Palmatine as an Agent Against Metabolic Syndrome and Its Related Complications: A Review

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy

Sophia Ogechi Ekeuku ¹
Kok-Lun Pang ¹
Kok-Yong Chin ^{1,2}

¹Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Cheras, Kuala Lumpur, Malaysia; ²State Key Laboratory of Oncogenes and Related Genes, Renji-Med X Clinical Stem Cell Research Center, Department of Urology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, People's Republic of China

Abstract: Palmatine is a naturally occurring isoquinoline alkaloid with various pharmacological properties. Given its antioxidant and anti-inflammatory properties, palmatine may be able to impede the effects of metabolic syndrome (MetS) and its related diseases triggered by inflammation and oxidative stress. This review summarises the existing literature about the effects of palmatine supplementation on MetS and its complications. The evidence shows that palmatine could protect against MetS, and cardiovascular diseases, osteoporosis and osteoarthritis, which might be associated with MetS. These protective effects are mediated by the antioxidant and anti-inflammatory properties of palmatine. Although preclinical experiments have demonstrated the efficacy of palmatine against MetS and its related diseases, no human clinical trials have been performed to validate these effects. This research gap should be bridged to validate the efficacy and safety of palmatine supplementation in protecting humans against MetS and its related diseases.

Keywords: anti-inflammation, antioxidant, myocardial reperfusion injury, obesity, osteoporosis, osteoarthritis

Introduction

Metabolic syndrome (MetS) is a strong risk factor for type 2 diabetes, cardiovascular diseases and stroke, which carry high morbidity and threaten global health.¹ Although the definition of MetS has evolved with time, it is usually identified by the presence of 3 out of 5 metabolic anomalies, which include obesity, hyperglycaemia, dyslipidaemia [high triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-c) and hypertension].^{2,3} Around 20–25% of the adult populations worldwide suffer from MetS.⁴ Data from National Health and Nutrition Examination Survey between 2003 and 2012 revealed that the prevalence of MetS in the United States was 36.5% for women and 30.3% for men.⁵ A systematic review reported that the incidence of MetS in the Asia-Pacific regions was between 11.9% and 37.1%.⁶

Oxidative stress, characterised by an imbalance between oxidants and antioxidants in the body, plays a major role in the development of MetS.⁷ Increased biomarkers of oxidative stress and diminished antioxidant defences were detected in the blood of patients with MetS, suggesting overproduction of oxidising agents in vivo.⁸ This imbalance involved enzymatic [superoxide dismutase (SOD) activity] and non-enzymatic antioxidant defences (circulating vitamin C and E), as well as increased oxidation products of proteins and lipids.^{8,9} Increased adipose/fat tissue observed in obesity can

Correspondence: Kok-Yong Chin
Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, Cheras 56000, Kuala Lumpur, Malaysia
Tel +60 3-9145 9573
Email chinkokyong@ppukm.ukm.edu.my

trigger oxidative stress.¹⁰ Tissue hypoxia due to the inadequate blood supply is a complication in obesity, which triggers cellular necrosis, and migration and phagocytosis of cellular debris by white blood cells.¹¹ The process will elevate oxidative stress due to the release of free radicals, such as nitric oxide and hydrogen peroxide, which may worsen the existing metabolic condition of the patients.^{12–14} For example, blood pressure was positively associated with oxidative stress biomarkers and negatively associated with the antioxidant status of patients.¹⁵ On the other hand, chronic inflammation instigated by a hyperactive immune response is another contributor to MetS.¹⁶ Increased circulating pro-inflammatory cytokines, such as interleukin-6 (IL-6), tumour necrosis factor- α (TNF- α), and C-reactive protein (CRP), were reported in patients with MetS.¹⁷ These cytokines, produced by adipocytes and white blood cells, bind to the respective receptors on target cells and trigger a cascade of signalling events leading to adverse metabolic changes.¹⁸

