (*C. nippon*) by means of CO₂ supercritical fluid extraction (SFE) with absolute ethanol as co-solvent. Under the optimized conditions, the extract yield reached 3.58%. Meanwhile, the SFE extract showed strong activity on the inhibition of MAO-B in a dose-dependent manner with the inhibition efficiency reaching 93.77%, when the concentration of extract was 278.15 mg/l, much higher than that of water and ethanol extracts. Estradiol, uracil, hypoxanthine, p-hydroxybenzaldehyde and phospholipids were identified in the SFE extract, and they were all reported previously to have the inhibitory effect on MAO. Xu et al. [57] developed a method based on CO₂ supercritical fluid extraction to extract sex hormone from antler velvets of sika deer (*C. nippon*). The optimized experimental condition was 85% ethanol used as co-solvent, temperature at 65 °C and extraction pressure at 30 MPa. The extraction efficiency of estradiol and progesterone in velvet antlers were 3.07 and 776.18 ng/g respectively. Zhou et al. [58] reported that CO₂ SFE coupled with ultrasonic technology was suitable to extract active sex hormones and insulin-like growth factor-1 (IGF-1) from antler velvets of sika deer (*C. nippon*). The results showed that, in presence of 75% ethanol as the co-solvent, the content of estradiol and progesterone was 1224.1 pg/g and 354.1 ng/g respectively, much higher than that obtained by the refluxing extraction method. Meanwhile, under the optimized conditions, IGF-1 in the residue kept high activity while little IGF-1

activity was found in traditional residue. By the same technique, Zhou et al. [59] also identified three biological bases and five phospholipids in velvet antlers of sika deer (*C. nippon*), which were uracil, hypoxanthine, uridine, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, lysophosphatidylcholine and lysophosphatidylethanolamine.

In another study [25], slices of velvet antlers of sika deer (*C. nippon*) were extracted sequentially with hexane, chloroform, and 70% ethanol, and the 70% ethanol extract was fractionated further by silica gel column chromatography. The final fraction that showed the highest proliferation activity on spleen cells in synergy with ConA was eluted with chloroform:methanol:H₂O (70:10:1.5). The result of structural analysis showed that the active components were phosphatidylcholines, which contained 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.

4. Detection and quantification of bioactive components

4.1. Classical techniques

The crude protein, fat (ether extracts), crude fiber, total ash and mineral contents are considered quality indexes for velvet antlers. All these parameters can be determined by the methods of the Association of Official Analytical Chemists [60]. On the other hand, several classical techniques for the determination of chemical compounds in velvet antlers have been described. The 'Davis method' was used for measuring amino acid [61]. Velvet antler sample was mixed with 6N HCl and then the N₂ gas was purged into the samples solution. After hydrolyzed in a dry oven at 110 °C for 24 h, the hydrolyzed samples were evaporated and a sodium-distilled buffer of pH 2.2 was added. Samples were then filtered by a syringe filter (0.45 mm) and analyzed by an amino acid autoanalyzer. The content of collagen was calculated by multiplying 7.25 with the content of hydroxyproline, which was determined by the method of Bergman and Loxley [30]. For GAG, uronic acid and sialic acid analyses, velvet antler sample was decalcified in 0.05 M Na₂EDTA (pH = 7.4, including 0.5 M Tris) for 2 days at 4 °C and the decalcified sample was centrifuged to obtain a precipitate. Then the sample was digested by activated papain for 16 h at 65 °C, and after that the upper liquid layer was removed. GAG content of antler was determined by a microtitre plate adaptation of a dimethylmethylene blue assay by Farndale et al. [62]. Uronic acid content of antler was determined by the method of Kosakaki and Yosizawa [63]. Sialic acid content of antler was determined by the method of Warren [64], as described by Jeon et al. [65]. Because the proportion of sialic acid in ganglioside are relative determinate, researchers can deduce the content of ganglioside in velvet antler. Ganglioside is an important component in velvet antler and can be considered as the quality index of velvet antler.

