The Americas Journal of Plant Science and Biotechnology ©2011 Global Science Books

Berberine: Biochemical Analysis and Cardiovascular Benefits

Yaw L. Siow[1,2,3*] • Jay Petkau[1,2] • Lindsei Sarna[2,4] • Jennifer Enns[2,3] • Karmin O[2,3,4]

[1] Agriculture and Agri-Food Canada, 351 Taché Avenue, Winnipeg, MB R2H 2A6, Canada
[2] Canadian Centre for Agri-Food Research in Health and Medicine, St Boniface Hospital Research Centre, 351 Taché Avenue, Winnipeg, MB R2H 2A6, Canada
[3] Department of Physiology, Faculty of Medicine, University of Manitoba, Canada
[4] Department of Animal Science, Faculty of Agricultural and Food Sciences, University of Manitoba, Canada

Corresponding author: * **YawChris.Siow@agr.gc.ca**

ABSTRACT

Berberine is an alkaloid found in plants such as goldenseal (*Hydrastis Canadensis* L.), European barberry or jaundice berry (*Berberis vulgaris* L.), and Chinese goldthread or *Huanglian* (*Coptis chinensis* Franch.). Goldenseal can be grown in Canada and natural health products derived from this plant have been found on consumer shelves. In this review, a UPLC-based analysis of berberine in a goldenseal product is presented. The linear range for berberine is from 10 to 500 μg/mL with a coefficient of determination (R^2) of 0.9996. Two major cardiovascular benefits of berberine are discussed: lipid lowering and antioxidant effects. Berberine has been shown to lower blood cholesterol through the stabilization of LDL receptor mRNA and to lower triglycerides through activation of AMPK, which in turn, targets two other enzymes involved in lipid biosynthesis, ACC and HMG CoA reductase. For its antioxidant effects, berberine has been shown to directly scavenge ROS/RNS species, to upregulate endogenous antioxidant defense mechanisms and to inhibit endogenous ROS/RNS-generating oxidases such as NADPH oxidase. Berberine has potential to be used as a complementary therapy for hyperlipidemia and for prevention and management of cardiovascular disease.

Keywords: AMPK, anti-oxidant, lipid-lowering, NADPH oxidase, UPLC analysis
Abbreviations: **ACC**, acetyl-CoA carboxylase; **AMPK**, AMP-activated protein kinase; **HMG-CoA**, 3-hydroxy-3-methylglutaryl coenzyme A; **LDL**, low density lipoproteins; **LDLR**, LDL receptor; **LPS**, lipopolysaccharide; **MAPK/ERK**, mitogen-activated protein kinase/extracellular-signal regulated kinase; **NADPH**, nicotinamide adenine dinucleotide phosphate; **NO**, nitric oxide; $\mathbf{O_2^-}$, superoxide anion; $\mathbf{OONO^-}$, peroxynitrite; **RNS**, reactive nitrogen species; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase; **SREBP**, sterol regulatory element binding proteins; **UPLC**, ultra performance liquid chromatography

CONTENTS

INTRODUCTION

Berberine is an isoquinolone alkaloid (**Fig. 1**) which is found in the fruit, root, rhizomes, stems and bark of plants such as goldenseal (*Hydrastis Canadensis* L.) (USDA ARS), European barberry or jaundice berry (*Berberis vulgaris* L.) (USDA ARS), and Chinese goldthread or *Huanglian* (*Coptis chinensis* Franch.) (USDA ARS). Previously in Canada, the native goldenseal was a common species found in the deciduous forest of southern and southwestern Ontario (Royal Ontario Museum) but it is now a threatened plant species (Ontario Gov 2009). Currently, goldenseal is listed under the Endangered Species Act of Ontario and there are restrictions in the production of this plant (Filotas *et al.* 2009). For a more detailed discussion on the cultivation of goldenseal, please refer to the reviews by Sinclair and Catling (Sinclair and Catling 2001; Sinclair *et al.* 2005).

Extracts and decoctions from the above plants have been used in Chinese and Ayurvedic medicine for many years as treatment for gastrointestinal disorders, liver dysfunction, gallbladder disease and urinary tract infections (Gruenwald 1998; Jellin *et al.* 2000). Recently, products

Received: 30 January, 2010. Accepted: 10 December, 2010.

Invited Mini-Review

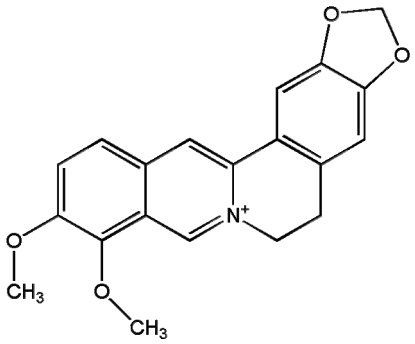

Fig. 1 Chemical structure of berberine.

based on goldenseal have appeared in the natural health products or supplements section of many retailers and grocers. As the list of potential therapeutic benefits of berberine grows, there is an increased need to provide valid science-based information to consumers so they are able to make informed choices in their personal healthcare regimen. In this review, the analysis of berberine using a more recent technology in chromatography is discussed. It will also review the lipid-lowering and antioxidant effects of berberine.

ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC) ANALYSIS

There are many reports on the chemical analysis of berberine, with most of them utilizing liquid chromatography combined with mass spectrometry (Weber *et al.* 2003). Brown and colleagues have just described a validation study of a rapid extraction and the quantitative determination of hydrastine and berberine using high pressure liquid chromatographic techniques in a variety of goldenseal products (Brown and Roman 2008; Brown *et al.* 2008). Recently, a new liquid chromatography technology – ultra-performance liquid chromatography (UPLC) has been introduced to the analytical and separation science community and it provides improved resolution, speed and sensitivity (Swartz 2005) when compared to traditional high performance liquid chromatography. This chromatography technique is used here to analyze an extract prepared from goldenseal.

Sample extraction

Extraction was performed on goldenseal capsules purchased at a local grocery store and the procedure was similar to that described previously (Brown *et al.* 2008) with some modifications. Briefly, for 100 mg of sample, 8 mL of extraction solvent was used. The solvent was comprised of 30% acetonitrile, 1% acetic acid, and 69% water. The sample was quickly mixed by vortex prior to sonication for 15 min then centrifuged for 10 min at 2500 × *g*. The supernatant was filtered with a Pall Acrodisc 0.8/0.2 µm syringe filter and stored at 4°C until analyzed by UPLC.

UPLC chromatography conditions

Samples and standards were analyzed using a Waters Acquity® UPLC system consisting of the photo-diode array detector, binary solvent manager, sample organizer and Empower2 software. Detection was at 280 nm after separation on a Waters UPLC BEH C18 1.7 µm 2.1 × 100 mm column set at 28°C. Samples were diluted in extraction solvent 1:1 and filtered using a Whatman Mini-UniPrep syringeless filter prior to 1 µL injection. The gradient started at 90% mobile phase A (25 mM ammonium formate in water with 0.1% formic acid) and 10% B (acetonitrile) and increased to 50% mobile phase B in 4 min. The gradient was run at 0.4 mL/min and the total gradient run time was 7 min. **Fig. 2** shows a typical UPLC chromatographic profile generated from an analysis of the goldenseal sample in comparison to standard compounds obtained from Sigma-Aldrich.

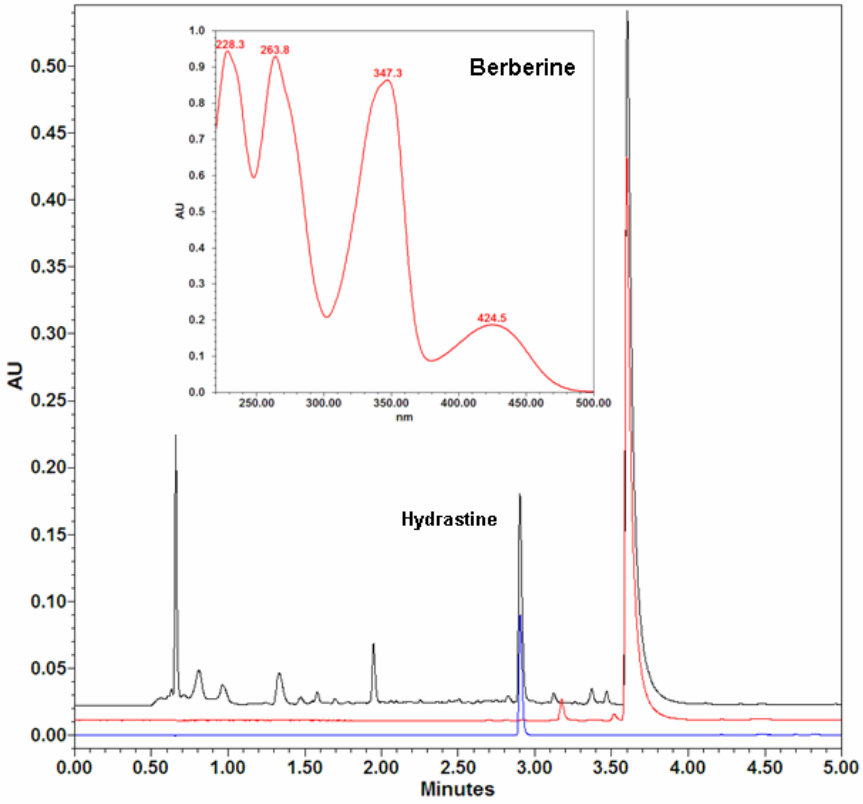

Fig. 2 UPLC Separation Profile of Goldenseal Extract on an UPLC BEH C18 column at 280 nm. Identification of marker compounds, berberine (red line) and hydrastine (blue line) was based on profile of injected standards. Inset shows spectrum of berberine standard using Waters Acquity PDA Detector (220-500 nm).

