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Preventive and treatment effects of a hemp seed (*Cannabis sativa* L.) meal protein hydrolysate against high blood pressure in spontaneously hypertensive rats

Abstract

Purpose

This work determined the ability of hemp seed meal protein hydrolysate (HMH)-containing diets to attenuate elevated blood pressure (hypertension) development in spontaneously hypertensive rats (SHRs). Effects of diets on plasma levels of renin and angiotensin I-converting enzyme (ACE) in the SHRs were also determined.

Methods

Defatted hemp seed protein meal was hydrolyzed using simulated gastrointestinal tract digestion with pepsin followed by pancreatin, and the resulting HMH used as a source of antihypertensive peptides. The HMH was substituted for casein at 0.5 and 1.0 % levels and fed to young growing rats for 8 weeks (preventive phase) or adult rats for 4 weeks (treatment phase).

Results

Feeding of young growing SHRs with HMH resulted in attenuation of the normal increases in systolic blood pressure (SBP) with an average value of ~120 mmHg when compared to the casein-only group of rats (control) with a maximum of 158 mm Hg ($p < 0.05$). Feeding adult rats (SBP ~145 mmHg) with same diets during a 4-week period led to significant ($p < 0.05$) reduction in SBP to ~119 mmHg in comparison with 150 mmHg for the control rats. Plasma ACE activity was significantly ($p < 0.05$) suppressed (0.047–0.059 U/mL) in HMH-fed rats when compared to control rats (0.123 U/mL). Plasma renin level was also decreased for HMH-fed rats (0.040–0.054 μg/mL) when compared to control rats that were fed only with casein (0.151 μg/mL).

Conclusions

The results suggest that HMH with strong hypotensive effects in SHRs could be used as a therapeutic agent for both the prevention and treatment of hypertension.

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ORIGINAL CONTRIBUTION

Preventive and treatment effects of a hemp seed (*Cannabis sativa* L.) meal protein hydrolysate against high blood pressure in spontaneously hypertensive rats

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Abstract
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Methods Defatted hemp seed protein meal was hydrolyzed using simulated gastrointestinal tract digestion with pepsin followed by pancreatin, and the resulting HMH used as a source of antihypertensive peptides. The HMH was substituted for casein at 0.5 and 1.0 % levels and fed to young growing rats for 8 weeks (preventive phase) or adult rats for 4 weeks (treatment phase).
Results Feeding of young growing SHRs with HMH resulted in attenuation of the normal increases in systolic blood pressure (SBP) with an average value of ~120 mmHg when compared to the casein-only group of rats (control) with a maximum of 158 mm Hg ($p < 0.05$). Feeding adult rats (SBP ~145 mmHg) with same diets during a 4-week period led to significant ($p < 0.05$) reduction in SBP to ~119 mmHg in comparison with 150 mmHg for the control rats. Plasma ACE activity was significantly ($p < 0.05$) suppressed (0.047–0.059 U/mL) in HMH-fed rats when compared to control rats (0.123 U/mL). Plasma renin level was also decreased for HMH-fed rats (0.040–0.054 μg/mL) when compared to control rats that were fed only with casein (0.151 μg/mL).
Conclusions The results suggest that HMH with strong hypotensive effects in SHRs could be used as a therapeutic agent for both the prevention and treatment of hypertension.

Keywords Hemp seed meal · Protein hydrolysate · Spontaneously hypertensive rats · Systolic blood pressure · Plasma ACE activity · Plasma renin activity

Introduction

Hypertension or elevated blood pressure (BP), defined as systolic blood pressure (SBP) >140 mmHg or diastolic blood pressure (DBP) >90 mmHg, forms an important risk factor for the development of cardiovascular diseases [1, 2]. Hypertension is a major public health problem, and its global prevalence is increasing at an alarming rate affecting over 20 % of the adult population [3]. Worldwide prevalence of hypertension is estimated to affect as much as one billion individuals with approximately 7.1 million associated deaths per year [4]. BP is regulated by several mechanisms, but the most significant and widely studied is the renin–angiotensin–aldosterone system (RAAS). In the RAAS, kidney-secreted renin cleaves angiotensinogen to produce an inactive decapeptide called angiotensin 1 (AT-I). AT-I is then hydrolyzed by angiotensin I-converting enzyme (ACE) to produce a potent vasoconstrictor octapeptide called angiotensin II (AT-II). ACE also breaks

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down bradykinin (a vasodilator) to produce inactive fragments leading to increases in arterial BP [5]. Independent of ACE, chymase is an enzyme that also converts AT-I–AT-II, and these combined enzyme actions ultimately are responsible for regulating BP. Excessive activities of these enzymes could lead to BP elevation that leads to hypertension if left untreated and may progress into cardiovascular complications that sometimes result in death. Bradykinin achieves its vasodilation properties by binding to the β-receptor with an eventual increase in Ca^{2+} levels. The binding of bradykinin to β-receptors and the increase in Ca^{2+} level stimulate nitric oxide synthase (NOS) to convert L-arginine to nitric oxide (NO), another potent vasodilator. Therefore, the hydrolytic action of ACE on bradykinin to produce inactive fragments indirectly inhibits the production of NO.