4.2. Chromatography

Early in the 1960s, thin-layer chromatography (TLC) was used in the chemical analysis of components from velvet antlers. For example, Zhou et al. [59] identified five phospholipids in velvet antlers of sika deer (*C. nippon*).

GC techniques are not so generalized for determining the component from velvet antlers, due to the analytical difficulty, even after derivatization, besides the long time required of these reactions and the low limits of detection (LOD) [66]. Nevertheless, Lu et al. [39] reported a method for the simultaneous determination of 11 sex hormones in antler velvet health products by GC-MS/MS.

The most widely used methods, for the determination of chemical components in velvet antlers, are based on high-performance

liquid chromatography (HPLC). In common, various types of stationary phase could be used to separate special components. Lu et al. developed a quantitative method for sex hormones in velvet antler by RP-HPLC using ethylene bridged hybrid (BEH) C18 column and UV detection [38]. Under the optimal conditions, the proposed method provides the good linearities and determination limits (0.2–1.0 $\mu\text{g}/\text{kg}$) of the sex hormones investigated. In addition, SCX (Zorbax 300) column has been used to separate sialic acid in velvet antler using acetonitrile/water as mobile phase and 0.1% phosphoric acid added to get better separation [67]. Moreover, size exclusion chromatograph (Sephacrose CL-4B) was also used to separate the proteoglycans isolated from velvet antlers (*C. elaphus*) and then three fractions were obtained [55]. Fraction I contained a high molecular mass (>1000 kDa) chondroitin sulfate proteoglycan and its amino acid composition resembled that of aggrecan. Fraction II contained proteoglycans with intermediate molecular weight which were recognized by monoclonal antibodies specific to chondroitin sulfate and keratan sulfate. Fraction III contained a low molecular mass (<160 kDa) proteoglycan, decorin.

4.3. Electrophoresis

A method based on SDS-PAGE coupled with MALDI-TOF MS was developed to analyze the velvet antler polypeptide isolated and purified by ion exchange chromatography and gel filtration [46]. This peptide showed a single band in SDS-PAGE and its molecular weight measured by MALDI-TOF MS was 3263.4 Da, in accordance with its primary structure, a single linear chain of 32 amino acid residues, VLSATDKTNVLAAWGKVGGNAPAFGAEALERM. Zhou et al. compared the chemical composition of polypeptides (PPs) isolated with same procedure from velvet antlers of sika deer (*C. nippon*) and red deer (*C. elaphus*) using SDS-PAGE and MALDI-TOF mass spectrometry [68]. The result showed that the graphs of SDS-PAGE and spectrum of MALDI-TOF MS of these two PPs are significantly different. Moreover, capillary electrophoresis-based two-dimensional separation system was developed for separation of soluble extracts of velvet antlers [69]. In the system, an on-column, etched fused-silica porous junction interface was designed and fabricated, and an integrated two-dimensional separation platform involving on-line coupling of capillary isoelectric focusing (CIEF) with capillary electrophoresis (CE) was constructed by the interface. The results indicated that the total separation time was less than 1 h, and the 2D CE separation system was found to increase the resolving power and overall peak capacity over single dimension mode.

4.4. Other methods

Immunoblotting was widely used to detect the special protein presented in velvet antlers. Sunwoo et al. reported that using Western blotting, they detected chondroitin sulfate and keratan sulfate from extracts of velvet antlers [55]. Rucklidge et al. investigated the collagen isotypes presented in velvet antlers using SDS-PAGE and Western blotting [70]. Finally, collagen type I was found to be the major collagen and collagen type II, X and XI were also identified. In addition, radioimmunoassay technique was also used in analysis of the estradiol concentrations of velvet antlers of Pere David's deer (*Elaphurus davidianus*), sika deer (*C. nippon*) and fallow deer (*Dama dama*). The result showed that estradiol concentrations were significantly different in the velvet tissue of these three cervids ($P < 0.05$) [71].