Berberine determination

Standard solution was prepared as described previously (Brown *et al.* 2008). To calculate berberine content in the samples, an eight-point standard curve ranging from 10 to 500 μg/mL was generated. Over this range, quantitation of berberine was linear (correlation coefficient, R^2=0.9996). The amount of berberine in the sample was determined to be an average of 3.68 (%w/w) with a standard deviation of ±0.01%.

LIPID-LOWERING EFFECTS

Berberine has recently begun to gain attention for its lipid-lowering properties, particularly in regards to its effect on cholesterol and triglycerides metabolism. Its potential as a novel therapy for hyperlipidemia is under investigation. The current most commonly prescribed and highly effective lipid-lowering drugs are the statins, which act by competetively inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis. The primary goal of statins is to lower plasma cholesterol, which is mainly carried by low-density lipoproteins (LDL) in the circulation. Cholesterol metabolism genes are under the control of a family of transcription factors, the sterol regulatory element binding proteins (SREBP), which are anchored in the endoplasmic reticulum in their inactive form. Under conditions of low intracellular cholesterol, SREBP are cleaved and the cytosolic N-termini enter the nucleus, where they bind target gene promoters and stimulate gene expression. HMG-CoA reductase and LDL receptor (LDLR) are among the genes up-regulated by SREBP (Horton *et al.* 2002). The inhibition of HMG-CoA reductase by statins and the subsequent decline in intrahepatic cholesterol results in an increase in LDLR expression via SREBP activation, which brings about improved clearance of plasma LDL-cholesterol through LDLR-mediated endocytosis (Goldstein and Brown 2009). Hypercholesterolemia is recognized as a major risk factor for atherosclerosis (Canto and Iskandrian 2003; Nabel 2003). Lowering plasma LDL-cholesterol has been strongly associated with a decreased risk of developing cardiovascular disease in humans (Grundy 1998; Ansell *et al.* 1999). Berberine has been shown to regulate lipid metabolism by two mechanisms different from the manner in which statins act.

Effects on cholesterol

Berberine's ability to lower cholesterol is believed to occur through the stabilization of LDLR mRNA, leading to an increase of LDLR on the hepatocellular membrane and enhanced clearance of LDL-cholesterol (Kong *et al.* 2004). The actions of berberine have been shown to be independent of intracellular cholesterol levels and have no effect on SREBP promoter activity, indicating that SREBP are not involved in the mechanism of berberine-mediated LDLR up-regulation. Regulatory sequences involved in turnover rate and stability have been identified in the 3′ untranslated region of the LDLR mRNA, and they appear to play an important role in the stabilization of LDLR mRNA by berberine (Kong *et al.* 2004). Another study identified two RNA binding proteins through which berberine may regulate LDLR mRNA stability (Li *et al.* 2009). Several studies also indicated that activation of the mitogen-activated protein kinase/extracellular-signal regulated kinase (MAPK/ERK) pathway might be a prerequisite event in berberine-mediated stabilization of the LDLR transcript (Kong *et al.* 2004; Abidi *et al.* 2005; Brusq *et al.* 2006). Kong *et al.* demonstrated these events in human hepatoma (HepG2) cells (using 7.5-15 μg/mL) and diet-induced hyperlipidemic hamsters (50 mg/kg berberine for 10 days). Additionally, a clinical trial in a small number of hyperlipidemic patients given oral berberine supplements (0.5 g berberine twice a day for 3 months) yielded promising results, with significant decreases in serum total cholesterol (-29%), triglycerides (-35%) and LDL-cholesterol (-25%), but no change in cardioprotective high density lipoprotein-cholesterol levels (Kong *et al.* 2004).

Effects on triglycerides

While this mechanism explains the role of berberine in lowering serum cholesterol, it does little to account for the decline in triglycerides observed in human subjects. Hypertriglyceridemia contributes to a number of metabolic diseases, including cardiovascular disease, metabolic syndrome and type II diabetes (Cannon 2008). Studies by Brusq *et al.* (2006) have revealed a second mechanism by which berberine lowers lipids, particularly triglycerides. The authors showed that berberine activated AMP-activated protein kinase (AMPK), a kinase known to be involved in regulating energy balance in the cell (Hardie 2008). The two primary targets of AMPK are acetyl-CoA carboxylase (ACC) and HMG-CoA reductase (Carling *et al.* 1989). Berberine caused AMPK phosphorylation, a finding which was supported by several other studies (Lee *et al.* 2006; Turner *et al.* 2008), and the discovery that activation of AMPK by berberine was MAPK/ERK-dependent was confirmed (Kong *et al.* 2004; Brusq *et al.* 2006). The subsequent phosphorylation of ACC by AMPK slowed triglyceride synthesis and promoted the oxidation of fatty acids (Hardie and Pan 2002). Other studies reported decreases in plasma triglycerides of similar magnitudes in humans (Yin *et al.* 2008; Zhang *et al.* 2008).