Natural compounds with anti-inflammatory and antioxidant properties, such as vitamin C, E and resveratrol, are hypothesised to prevent or reverse MetS complications.^{19–21} Palmatine is one of the candidate agents being investigated for its anti-MetS properties. Palmatine is a naturally-occurring quaternary protoberberine in the class of isoquinoline alkaloids.^{22,23} Structurally, it is an ammonium salt with four methoxyl moieties attached to the phenyl rings at C2 and C3 position.²⁴ The preventive and curative actions of palmatine on cancer, cardiac hypertrophy, diabetes and its complications, osteoporosis, osteoarthritis, Alzheimer's disease and atopic dermatitis have been reported.^{24–26} The biological effects of palmatine could be attributed to its anti-inflammatory^{27–29} and antioxidant properties.^{30–32} Of note, palmatine is shown to suppress Toll-like receptor (TLR) signalling by downregulating the expression of TLR4, Toll/IL-1 receptor-domain-containing adaptor protein inducing interferon- β and nuclear factor-kappa B in goat endometrial epithelial cells.²⁹ This event leads to increased synthesis of prostaglandins E2 and IL-10 but reduced synthesis of TNF- α , IL-1 β , IL-6, nitric oxide, matrix metalloproteinase (MMP)-9 and MMP-2.²⁹ Therefore, the current review aims to summarise the effects of palmatine on MetS and its associated diseases.

The Effects of Palmatine on Components of MetS

Central obesity is a key feature of MetS. The excess nutrients are stored in the adipose tissue as lipids, leading to hypertrophy of adipocytes and increased fat mass.³³ Weight

reduction through lifestyle, dietary, pharmacological and surgical interventions are often prescribed to patients with MetS.^{34,35} The antiadipogenic activities of palmatine have been demonstrated in *in vitro* and *in vivo* models. Choi et al reported that 3T3-L1 pre-adipocytes treated with palmatine (12.5, 25 and 50 μ M) for 24 hrs showed reduced lipid accumulation compared to the untreated cells during differentiation.³⁶ Expression of proliferator-activated receptor- γ (PPAR- γ) and CCAAT/enhancer-binding protein- α (C/EBP- α) was also reduced with palmatine treatment, which signifies a reduction in adipogenesis. These positive findings justify further investigation into the anti-obesity effects of palmatine.

In an animal study by Ning et al, 4-week old Syrian golden hamsters with hyperlipidemia induced by high-fat diet (HFD) showed reduced body weight, serum total cholesterol (TC), TG, and low-density lipoprotein cholesterol (LDL-c) after being treated with palmatine (23.35, 46.70 and 70.05 mg/kg/day) for four weeks.³⁷ Palmatine increased the expression of low-density lipoprotein receptor (LDLR) and cholesterol 7 α -hydroxylase (CYP7A1), which mediate the endocytosis of cholesterol and the conversion of cholesterol to bile acid, respectively.^{38–41} It also decreased apical sodium-dependent bile acid transporter (ASBT) mRNA and protein expression in these hamsters, which subsequently reduces the bile acid reabsorption in the intestine and increases the excretion of total bile acids in the faeces.^{42,43} Besides, CYP7A1 also will be upregulated to compensate for the low bile acid level by converting it to cholesterol.⁴⁴

Similarly, in another study by He et al, 5-week old Syrian golden hamsters with hyperlipidemia induced by high-fat high-cholesterol diet were treated with palmatine (46.7 mg/kg/day for 140 days).⁴⁵ The treated hamsters showed reduced body weight and epididymal adipose weight, improved lipid profile and increased LDLR and CYP7A1 mRNA and protein expression. Additionally, palmatine supplementation also reduced 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGCR) mRNA and protein expression and increased uncoupling protein (UCP)-2 mRNA expressions.⁴⁵ HMGCR is the rate-limiting enzyme in the mevalonate pathway responsible for cholesterol synthesis.⁴⁶ A decrease in HMGCR expression suggests a reduction in cholesterol production.^{47,48} UCP-2 is a mitochondrial protein involved in thermogenesis and energy expenditure.⁴⁹ An increase in UCP-2 expression is associated with a reduction in body weight due to improved energy metabolism.⁵⁰