Moreover, the structures of a newly isolated mixture of monoacyldiglycerides were determined by various spectroscopic methods, FAB MS, CID tandem MS, and 1D and 2D NMR. Finally, at least nine inseparable sn-3-monoacyldiglycerides was identified [72].

5. Pharmacological effects

2000 years ago, the medicinal document recorded the medical treatments and prescriptions for 52 different diseases using velvet antlers [7]. In recent decades, a number of studies have proved that velvet antlers have various pharmacological effects, such as modulatory effect on the immune system, blood system, bone metabolism, and glucose metabolism, together with anticancer, anti-fatigue, anti-inflammatory, analgesic, anti-bacterial, anti-viral, anti-stress, and anti-oxidant activities. An overview on the current status of modern pharmacological evaluations is summarized in Table 2.

5.1. Immunomodulatory

Improving immune system function has long been a historical application of velvet antlers. The injection of polypeptides of velvet antlers could affect the phagocytic ability of macrophages and regulate immune function. The up-regulation of monocytes and the enhancement of the phagocytic activity are considered to play key roles in the health benefits [73].

Zhao et al. [74] reported that a fraction separated from aqueous extract of velvet antler by gel-chromatography and RP-HPLC, mainly composed of small peptides, showed effective promotion activity on the proliferation of murine splenocyte in vitro. Suh et al. [73] found that the administration of the ethanolic fraction of velvet antlers of sika deer (*C. nippon*) (100 mg/kg, p.o.) could enhance lucigenin chemiluminescence and the engulfment of fluorescein-conjugated *E. coli* particles in murine peritoneal macrophages. Meanwhile, phagocytic activity was suppressed by the treatment of S-nitrosoglutathione (GSNO), an exogenous nitric oxide donor depending on the concentration of dose. The mechanism might be that the ethanolic fraction of velvet antlers suppressed the production of nitric oxide, and increased the concentration of Ca^{2+} . Kim et al. [75] prepared a fraction from the 70% ethanol extract of velvet antlers (*C. elaphus*), which showed mitogenic activity on the proliferation of spleen cells in combination with concanavalin A (1 $\mu\text{g}/\text{mL}$), compared with the control (PBS) at the dose of 100 $\mu\text{g}/\text{mL}$. According to the structural analysis, phosphatidylcholines were identified as the main bioactive substance. Furthermore, the relationship between the stimulant activity and the fatty acid residues of phosphatidylcholines was investigated extensively. The data collectively suggested that chain length and saturation of the fatty acids might play important roles in the promotion activity of splenocyte proliferation. However, another study [76] showed that the aqueous extract of velvet antlers (10–400 $\mu\text{g}/\text{mL}$) had inhibitory effects on concanavalin A (ConA)-stimulated splenocyte proliferation, while no difference was observed when lipopolysaccharide (LPS) was used as stimulus.

5.2. Anti-cancer

Velvet antlers have been considered as candidates for inhibiting cancer growth. For example, oral administration of 1 g/kg/day of velvet antlers for 26 weeks could effectively reduce the severity of colon cancer induced by azoxymethane [77]. In the velvet antler treated group, none of the colon tumors involved lymph nodes. Moreover, the higher proportion of colon tumors was of a low grade following velvet antler treatment, compared with the control treatment (0.96 vs. 0.84, respectively). When the analysis was expanded to include tumors in all locations, the analogous proportions were 0.91 for DV-treated rats, and 0.66 for control rats, and the difference was even more statistically significant ($P < 0.0001$). In a different study [78], purified protein fraction separated from crude proteins of velvet antlers (red deer and sika deer) could obviously prolong the survival time of mice inoculated

Table 2
Summary of the studies conducted on the pharmacological effects of deer antlers.