A recent study examined the effect of a combination of berberine and simvastatin as cholesterol-lowering therapy in rodents and humans (Kong *et al.* 2008). The combination therapy decreased human and rat plasma LDL-cholesterol more effectively than either treatment alone, and boasts the advantage of requiring a low dose of both berberine and statin to achieve significant lipid lowering results.

ANTIOXIDANT EFFECTS

The growing list of therapeutic benefits that have become associated with berberine, including its anti-diabetic and cardioprotective properties, may be explained in part by its remarkably diverse antioxidant capabilities. Berberine has been shown to directly scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) effectively *in vitro*. In biological systems, berberine appears to minimize the severity of oxidative stress by conserving the integrity of the innate antioxidant defense system and minimizing the extent of ROS generation.

Direct mechanism

The *in vitro* ROS/RNS scavenging abilities of berberine have been shown to be diverse, ranging from superoxide (O_2^-) and nitric oxide (NO) quenching to limiting the formation of precariously reactive molecules such as peroxynitrite ($OONO^-$) and the hydroxyl radical ($OH\cdot$). At low concentrations, berberine (50-100 μM) was found to inhibit DNA cleavage under pro-oxidative conditions namely due to its strong O_2^- scavenging abilities (Choi *et al.* 2001). Berberine has also been shown to be an effective peroxynitrite scavenger (Jung *et al.* 2009). The direct quenching of O_2^- and NO *in vitro* using colorimetric analysis was shown to be concentration dependant, although the efficacy of berberine (2-512 μg/ml) to directly scavenge ROS/RNS was found to be inferior to the well known antioxidant ascorbic acid (Shirwaikar *et al.* 2006). Using electron spin resonance spectroscopy, berberine (1 mM) was shown to actively scavenge the precarious $OH\cdot$ radical, a capability that is lost at lower concentrations (1-100 μM) (Choi *et al.* 2001).

Free iron in its oxidized ferrous form is extremely reactive under aerobic conditions, generating cytotoxic lipid peroxides via Fenton chemistry (Gutteridge 1995). Although berberine (50 μM-1 mM) has the ability to scavenge various ROS/RNS, its ability to chelate Fe(II) remains

debatable. Berberine reduces lipid peroxidation in rat brain homogenate, a property accredited to its direct chelation of Fe(II) under experimental conditions (Shirwaikar *et al.* 2006). However, Jang and colleagues (2009) using slightly different analytical techniques, failed to observe similar ferrous chelation, a property that was attributed to berberine's inferior A ring positioning of the methylenedioxy functional group. Among structurally similar alkaloids such as berberrubine, it appears that the D ring positioning of the methylenedioxy group is critical for facilitating optimal Fe(II) chelation (Jang *et al.* 2009). While berberine directly scavenges unstable species under experimentally simulated oxidative conditions, it is evident that berberine's antioxidant capabilities in biological systems go beyond the incidental interaction and inactivation of reactive molecular species.

Indirect mechanisms

1. Berberine up-regulates endogenous antioxidant defense mechanisms

In biological systems, berberine offers a complex array of antioxidant protection which has recently been brought to light. One mechanism whereby berberine regulates oxidative stress is through the conservation of non-enzymatic antioxidants, a phenomenon observed *in vivo*. Berberine (30 mg/kg body weight) was shown to prevent the decrease in the colonic supply of non-enzymatic antioxidants in a rat model of colon cancer. Colonic levels of glutathione, vitamin C and vitamin E were all conserved at relatively normal levels when berberine was co-administered with the carcinogen azoxymethane. Such maintenance was associated with reduced lipid peroxide generation, prevention of malignant morphological changes, and reduced cellular apoptosis. Significant protection was also achieved when affected animals were treated post exposure with berberine (30 mg/kg body weight), although the final protective outcomes were less obvious. The observed "sparing" of innate antioxidants may be a physiological consequence of berberine's direct scavenging *in vivo* or a result of the elaborate modulation of the endogenous antioxidant defense system altogether (Thirupurasundari *et al.* 2009). Nonetheless, a more complete investigation is warranted.

Besides maintaining the supply of endogenous antioxidant molecules, berberine has also been shown to regulate the activity of enzymes that catalyze the decomposition and removal of ROS from cell systems. These enzymes are integral for the maintenance of cellular redox as they are considered the first line of defense against oxidative stress. In cell culture studies, pre-treatment with berberine (25 μM) significantly increased superoxide dismutase (SOD) activity in lipopolysaccharide (LPS) stimulated human monocyte derived macrophages and contributed to an overall reduction in intracellular superoxide (Sarna *et al.* 2010). Similar enhancement of SOD activity has also been observed in primary cell culture studies. For instance, berberine (10-1000 μM) restored SOD activity in a concentration dependant manner in isolated rabbit corpus cavernosum smooth muscle cells injured with hydrogen peroxide. The enhancement of SOD activity was related to a reduction in the generation of lipid peroxides and restoration of nitric oxide levels (Tan *et al.* 2007).