In another study by Ma et al, female KK-Ay mice with hyperlipidemia and hyperglycemia induced by HFD showed a decrease in serum TC, TG and LDL-c but an increase in HDL-c after being treated with palmatine (25, 50, 100 mg/kg/day) for 40 days.⁵¹ 11-hydroxypalmatine (100 mg/kg/day) derived from tubers of *Stephania glabra* also reduced blood glucose level in alloxan-induced diabetic Swiss albino mice.⁵² Palmatine (10 mg/kg for 6 weeks) also reduced blood glucose and increased insulin in streptozocin-induced diabetic rats.³² These changes were attributed to reduced oxidative stress, marked by the lowering of malondialdehyde (MDA) and nitrite levels and the increasing of SOD activity.³² However, 24 hrs of palmatine treatment (up to 14.6 μ M) inhibited the glucose uptake in HepG2 cells via a non-concentration-dependent manner⁵¹ which suggest the hypoglycaemic properties of palmatine may not be related to glucose transportation or insulin sensitivity.

An overview of the effects of palmatine on components of MetS is presented in Table 1. Overall, palmatine supplementation attenuated the adverse effects associated with MetS. The cellular studies showed that palmatine could suppress the formation of adipocytes. Palmatine could also reduce cholesterol production and reabsorption, as well as enhancing energy metabolism, thereby improving lipid profile in animals. A general limitation for the in vivo studies is the absence of a positive control group, ie, pharmacological treatment for MetS. Therefore, the effects of palmatine and the standard treatment cannot be compared. Besides, clinical trial validating the effect of palmatine on MetS is absent in the literature. These research gaps should be considered in future studies.

The Effects of Palmatine on Cardiovascular Diseases Associated with MetS

MetS is a significant risk factor for myocardial infarction, a common obstructive heart disease and one of the leading causes of death in developed countries.⁵³ Apart from the pro-atherogenic environment, the pro-inflammatory and pro-oxidative conditions of MetS could damage the blood vessel wall, triggering coronary microvascular dysfunction.^{54,55} Insufficient supply of blood and oxygen to the heart is the initial cause of cardiac damage, thus an immediate blood supply restoration is crucial in minimising myocardial injury. However, sudden reperfusion causes

tissue damage despite the restored blood flow, thereby worsening the initial ischemic injury.⁵⁶

The cardioprotective effects of palmatine have been reported in several studies. In a study by Kim et al, lipopolysaccharides (LPS)-stimulated RAW 264.7 cells treated with palmatine (1, 5, 10 μ M for 16 hours) showed decreased high mobility group box 1 (HMGB1) release compared to the untreated LPS-stimulated cells.³¹ HMGB1 regulates the activation of neutrophils during inflammation.⁵⁷ The reduction in HMGB1 by palmatine suggests that it could reduce inflammation during MI by lowering the influx of neutrophils into the dying tissues.³¹ Human aortic endothelial cells (HAEC) treated with palmatine (1, 2, 5 and 10 μ M for 8 hours) also showed increased heme oxygenase-1 (HO-1) with antioxidant and anti-inflammatory properties.³¹ Together, these observations imply the potential effects of palmatine in reducing inflammatory damage to the myocardium during myocardial infarction.

In an animal study by Kim et al, male Sprague Dawley rats with ischemic reperfusion (I/R)-mediated acute myocardial infarction were treated with palmatine (25 and 50 mg/kg) 1 hr before ischemia.³¹ Reduced MDA and increased SOD and catalase (CAT) activities suggestive of reduced oxidative stress were observed in I/R rats with palmatine treatment.³¹ Palmatine treated I/R rats also showed reduced lactate dehydrogenase (LDH) and creatine phosphokinase (CK), which are intracellular metabolic enzymes of cardiomyocytes elevated after infarction.³¹ Palmatine also increased the first derivative ($\pm dp/dt$) of left ventricular pressure and reduced infarct size of the heart.³¹ In the myocardial tissues of I/R rats, cyclooxygenase-2 (COX-2) and inducible NO synthase (iNOS) expression were reduced with palmatine supplementation, suggestive of decreased inflammation in I/R-mediated myocardial infarction.³¹

Taken together, palmatine is effective in reducing myocardial injury by suppressing inflammatory and oxidative damage associated with reperfusion in animal models. It could be used as an adjuvant to the standard therapy in the management of myocardial infarction. However, its putative use should be validated in a human clinical trial.

The Effects of Palmatine on Musculoskeletal Disorder Associated with MetS

Osteoporosis is a degenerative bone disease that can be triggered by low-grade inflammation and oxidative stress.