Property/condition	Formulation/extract/dosage	Model	Mode of action	Effect	Reference
Immune system	Ethanol extract of velvet antler (100 mg/kg/day, p.o.)	Mice (in vitro)	The phagocytic activity of murine peritoneal macrophages	Stimulatory	[73]
	Simulated gastrointestinal digest of aqueous extract of velvet antler (0.05–0.4 mg/ml)	Murine splenocytes	Proliferation of resting murine splenocytes Proliferation of ConA-stimulated murine splenocytes	Stimulatory Antagonism	[74]
	Phosphatidylcholines isolated from deer antler and 70% ethanol extract	Murine splenocytes	Proliferation of splenocytes	Stimulatory	[75]
	Aqueous extract of velvet antler (AVA, 10–400 µg/mL) Simulated gastrointestinal digests of AVA (200 µg/mL)	Murine splenocytes and macrophages	Proliferation of splenocytes, and neutral red pinocytosis activity of macrophages Proliferation of splenocytes	Antagonism Antagonism	[76]
Anti-cancer	Velvet antler (1 g/kg/day, p.o., for 26 weeks)	Mice (in vivo)	Metastasis and tumor grade	Antagonism	[77]
	Water soluble proteins of velvet antler (200–400 mg/kg/day, i.g., for 8 days)	Mice (in vivo)	Survival time of mice inoculated abdominally with S180	Stimulatory	[78]
	Water extract of velvet antler (400 mg/kg, i.g., for 10 days)	Mice (in vivo)	Efficiency of tumor inhibition	Stimulatory	[79]
Anti-fatigue	Water extract of velvet antler (80 mg/kg/day, i.g., for 30 days)	Mice (in vivo)	Burden swimming time, the levels of serum liver glycogen and muscle glycogen and activity of lactate dehydrogenase The levels of blood lactic acid and blood urea nitrogen	Stimulatory Antagonism	[80]
	Velvet antler polypeptides (30 mg/kg/day, i.g., for 30 days)	Mice (in vivo)	Climbing time and Burden swimming time The level of serum liver glycogen	Stimulatory Antagonism	[81]
Anti-osteoporosis	Total velvet antler polypeptides (60 mg/kg/d, S.C., for 29 days)	Rats (in vivo)	Bone mineral density, bone weight coefficient and bone formation parameters	Stimulatory	[83]
	Velvet antler powder (p.o. for 13 month)	Mice (in vivo)	The levels of plasma phosphorus and calcitonin, femoral bone density and calcium content Serum level of plasma parathyroid hormone	Antagonism Stimulatory	[82]
	Chloroform extract of velvet antler (10 µg/ml)	Bone marrow macrophages	Osteoclast differentiation and bone-resorption	Antagonism	[84]
	Velvet antler polypeptides (25 µg/ml)	Osteoarthritic rabbit chondrocytes	The expression of glycosaminoglycan and collagen type II Matrix metalloproteinases secretion	Stimulatory Antagonism	[85]
	Velvet antler polypeptides (25 µg/ml)	Osteoarthritic rabbit chondrocytes	Content of reactive oxygen species in chondrocytes and NO content in cell culture supernatant Activities of superoxide dismutase and glutathione peroxidase -Px in cell culture supernatant	Antagonism Stimulatory	[86]
Anti-inflammatory	Velvet antler powder (10 mg/day, p.o., for 28 days)	Mice (in vivo)	Airway inflammation and the development of airway hyperresponsiveness	Antagonism	[87]
	Water extract of deer antler (100 µg/kg/day, aqua-acupuncture, for 18 or 23 days)	Rats (in vivo)	Arthritis score, body weight loss, bone destruction score	Antagonism	[88]
	Water extract of deer antler (10 mg/day, p.o., for 23 days)	Mice (in vivo)	Incidence of arthritis and arthritis index, the level of inflammatory mediators in arthritic joints, and the levels of plasma total IgG and type II collagen-specific IgG	Antagonism	[89]

Table 2 (Continued)