The observed findings pertaining to the activation of antioxidant enzymes in cell systems have been demonstrated *in vivo* as well. Daily oral ingestion of berberine (30 mg/kg body weight) effectively conserved the colonic antioxidant enzyme defense system in a rat model of colon cancer. Specifically, among the rats exposed to azoxymethane, the group receiving the berberine-enriched diet maintained the activity of SOD, catalase, glutathione *S*-transferase, and glutathione peroxidase at levels comparable to healthy rats. Such protection was associated with a reduction in the severity of melanogenesis as compared to the azoxymethane group that received no berberine treatment. Although less significant, feeding rats post-exposure with berberine (30 mg/kg body weight) also effectively increased the activity of each antioxidant enzyme tested (Thirupurasundari *et al.* 2009). In a rat model of Type I diabetes mellitus, SOD activity was restored to levels comparable to normoglycemic rats following the daily consumption of berberine (150-300 mg/kg). The increased activity of SOD in the diabetic rats was associated with reduced lipid peroxidation and the prevention of pathologically associated cellular morphologies (Zhou *et al.* 2009). Daily administration of berberine (20 mg/kg) via stomach tube prevented the decline in serum SOD activity in rats experiencing a severe immune challenge (LPS administration during kidney ischemia reperfusion). Likewise, catalase and glutathione peroxidase activity were similarly restored, although these enzymes displayed a dose-dependent activation at both 10 and 20 mg/kg berberine. The restoration of enzymatic antioxidants in this particular model was associated with a reduction in serum superoxide and nitric oxide and an even more remarkable reduction in circulating $OONO^-$ (Yokozawa *et al.* 2004). Overall, berberine reduces the oxidative burden associated with pathologies such as cancer and diabetes, and conditions where critical immune challenge is present. The oxidative relief appears to be facilitated by the ability of berberine to conserve endogenous antioxidants and to increase the activity of various antioxidant enzymes. However, the molecular mechanisms responsible for such effects are awaiting clarification.

2. Berberine suppresses endogenous ROS/RNS-generating Oxidases

Besides the endogenous antioxidant defense system, another deciding factor that determines the redox status of a cell is the rate at which oxidative species are generated. Although ROS/RNS can be produced spontaneously, in biological systems, reactive molecules can also be accidentally or deliberately generated by specific oxidase enzymes. These oxidases are capable of generating ROS such as O_2^- and RNS such as NO with high efficiency and therefore play a major role in determining the cellular redox context. While enzymatic antioxidants such as SOD tend to be suppressed in various pathological conditions, enzymatic oxidases like the O_2^- generating reduced nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase tend to be activated. Therefore when investigating the antioxidant capacity of therapeutic agents in oxidative stress mediated disorders, it is equally important to consider their efficacy in modulating ROS/RNS generating systems.

A recent study demonstrated that berberine (25 μM) significantly inhibited NADPH oxidase mediated O_2^- production in LPS stimulated human monocyte-derived macrophages (Sarna *et al.* 2010). Berberine was found to regulate NADPH oxidase activity by selectively inhibiting gene expression of the enzyme's catalytic subunit, $gp91^{phox}$ (Sarna *et al.* 2010). Likewise, berberine (5 μM) inhibited the expression of inducible nitric oxide synthase (iNOS) in LPS treated murine macrophages, significantly reducing NO production in these cells (Jeong *et al.* 2009). Such inhibition of NO production was found to be mediated via an AMPK signaling pathway. Inhibition of ROS/RNS generating oxidase enzymes serves as one of the mechanisms through which berberine offers indirect antioxidant effect.

BERBERINE AND ATHEROSCLEROSIS

Hyperlipidemia is one of the major risk factors for cardiovascular disease. Apart from healthy life style, current pharmacotherapy is mainly achieved by lipid lowering medications. The majority of patients will be able to achieve target blood lipid levels on monotherapy or combined medications (Genest *et al.* 2009). However, some patients cannot tolerate medications such as statins and suffer from side effects (i.e. muscle pain and a decline in liver function). Berberine has now been shown to possess potent lipid lowering effects and it may be considered a future treatment for patients

with hyperlipidemia.

Besides its lipid lowering properties, the diverse range of both direct and indirect antioxidant actions displayed by berberine attribute to its potential therapeutic application in the prevention of atherosclerosis. Atherosclerosis is characterized by thickening, hardening and loss of elasticity of the arterial wall, which can lead to fatal blockage of arteries. It is increasingly recognized that oxidative stress can elicit systemic inflammation, which in turn contributes to the development of cardiovascular disease. Atherosclerosis is a chronic inflammatory condition potentiated by the enhanced generation of ROS/RNS throughout the initiating and progressive stages of the disease (Madamanchi *et al.* 2005). One mechanism whereby berberine could retard the progression of atherosclerosis is through the inhibition of LDL oxidation. *In vitro* analysis of copper induced LDL oxidation demonstrated that berberine (25-100 μM) could effectively reduce such undesirable modifications in a concentration dependant manner (Hsieh *et al.* 2007).