Table 1 Protective Effect of Palmatine on MetS Components

Researcher	Study Design	Findings
Cell Culture Studies		
Choi et al 2014 ³⁶	Cell line: 3T3-L1 mouse pre-adipocytes Mode of disease/model induction: Adipocyte differentiation Treatment: 12.5, 25 and 50 μ M palmatine for 24 hrs NC: Untreated cells DC: Cells with adipocyte differentiation media PC: n.a.	↓ lipid accumulation, PPAR- γ and C/EBP- α compared to DC
Ma et al 2016 ⁵¹	Cell line: Human liver cancer HepG2 cells Mode of disease/model induction: none Treatment: 0.584, 2.92, 14.6 μ M of palmatine for 24 hrs NC: Untreated cells PC: Metformin (0.2–5 μ g/mL)	Inhibit glucose uptake in non-concentration dependent manner
Animal Studies		
Ning et al 2015 ³⁷	Animals: 60 male Syrian golden hamsters (4 weeks old, 100 $\pm$ 5 g) Mode of disease induction: HFD-induced hyperlipidaemia Treatment: HFD with 23.35, 46.70 and 70.05 mg/kg/day of palmatine for 4 weeks NC: Distilled water DC: HFD group PC: 1.2 mg/kg/day of simvastatin for 4 weeks	↓ HFD-induced body weight gain and upregulation of serum TC, TG & LDL-c in a dose-dependent manner NS for HDL-c level compared to DC Further ↑ faecal excretion of TC and TBA compared to DC Restore HFD-induced downregulation of LDLR and CYP7A1 mRNA and protein expression ↓ HFD-induced upregulation of ASBT NS for HMGCR mRNA and protein expression on DC or palmatine groups compared to NC
He et al 2016 ⁴⁵	Animals: 54 male Syrian golden hamsters (5 weeks old, 100 $\pm$ 10 g) Mode of disease induction: HFHC-induced hyperlipidemia Treatment: 46.70 mg/kg/day of palmatine for 140 days NC: Distilled water DC: HFHC group PC: 1.2 mg/kg/day of orlistat for 140 days	↓ body weight and epididymal adipose weight gain compared to DC ↓ HFHC-induced upregulation of serum TC and LDL-c Further ↑ serum HDL-c and faecal excretion of TC & TBA compared to DC ↓ HMGCR and ↑ LDLR, CYP7A1 and UCP-2 mRNAs and proteins expression compared to DC
Ma et al 2016 ⁵¹	Animals: Female KK-Ay mice (8 weeks old, 40 $\pm$ 5 g) Mode of disease induction: HFD-induced diabetes Treatment: 25, 50, 100 mg/kg/day of palmatine for 40 days NC: n.a. DC: 0.9% saline PC: 0.32 mL of 225 mg/kg/day of metformin for 40 days	↓ serum TC, TG and liver/body weight ratio and ↑ HDL-c compared to DC ↓ water intake but NS for body weight gain, food consumption, urine output, fasting blood glucose, postprandial blood glucose and LDL-c levels compared to DC
Semwal et al 2010 ⁵²	Animals: Swiss albino mice of either sex (35–50 g) Mode of disease induction: Alloxan-induced diabetes Treatment: 25, 50, and 100 mg/kg 11-hydroxypalmatine NC: 0.9% saline without alloxan DC: 60 mg/kg alloxan intravenous injection PC: 5 mg/kg/day of glibenclamide for 24 hours on diabetic mice	↓ blood glucose level compared to NC
Pakseresht et al 2016 ³²	Animals: 32 male Wistar rats (35–50 g) Mode of disease induction: STZ-induced diabetes Treatment: 10 mg/kg/day of palmatine for 6 weeks NC: no treatment DC: Intraperitoneal STZ injection (55 mg/kg) PC: n.a.	↓ STZ-induced hyperglycaemia and ↑ STZ-induced hypoinsulinemia Restore STZ-induced upregulation of nitric oxide activity and downregulation of SOD level