Property/condition	Formulation/extract/dosage	Model	Mode of action	Effect	Reference
Anti-oxidation	Velvet antler polypeptide (30 mg/kg/d, i.v. in tail, for 2 day)	Rats (in vivo)	Serum and myocardial superoxide dismutase activity, the serum levels of creatine kinase, lactate dehydrogenase and aspartate transaminase	Antagonism	[90]
	Supercritical extract of antler velvet	Enzymatic reaction	The radical-scavenging activities of lipid peroxidation system, phenanthroline-Fe (II) system, and 2-deoxy-D-ribose-Fe (III) system	Stimulatory	[92]
Wound healing	Velvet antler extract ointment (embrocation every four days for 60 days)	Rats (in vivo)	The expression levels of IGF-I, TGF-beta and EGF in wound skin	Stimulatory	[93]
	3.2 kDa polypeptide from velvet antler (10–40 µg/ml)	Rat epidermal cells	Proliferation of cells	Stimulatory	[32,48]
	Slow release-membrane containing 15 mg/g velvet antler peptides (for 4 weeks)	Leghom chickens (in vivo)	The level of adhesion of tendon Tensile strength of restoring tendon	Antagonism Stimulatory	[94]
Regeneration promoting	Slow release-membrane containing 15 mg/g velvet antler peptides (for 6 weeks), velvet antler peptides (10 µg/kg/every other day, i.m., for 6 weeks)	Rats (in vivo)	The expression of myelin sheath TGF-β1 and IGF, the number of myelinated nerve fiber The level of nervous adherence	Stimulatory Antagonism	[95]
Anti-heart failure	Velvet antler polypeptide (30 mg/kg/d, i.v. in tail, for 2 day)	Rats (in vivo)	Ischemic size, ST elevation	Antagonism	[90]
	Velvet antler powder (200 mg/kg/d, i.g., for 4 weeks)	Rats (in vivo)	Cardiac functional parameters Serum brain natriuretic peptide level	Stimulatory Antagonism	[96]
	Velvet antler peptides (50 mg/kg/d, s.c.or., for 5 days)	Mice (in vivo)	The contents of malondialdehyde in liver and the contents of aspartate aminotransferase, glutamic-oxal(o)acetic transaminase in the serum The content of glutathione and superoxide dismutase activity in the liver	Antagonism Stimulatory	[97]
	Velvet antler peptides (120 mg/kg, i.v. from tail, two times)	Rats (in vivo)	The size of myocardial ischemia, the levels of lactate dehydrogenase, glutamic-oxal(o)acetic transaminase, malondialdehyde in serum and myocardial tissues The superoxide dismutase activity in serum and myocardial tissue	Antagonism Stimulatory	[98]
Anti-aging	70% ethanol extract of velvet antler (2–4 g/kg/d, i.g., for 30 days)	Mice (in vivo)	The serum superoxide dismutase activity Serum content of malondialdehyde	Stimulatory Antagonism	[99]
	70% ethanol extract of velvet antler (2–4 g/kg/d, i.g., for 14 days)	Mice (in vivo)	Latency of senile response and frequency of the continual three right responses The monoamine oxidase-B activity in brain Correct response rate	Antagonism Stimulatory	[100]
Development promoting	Standard chow containing 10% Elk velvet antler (p.o., for 90 days)	Rats (in vivo)	Duration of supported and unsupported standing of rat offspring, acquisition of neurological reflex parameters	Stimulatory	[101]

abdominally with S180 cell. The mean living time of model mice orally administrated with purified protein fraction (200 $\mu\text{g}/\text{kg}/\text{day}$) from velvet antlers (red deer and sika deer) was 18 d and 20 d respectively, while that of the control group was 16 d. Xiong et al. [79] reported that the intragastric administration of 400 mg/kg/day of aqueous extract of velvet antlers to mice, following injection with sarcoma 180 cell suspensions at the right armpit of mice, significantly reduced tumor weight compared with the mice injected with cell suspensions only. Moreover, proteoglycan separated from antler growing tip possessed obvious immuno-regulation effect in a dose dependent manner.