Berberine has also been shown to attenuate oxidative stress in various vascular cells which could confer protection against the onset and development of atherosclerotic plaques. Berberine's antioxidant properties may preserve the integrity of endothelial cells following exposure to oxidized LDL. Berberine has been shown to restore cell proliferation and viability, and subsequently prevent apoptosis in a concentration dependant manner in cultured endothelial cells. Such a protective effect was mediated by berberine's (25-75 μM) ability to reduce endothelial oxidative stress and at a higher concentration (100 μM), completely abolish oxidative injury (Hsieh *et al.* 2007). NADPH oxidases, particularly the phagocytic isoform expressed in macrophages, are a key source of oxidative stress in human atherosclerotic plaques, contributing roughly 60% of the ROS/RNS generated in the atheroma (Sorescu *et al.* 2002; Guzik *et al.* 2006). Berberine has been shown to attenuate O_2^- production in stimulated macrophages. Such inhibition was mediated by berberine's ability to reduce the expression of the catalytic subunit of NADPH oxidase as well as enhance SOD activity in activated macrophages (Sarna *et al.* 2010). In addition, berberine's antioxidant capabilities may prevent the chronic release of inflammatory cytokines. It was shown to attenuate the release of TNF-α, IL-6 and MCP-1 in stimulated macrophages (Chen *et al.* 2008), a property associated with berberine's suppression of ROS/RNS generation in these cells (Jeong *et al.* 2009).

FUTURE PERSPECTIVES

In conclusion, berberine is effective both in lipid-lowering and as an antioxidant. This compound has been shown to have potent lipid-lowering abilities both in animal models and in clinical trials of hyperlipidemic patients. Berberine has been well tolerated by virtually all subjects and has proven to be an effective compound for lowering cholesterol and triglycerides. Thus far, berberine has been shown to act via two complementary mechanisms distinct from the manner in which statins bring about a reduction in lipids. In light of these findings, we believe that berberine has great potential for use as an alternative or additional therapy for hyperlipidemia.

Berberine has displayed both a direct and indirect means of attenuating oxidative stress in various disease states. Future research should be directed towards deciphering the molecular mechanisms responsible for berberine's ability to conserve intrinsic antioxidants and to regulate the many antioxidant enzymes. The therapeutic relevance of berberine in the prevention and management of atherosclerosis remains under investigation.

In many instances, enriched and standardized plant extracts of goldenseal, barberry or golden thread may be presented as alternative therapies for various physiological dysfunctions, especially in the age of rising healthcare costs. Placebo-controlled human clinical trials for these products should be encouraged and conducted and any adverse effects should be promptly reported. Otherwise, subscription to these products should be under the guidance of a primary healthcare provider.

ACKNOWLEDGEMENTS

This study article was supported in part by grants (to Y.L.S.) from the Heart & Stroke Foundation of Manitoba, Canadian Foundation for Innovation and the Manitoba Research Innovation Fund. L.K.S. and J.E. are recipients of graduate studentships from the Manitoba Health Research Council.

REFERENCES

Abidi P, Zhou Y, Jiang JD, Liu J (2005) Extracellular signal-regulated kinase-dependent stabilization of hepatic low-density lipoprotein receptor mRNA by herbal medicine berberine. *Arteriosclerosis, Thrombosis, and Vascular Biology* **25**, 2170-2176

Ansell BJ, Watson KE, Fogelman AM (1999) An evidence-based assessment of the NCEP Adult Treatment Panel II guidelines. National Cholesterol Education Program. *Journal of the American Medical Association* **282**, 2051-2057

Brown PN, Paley LA, Roman MC, Chan M (2008) Single-laboratory validation of a method for the detection and/or quantification of select alkaloids in goldenseal supplements and raw materials by reversed-phase high-performance liquid chromatography. *Pharmaceutical Biology* **46**, 135-144

Brown PN, Roman MC (2008) Determination of hydrastine and berberine in goldenseal raw materials, extracts, and dietary supplements by high-performance liquid chromatography with UV: Collaborative study. *Journal of AOAC International* **91**, 694-701

Brusq JM, Ancellin N, Grondin P, Guillard R, Martin S, Saintillan Y, Issandou M (2006) Inhibition of lipid synthesis through activation of AMP kinase: An additional mechanism for the hypolipidemic effects of berberine. *Journal of Lipid Research* **47**, 1281-1288

Cannon CP (2008) Mixed dyslipidemia, metabolic syndrome, diabetes mellitus, and cardiovascular disease: clinical implications. *American Journal of Cardiology* **102**, 5L-9L

Canto JG, Iskandrian AE (2003) Major risk factors for cardiovascular disease: Debunking the "only 50%" myth. *Journal of the American Medical Association* **290**, 947-949