Elevated BP is routinely treated using a combined therapy of antihypertensive drugs, such as captopril (C), lisinopril, enalapril, etc. [6]. However, these synthetic drugs are believed to have certain side effects, such as cough, taste disturbances, skin rashes, or angioneurotic edema, which limit their use in some patients such as pregnant women and the elderly who are easily susceptible to health complications. While there are many commercially available synthetic ACE inhibitors, only one known commercial renin inhibitor (Aliskiren) is available for human therapy [7]. Renin, an aspartyl protease, has been found to produce highly selective inhibition in RAAS by catalyzing the first and rate-limiting step that converts angiotensinogen to angiotensin I. This selective behavior makes renin a very difficult enzyme to inhibit due to its high substrate specificity [8]. Therefore, it has been suggested that research and development to find safer, innovative, and economical ACE and renin inhibitors from food-based sources are necessary for expanding hypertension treatment and prevention strategies [6]. Research has shown that some food proteins possess the ability to release both ACE and renin inhibitory peptides after enzymatic hydrolysis, exhibiting multifunctional properties [9]. Such peptides may serve as ingredients for functional foods or nutraceuticals and could be used as alternative or complementary treatment tools for reducing high BP. Therefore, bioactive antihypertensive peptides of food origin are increasingly gaining recognition as alternatives or compliments to synthetic drugs in hypertension therapy. Preliminary in vitro studies have shown that industrial hemp proteins (principally identified as edestin and albumin) and high digestibility promotes their efficacy as a source of health-enhancing bioactive peptides [14]. Hemp seed proteins are currently sold for food product formulation in Canada mostly in the form of protein concentrates or hemp seed protein powders. Short-term (24 h) oral administration (200 mg/kg body weight) of hemp seed protein hydrolysate (HPH) to spontaneously hypertensive rats (SHRs) was shown to reduce SBP (−30 mmHg after 8 h) and was positively correlated with the in vitro ACE and renin inhibitions [13]. Having previously established the ability of a hemp seed hydrolysate to reduce SBP on a short-term basis, the primary objective of this study was to evaluate the ability of a hemp seed hydrolysate to attenuate hypertension (prevention) in growing SHRs during an 8-week feeding experiment. We also determined the BP-lowering effect (treatment) of the hydrolysate in SHRs with established hypertension during a 4-week secondary study while normotensive rats were used for comparison.

Materials and methods

Materials

Defatted coarse hemp seed protein meal (HPM, 25 % protein content) was a gift from Hemp Oil Canada (Ste. Agathe, Manitoba, Canada). Briefly, the hemp seed is mechanically pressed to extract oil, and the resulting product is the defatted hemp seed cake, which is then milled in a classifier milling system to the desired particle size. The milled powder is sifted using various screens to obtain products sold as high-value protein powders. The by-product that does not pass through the sieves is the HPM, which is normally considered a waste product. Renin enzyme and renin substrate were purchased from Cayman (Cayman Chemical, Ann Arbor, MI). Pepsin (from porcine gastric mucosa, EC 3.4.23.1 with activity ≥250 U/mg solid), pancreatin (from porcine pancreas; digests not less than 25 times its weight of casein in 60 min at pH 7.5 and 40 °C), *N*-(3-[2-furyl]acryloyl)-phenylalanylglycylglycine (FAPGG), captopril, and rabbit lung ACE (E.C.3.4.15.1) were purchased from Sigma-Aldrich (St. Louis, MO).

Preparation of hemp seed protein isolate (HPI) and hemp seed meal hydrolysate (HMH)

seed peptides possess both antioxidant [10–12] and antihypertensive properties [13]. The antioxidant and antihypertensive activities may be due to the presence of high levels of negatively charged amino acids for electron donation to reactive oxygen species and arginine for the production of NO, a vasodilating agent, respectively. The presence of superior amino acid profile in hemp seed

HPI was prepared according to a previously described protocol [10]. Briefly, the HPM was extracted for 2 h at 37 °C with alkaline water (pH 10) followed by centrifugation (7,000*g* for 1 h at 4 °C). The supernatant was adjusted to pH 5.0 with 2 M HCl, centrifuged, and the precipitate was neutralized to pH 7.0 with 2 M NaOH

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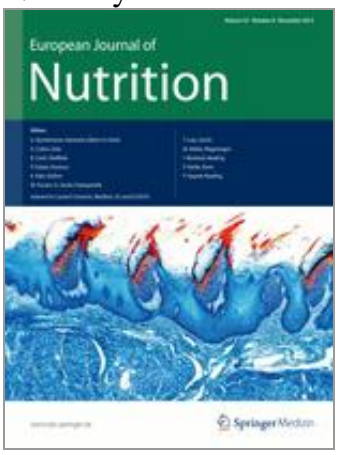

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