5.3. Anti-fatigue

Many studies suggest that velvet antler extracts have anti-fatigue effects. The intragastric administration of water extract of velvet antlers [80], at a dose of 80 mg/kg/day for 30 days, markedly prolonged the burden swimming time (from 5.8 ± 2.3 to 8.6 ± 2.5 min), increased the content of liver glycogen, muscle glycogen and the effect of lactate dehydrogenase, and decreased the content of blood lactic acid and serum urea nitrogen. These results suggested that the anti-fatigue effect of water extract of velvet antler might be related to the high efficiency of elimination of blood lactic acid and serum urea nitrogen. Luo et al. [81] treated the mice with 30 mg/kg/day of velvet antler polypeptide (VAP) for 30 days. After that, the index, such as anti-anoxia survival time, pant time after decapitation, continuously climbing time and burden swimming time were analyzed to evaluate the status of the mice. The results showed that the oral administration of velvet antler polypeptide (VAP) could significantly increase the index mentioned above, and decrease the lactate in serum compared with the control group, indicating VAP could improve the ability of anti-anoxia and anti-fatigue *in vivo*.

Based on the above results, it can be seen that proteins and polypeptides of velvet antlers are the major anti-fatigue substances, but further investigations are needed to elucidate the mechanisms.

5.4. Anti-osteoporosis

Chen et al. [82] reported that long-term velvet antler administration (13 months) showed positive effects on bone status of 2 month-old senescence-accelerated mice with ovarian function deficiency. The results also revealed that velvet antler treatment could moderately decreased plasma phosphorus and calcitonin levels, and femoral bone density and calcium content, and increased plasma parathyroid hormone (PTH) and alkaline phosphates (ALP) activity levels associated with an ovariectomy. Duan et al. [83] investigated the effect of total velvet antler polypeptides (TVAP) on the osteoporotic model rats induced by retinoic acid. Their study showed the content of Ca in bone, bone mineral density and bone weight coefficient were significantly increased in TVAP group rats, compared with the control. Meanwhile, the mean trabecular plate thickness, the mean trabecular plate interspace and the trabecular bone volume were improved obviously, compared with that of the osteoporotic model rats. Therefore, TVAP has a preventive and therapeutic effect on osteoporosis due to its remedial activity against negative-balance of bone remodeling induced by retinoic acid.

Li et al. [84] investigated the effects of chloroform extract of velvet antlers on osteoclast differentiation and bone-resorption *in vitro*. This chloroform extract inhibited osteoclast differentiation in mouse bone marrow cultures stimulated by receptor activator of NF- κ B ligand (RANKL) and macrophage-colony stimulating factor (M-CSF). It also inhibited the bone resorptive activity of differentiated osteoclasts, accompanied by the disruption of actin rings and the induction of the apoptosis. The effect of chloroform

extract on the inhibition of the phosphorylation of Akt, ERK and I- κ B in response to receptor activator of NF- κ B ligand might be the mechanism of the suppression of osteoclast differentiation and bone-resorption. The effects of velvet antler polypeptides (VAPs) on osteoarthritic chondrocytes (OCs) isolated from osteoarthritic rabbit model were investigated by Zhang et al. [85]. They found that VAPs inhibited the expression of collagen I, collagen X, MMP-1 and MMP-13 mRNA, and increased the glycosaminoglycan (GAG) and collagen type II expression levels in the extracellular matrix. Meanwhile, the proportion of early apoptotic cells was significantly reduced by VAPs in a dose-dependent manner. It is mainly because that the treatment of VAPs could inhibit matrix metalloproteinases (MMPs) secretion, keep the balance of cartilage matrix metabolism, and sustain an external environment where cartilage cells could survive. Li et al. [86] reported that the VAPS had anti-oxidative damage effect on osteoarthritis cartilage cells isolated from 5 month old rabbit subjected to Hulth osteoarthritis in a dose-dependent manner. For VAPs treated group, the content of NaNO_2 and GSH-Px decreased significantly compared with control group. Meanwhile, the content of reactive oxygen species (ROS) in VAPs treated group was much lower than that of control group.