Carling D, Clarke PR, Zammit VA, Hardie DG (1989) Purification and characterization of the AMP-activated protein kinase. Copurification of acetyl-CoA carboxylase kinase and 3-hydroxy-3-methylglutaryl-CoA reductase kinase activities. *European Journal of Biochemistry* **186**, 129-136

Chen FL, Yang ZH, Liu Y, Li LX, Liang WC, Wang XC, Zhou WB, Yang YH, Hu RM (2008) Berberine inhibits the expression of TNFalpha, MCP-1, and IL-6 in AcLDL-stimulated macrophages through PPARgamma pathway. *Endocrine* **33**, 331-337

Choi DS, Kim SJ, Jung MY (2001) Inhibitory activity of berberine on DNA strand cleavage induced by hydrogen peroxide and cytochrome c. *Bioscience, Biotechnology, and Biochemistry* **65**, 452-455

Filotas M, Todd J, Westerveld S, Prinold H (2009) *Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) Factsheet: Growing Non-traditional Crops in Ontario*. ORDER NO. 09-043W

Genest J, McPherson R, Frohlich J, Anderson T, Campbell N, Carpentier A, Couture P, Dufour R, Fodor G, Francis GA, Grover S, Gupta M, Hegele RA, Lau DC, Leiter L, Lewis GF, Lonn E, Mancini GB, Ng D, Pearson GJ, Sniderman A, Stone JA, Ur E (2009) 2009 Canadian Cardiovascular Society/Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult - 2009 recommendations. *Canadian Journal of Cardiology* **25**, 567-579

Goldstein JL, Brown MS (2009) The LDL receptor. *Arteriosclerosis, Thrombosis, and Vascular Biology* **29**, 431-438

Gruenwald J (1998) *PDR for Herbal Medicines* (1st Edn), Medical Economics Co., Inc., Montvale, NJ, 1244 pp

Grundy SM (1998) Statin trials and goals of cholesterol-lowering therapy. *Circulation* **97**, 1436-1439

Gutteridge JM (1995) Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clinical Chemistry* **41**, 1819-1828

Guzik TJ, Sadowski J, Guzik B, Jopek A, Kapelak B, Przybylowski P, Wierzbicki K, Korbut R, Harrison DG, Channon KM (2006) Coronary artery superoxide production and nox isoform expression in human coronary artery disease. *Arteriosclerosis, Thrombosis, and Vascular Biology* **26**, 333-339

Hardie DG (2008) AMPK: A key regulator of energy balance in the single cell and the whole organism. *International Journal of Obesity (London)* **32 Suppl 4**, S7-S12

Hardie DG, Pan DA (2002) Regulation of fatty acid synthesis and oxidation by the AMP-activated protein kinase. *Biochemical Society Transactions* **30**, 1064-1070

Horton JD, Goldstein JL, Brown MS (2002) SREBPs: Activators of the complete program of cholesterol and fatty acid synthesis in the liver. *Journal of*

Clinical Investigations **109**, 1125-1131

Hsieh YS, Kuo WH, Lin TW, Chang HR, Lin TH, Chen PN, Chu SC (2007) Protective effects of berberine against low-density lipoprotein (LDL) oxidation and oxidized LDL-induced cytotoxicity on endothelial cells. *Journal of Agricultural and Food Chemistry* **55**, 10437-10445

Jang MH, Kim HY, Kang KS, Yokozawa T, Park JH (2009) Hydroxyl radical scavenging activities of isoquinoline alkaloids isolated from *Coptis chinensis*. *Archives of Pharmacal Research* **32**, 341-345

Jellin JM, Batz F, Hitchens K (2000) *Natural Medicines Comprehensive Database* (3rd Edn), Therapeutic Research Faculty, Stockton, CA, 1529 pp

Jeong HW, Hsu KC, Lee JW, Ham M, Huh JY, Shin HJ, Kim WS, Kim JB (2009) Berberine suppresses proinflammatory responses through AMPK activation in macrophages. *American Journal of Physiology - Endocrinology and Metabolism* **296**, E955-E964

Jung HA, Min BS, Yokozawa T, Lee JH, Kim YS, Choi JS (2009) Anti-Alzheimer and antioxidant activities of *Coptidis rhizoma* alkaloids. *Biological and Pharmaceutical Bulletin* **32**, 1433-1438

Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, Wang Y, Wang Z, Si S, Pan H, Wang S, Wu J, Li Z, Liu J, Jiang JD (2004) Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. *Nature Medicine* **10**, 1344-1351

Kong WJ, Wei J, Zuo ZY, Wang YM, Song DQ, You XF, Zhao LX, Pan HN, Jiang JD (2008) Combination of simvastatin with berberine improves the lipid-lowering efficacy. *Metabolism* **57**, 1029-1037

Lee YS, Kim WS, Kim KH, Yoon MJ, Cho HJ, Shen Y, Ye JM, Lee CH, Oh WK, Kim CT, Hohnen-Behrens C, Gosby A, Kraegen EW, James DE, Kim JB (2006) Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. *Diabetes* **55**, 2256-2264