Abbreviations: ↓, decrease or downregulate; ↑, increase or upregulate; ASBT, apical sodium-dependent bile acid transporter; C/EBP- α , CCAAT/enhancer-binding protein- α ; CYP7A1, cholesterol 7 α -hydroxylase; DC, disease control/model; HDL-c, high-density lipoprotein cholesterol; HFD, high-fat diet; HFHC, high-fat high-carbohydrate diet; HMGCR, 3-hydroxy-3-methyl glutaryl coenzyme A reductase; LDL-c, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; MDA, malondialdehyde; n.a., not available; NC, negative control; NS, not significant; PC, positive control; PPAR- γ , proliferator-activated receptor- γ ; SOD, superoxide dismutase; STZ, streptozotocin; TBA, total bile acids; TC, total cholesterol; TG, triglyceride; UCP-2, uncoupling protein-2.

Pro-inflammatory cytokines, such as TNF- α and IL-1, increase the production of receptor activator of nuclear factor- κ B ligand (RANKL), which promote osteoclast formation and activation.^{58,59} They also suppress apoptosis and prolong the lifespan of osteoclasts.⁶⁰ Reactive oxygen species (ROS) trigger phosphorylation and degradation of nuclear factor- κ B inhibitor, leading to the activation of nuclear factor- κ B, thereby promoting osteoclast formation and activity.⁶¹ ROS also induce apoptosis of osteoblasts by activating mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinases, c-Jun-N terminal kinase and p38.^{62,63} Altogether, these changes lead to altered bone remodelling favouring bone loss.

The simultaneous presence of inflammation and oxidative stress in MetS could potentially result in osteoporosis. The increase in adipocyte differentiation in bone marrow prevents osteoblast differentiation because they are derived from a common progenitor, the multipotential mesenchymal stem cells.⁶⁴ Adipose tissue is a significant source of pro-inflammatory cytokines which triggers bone resorption via mechanisms mentioned above.^{65,66} Products of lipid oxidation in dyslipidaemia induce oxidative stress on osteoblast and prevent their differentiation but activate PPAR- γ signalling and promote adipocyte differentiation.¹⁶ In hypertension, competition between sodium and calcium ions reduces calcium reabsorption in the renal proximal tubule and contributes to increased urinary excretion of calcium.¹⁶ In hyperglycaemia, the accumulation of advanced glycosylation end products (AGEs) in collagen could suppress bone remodelling and reduce bone quality, leading to increased fracture risk.^{16,67,68}

In a study by Ishikawa et al, palmitate (1, 5, 10, 40 and 100 μ M for five days) decreased the number of osteoclasts formed from RAW 264.7 macrophages and bone resorption activity indicated by the reduction of total pit formation and fluorescent intensity of conditioned media.⁶⁹ Ishikawa et al also studied the effects of palmitate (1, 5, 10, 40, 100 and 200 μ M for 3 days) on LPS-stimulated MC3T3-E1 pre-osteoblasts.⁷⁰ Palmitate decreased RANKL and osteoprotegerin (OPG) levels in culture supernatants of LPS-stimulated MC3T3-E1 pre-osteoblasts.⁷⁰ Similarly, the RANKL and OPG mRNAs expression of palmitate-treated pre-osteoblasts also reduced compared to untreated LPS-stimulated pre-osteoblasts.⁷⁰ In another study, RAW264.7 cells, mouse clonal stromal cells from bone marrow (ST2 cells), bone marrow cells (BMCs) and bone marrow macrophages (BMMs) were induced with RANKL or 1 α ,25-

dihydroxyvitamin D3 to form osteoclasts followed by palmitate treatment (1, 10, 20 and 40 μ M) for 1 hr.²⁷ Palmitate lowered pit formation and actin ring formation in 1 α ,25-dihydroxyvitamin D3-treated BMC and ST2 co-culture.²⁷ RANKL (but not OPG and macrophage colony-stimulating factor) mRNA expression was also reduced upon palmitate treatment in 1 α ,25-dihydroxyvitamin D3-stimulated ST2 cells.^{27,71} Palmitate, however, did not significantly reduce the osteoclast formation in RANKL-treated BMMs.²⁷ Ishikawa et al validated the in vitro findings using ICR female mice with bilateral ovariectomy (OVX)-induced osteoporosis.⁷⁰ They were treated with 1 and 10 mg/kg/day of palmitate for 13 weeks.⁷⁰ The results showed that palmitate treatment reduced osteoclast number, serum RANKL, OPG and RANKL/OPG ratio in OVX-treated mice.⁷⁰ These results suggest that palmitate may be able to prevent osteoporosis by reducing bone turnover.⁷⁰ However, the effects of palmitate on bone in animal models of MetS have not been established. Further studies should consider validating the antiosteoporotic effects of palmitate using animal models induced by high-fat high-carbohydrate diet, which has been shown to induce deterioration of bone mass, structure and strength.^{18,20}