5.5. Anti-inflammatory

The water extract of velvet antlers also showed anti-allergic effect on ovalbumin-sensitized mouse model after administrated for 4 weeks [87]. In this study, all the features of the asthmatic phenotype, including airway inflammation and the development of airway hyperresponsiveness, were reduced by treatment with velvet antler extracts. The experimental group showed the strong inhibition of Th2 cytokine and proinflammatory cytokine production in bronchoalveolar fluid, compared to control mice, and the regulatory T-cell population of splenocytes in the allergic asthma mice increased after the oral administration of VA. In a different study [88], the water extract from velvet antlers was also identified as the inhibitor of dihydroorotate dehydrogenase (DHO-DHase). Administration of water extract by acupuncture five times per week (300 mg/kg/day) could completely prevent the development of collagen-induced arthritis based on the reduction of the arthritis score, and the 50% effective dose (ED50) of water extract on arthritis score was 64 mg/kg. The reason is that the water extract could effectively inhibit DHO-DHase activity in a concentration dependent manner, which might be mixed with competitive and noncompetitive inhibition. Suh et al. [89] determined the effect of injection with water extract of velvet antlers on collagen-induced arthritis in mice. In three independent experiments, mice treated with extract exhibited significantly reduced incidence of arthritis (30–45%), as compared with mice not given DAA in water (86–98%). The arthritis index was also significantly low in extract-treated animals. Meanwhile, a marked reduction in the expression of inflammatory mediators, such as cyclooxygenase 2, IFN- γ , and tumor necrosis factor alpha, was found in arthritic joints of extract-treated mice. Additionally, total IgG and type II collagen-specific IgG levels were lower in serum and arthritic joints of extract-treated mice.

5.6. Anti-oxidation

Chen et al. [90] investigated the cardioprotective effects of peptides, VAP, extracted from velvet antlers on acute ischemic myocardial injury induced by isoproterenol in rats. It was found that VAP could significantly decrease ST elevation, infarct size, serum levels of creatine kinase, lactate dehydrogenase, aspartate transaminase, superoxide dismutase and myocardial malondialdehyde, and increase serum and myocardial SOD activity in the rats of acute ischemic myocardial injury induced by isoproterenol. These results indicated that VAP exerted significant cardioprotective

effects against acute ischemic myocardial injury induced by isoproterenol in rats, likely through its antioxidant and anti-lipid peroxidation properties.

Recently, many industrial commercial enzymes, such as alpha-chymotrypsin, pepsin, papain and trypsin were used to produce a number of effective extracts. In addition, the enzymatic extracts from velvet antlers have shown antioxidative activity. Therefore, they have the potential to be developed into new health foods. Zhao et al. [91] prepared crude protein hydrolysates from aqueous extract of velvet antlers (AEVA) by simulating gastrointestinal digestion and separating the hydrolysates into four fractions according to molecular weight. Then, the antioxidant activities of peptide fractions were evaluated. Generally, low-MW peptide fractions had higher 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity and Fe (II)-chelating ability, and high-MW peptide fractions were more effective in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and reducing power. In another study, the antioxidant activity of supercritical extract of antler velvets in lipid peroxidation system and its hydroxyl radical-scavenging activities in phenanthroline-Fe (II) system and 2-deoxy-D-ribose-Fe (III) system were analyzed by Zhou et al. [92]. They found that all velvet antler extracts had higher activities in the above three antioxidant activity assays compared with vitamin C solution as control.

5.7. Wound healing and regeneration promoting

Weng et al. [32] reported a novel 3.2 kDa polypeptide from VA of red deer (*C. elaphus Linnaeus*), which could stimulate the growth of rat epidermal cells in a dose-dependent manner. Another homologous polypeptide from sika deer (*C. nippon Temminck*) also had a similar activity on rat epidermal cells proliferation [48]. Gu et al. [93] also reported that the positive effects of velvet antler extract on skin wound healing of rats. In this experiment, wound healing was significantly more rapid in velvet antler extract treated skins. The wound treated with a high concentration antler ointment, a low concentration antler ointment, and the control closed completely at post-injury day 40, day 44 and day 60, respectively. It could be partially explained by the founding that the expressions of IGF-I (day 8 and day 16), TGF-beta (day 8, day 16 and day 20) and EGF (day 4, day 8, day 16, and day 32) were obviously up-regulated in high concentration antler-treated skins compared to control skins.