Li H, Chen W, Zhou Y, Abidi P, Sharpe O, Robinson WH, Kraemer FB, Liu J (2009) Identification of mRNA binding proteins that regulate the stability of LDL receptor mRNA through AU-rich elements. *Journal of Lipid Research* **50**, 820-831

Madamanchi NR, Vendrov A, Runge MS (2005) Oxidative stress and vascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology* **25**, 29-38

Nabel EG (2003) Cardiovascular disease. *New England Journal of Medicine* **349**, 60-72

Ontario Gov (2009) *The Ontario Gazette*. **Vol. 142-39** Report No. 0030-2937 Ministry of Government Services p 2597

Royal Ontario Museum *Distribution : Goldenseal*. Royal Ontario Museum and the Ontario Ministry of Natural Resources Available online: http://www.rom.on.ca/ontario/risk.php?doc_type=map&id=45

Sarna LK, Wu N, Hwang S-Y, Siow YL, O K (2010) Berberine inhibits NADPH oxidase mediated superoxide anion production in macrophages. *Canadian Journal of Physiology and Pharmacology* **88**, 369-378

Shirwaikar A, Rajendran K, Punitha IS (2006) *In vitro* antioxidant studies on the benzyl tetra isoquinoline alkaloid berberine. *Biological and Pharmaceutical Bulletin* **29**, 1906-1910

Sinclair A, Catling PM (2001) Cultivating the increasingly popular medicinal plant, goldenseal: Review and update. *American Journal of Alternative Agriculture* **16**, 131-140

Sinclair A, Nantel P, Catling PM (2005) Dynamics of threatened goldenseal populations and implications for recovery. *Biological Conservation* **123**, 355-360

Sorescu D, Weiss D, Lassegue B, Clempus RE, Szocs K, Sorescu GP, Valppu L, Quinn MT, Lambeth JD, Vega JD, Taylor WR, Griendling KK (2002) Superoxide production and expression of nox family proteins in human atherosclerosis. *Circulation* **105**, 1429-1435

Swartz ME (2005) UPLC: An introduction and review. *Journal of Liquid Chromatography and Related Technologies* **28**, 1253-1263

Tan Y, Tang Q, Hu BR, Xiang JZ (2007) Antioxidant properties of berberine on cultured rabbit corpus cavernosum smooth muscle cells injured by hydrogen peroxide. *Acta Pharmacologica Sinica* **28**, 1914-1918

Thirupurasundari CJ, Padmini R, Devaraj SN (2009) Effect of berberine on the antioxidant status, ultrastructural modifications and protein bound carbohydrates in azoxymethane-induced colon cancer in rats. *Chemico-Biological Interactions* **177**, 190-195

Turner N, Li JY, Gosby A, To SW, Cheng Z, Miyoshi H, Taketo MM, Cooney GJ, Kraegen EW, James DE, Hu LH, Li J, Ye JM (2008) Berberine and its more biologically available derivative, dihydroberberine, inhibit mitochondrial respiratory complex I: A mechanism for the action of berberine to activate AMP-activated protein kinase and improve insulin action. *Diabetes* **57**, 1414-1418

USDA ARS, National Genetic Resources Program *Berberis vulgaris L.* National Germplasm Resources Laboratory, Beltsville, Maryland. Available online: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?6992

USDA ARS, National Genetic Resources Program *Coptis chinensis Franch.* National Germplasm Resources Laboratory, Beltsville, Maryland. Available online: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?402553

USDA ARS, National Genetic Resources Program *Hydrastis canadensis L.* National Germplasm Resources Laboratory, Beltsville, Maryland. Available online: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?19478

Weber HA, Zart MK, Hodges AE, White KD, Barnes SM, Moody LA, Clark AP, Harris RK, Overstreet JD, Smith CS (2003) Method validation for determination of alkaloid content in goldenseal root powder. *Journal of AOAC International* **86**, 476-483

Yin J, Xing H, Ye J (2008) Efficacy of berberine in patients with type 2 diabetes mellitus. *Metabolism* **57**, 712-717

Yokozawa T, Ishida A, Kashiwada Y, Cho EJ, Kim HY, Ikeshiro Y (2004) *Coptidis rhizoma*: Protective effects against peroxynitrite-induced oxidative damage and elucidation of its active components. *Journal of Pharmacy and Pharmacology* **56**, 547-556

Zhang Y, Li X, Zou D, Liu W, Yang J, Zhu N, Huo L, Wang M, Hong J, Wu P, Ren G, Ning G (2008) Treatment of type 2 diabetes and dyslipidemia with the natural plant alkaloid berberine. *Journal of Clinical Endocrinology and Metabolism* **93**, 2559-2565

Zhou J, Zhou S, Tang J, Zhang K, Guang L, Huang Y, Xu Y, Ying Y, Zhang L, Li D (2009) Protective effect of berberine on beta cells in streptozotocin- and high-carbohydrate/high-fat diet-induced diabetic rats. *European Journal of Pharmacology* **606**, 262-268