Xu et al. [94] investigated the effect of poly (lactide-co-glycolid) (PLGA) fibers containing Velvet Antler Peptide (VAP) on tendon repair and reconstruction of Leghorn chickens. Their results indicated that VAP/PLGA fibers could prevent tendon adhesion and promote tissue repair and reconstruction. Lu et al. [95] investigated the effect of VAP on sciatic nerve regeneration in rats through local administration and VAP-PLGA compound membrane for 6 weeks. All rats survived to the end of the experiment, without foot ulcer or neuroma. Compared with the control group, VAP can significantly reduce nervous adherence. For the regenerative fiber axon and the expression of myelin sheath TGF-beta 1 and IGF antigen, the staining intensity in VAP-treated group was higher than that in control group. The HRP retrograde tracing also showed that applying VAP through either local administration or VAP-PLGA compound membrane around the attached site of nerve anastomosis was capable of promoting nerve regeneration.

5.8. Others

Shao et al. [96] investigated therapeutic effects of VAD on cardiac functions in rats with heart failure following myocardial infarction. One week after the surgery, rats with heart failure received daily treatment of VAD by gavage for consecutively four weeks. It was found that VAD partially reversed changes in cardiac functional

parameters and serum BNP levels in rats with heart failure. These results provided further evidence for the heart-kidney-related theory, and suggested that VAD might be a potentially alternative and complementary medicine for the treatment of heart failure. The protective effects of VAP on acute hepatic injury induced by carbon tetrachloride (CCl₄) in mice were studied by Li et al. [97]. VAP was subcutaneously administrated into mice at a dose of 50 mg/kg/day for 5 days followed by intraperitoneally injection with 0.2% CCl₄ solution to induce acute hepatic injury. The results showed that, compared with the model group, the contents of ALT, AST in the serum of VAP treated mice was obviously decreased, the content of MDA in the liver was also lowered, and the activity of GSH and SOD in the liver was markedly improved after preventive treatment with VAP. Chen et al. [98] investigated the cardioprotective effects of VAP on acute ischemic myocardial injury in rats and its underlying mechanisms. The VAP was injected into the tail vein of each of the experimental rats 12 h and 23 h respectively after the establishment of myocardial ischemia injury mode. Meanwhile, compared with the MI control rats, VAP also significantly decreased levels of CK, LDH, AST, and MDA in serum and myocardial tissue, increased SOD activity in serum and myocardial tissue in MI rats.

Qin et al. [99] reported the long term (30 days) administration of 70% ethanol extract of velvet antler antlers (*E. davidianus*) on subacute aging model mice induced by D-galactose showed anti-aging effect. In different study, Yang et al. [100] found that 70% ethanol extract of velvet antler antlers (*E. davidianus*) could improve the behavior and immune function of aging mice to delay aging process. Chen et al. [101] reported that long term maternal velvet antler (*E. davidianus*) supplementation prior to and during pregnancy and lactation accelerated certain physical, reflexologic, and neuromotor developmental milestones, and caused no discernible adverse effects on developing off spring.

6. Conclusions

Velvet antlers have been widely used in East Asia for many centuries, which could nourish Yin, tonify the kidney, strengthen bones and muscles, and promote blood flow. In recent decades, pharmacological studies have validated the traditional uses of velvet antlers both in vitro and in vivo. Pharmacological studies demonstrate that velvet antlers possess multi-functions, such as immunomodulatory, anti-cancer, anti-fatigue, anti-osteoporosis, anti-inflammatory, anti-oxidant, wound healing and regeneration promoting activities. However, studies on the extraction and isolation of bioactive components of velvet antlers, as well as the physiological mechanisms associated with therapeutic effects should be further paid much more attention to make velvet antler products acceptable as curative medicines.

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