Osteoarthritis is a degenerative joint disease marked by deterioration in articular cartilage, subchondral bone sclerosis and formation of osteophyte with significant clinical signs, such as chronic pain, joint instability, stiffness, and radiographic joint space narrowing.⁷² In 2015, the World Health Organization estimated that 9.6% of people ≥ 60 years suffered from symptomatic osteoarthritis.⁷³ MetS is one of the primary risk factors of osteoarthritis, apart from age and joint injury.⁷⁴ Apart from increasing mechanical loading to the joint, obesity is associated with increased leptin production, which promotes inflammation and MMP synthesis.^{75,76} Cartilage degradation at the joint leads to localised inflammation and promote the further breakdown of cartilage through MMPs, forming a vicious cycle.⁷⁷

According to Zhou et al, IL-1 β -activated proline chondrocytes treated with palmitate (10, 25, 50, and 100 mg/L for 6 hrs) showed decreased MMP-1, MMP-3, and MMP-13, and increased expression of tissue inhibitor of MMP (TIMP)-1, collagenase II, aggrecan and Dickkopf-related protein 1 (DKK-1).⁷⁸ These changes were attributable to decreased expression of β -catenin, glycogen synthase kinase-3 β (GSK-3 β), Indian hedgehog (Ihh), sonic hedgehog (Shh) and Gli-2 caused by palmitate treatment.⁷⁸ These effects of palmitate were found similar when

Table 2 Health Benefits of Palmatine Related to the Cardiovascular and Musculoskeletal System

Researcher	Study Design	Findings
Cardioprotective activity		
Kim et al 2009 ³¹	Cell lines: Human aortic endothelial HAEC and murine macrophage RAW 264.7 cells Mode of disease induction: LPS-induced inflammation (RAW 264.7 cells) Treatment: 1, 2, 5, 10 μ M of palmatine for 8 hrs (HAEC) or 16 hrs (RAW 264.7 cells) NC: Untreated cells PC: 1 μ g/mL LPS-induced cells (RAW 264.7 cells) Negative: no treatment PC: n.a.	$\uparrow$ HO-1 expression in HAEC cells via a concentration-dependent manner $\downarrow$ LPS-induced HMGB1 release in RAW264.7 cells via a concentration-dependent manner and restore to baseline level at 10 μ M
Kim et al 2009 ³¹	Animals: Male Sprague–Dawley rats (200–250 g) Mode of disease induction: I/R-mediated acute myocardial injury Treatment: 25 and 50mg/kg of palmatine 1 hr before ischemia NC: Sham with 0.25 mL DMSO 1 hr before ischemia DC: 30 mins ischemia followed by either 6h reperfusion (biochemical analysis) or 24 hrs reperfusion (infarct size and heart function) PC: n.a.	Restore I/R-increased LVEDP to baseline $\uparrow$ I/R-reduced $\pm$ dp/dt of left ventricular pressure $\downarrow$ infarct size compared to DC $\downarrow$ I/R-upregulated serum MDA, LDH, CK and $\uparrow$ I/R-reduced SOD and CAT activities $\downarrow$ I/R-upregulated COX-2 and iNOS protein expressions in myocardial tissues
Antiarthritic activity		
Zhou et al 2016 ⁷⁸	Cell line: Primary rabbit chondrocytes Mode of disease induction: IL-1 β -induced OA Treatment: 29.20, 73.01, 146.02, and 292.05 μ M palmatine for 6 hrs pre-treatment NC: Untreated cells DC: 24 hrs of 10 ng/mL IL-1 β PC: n.a.	$\uparrow$ IL-1 β -downregulated TIMP, collagenase II, aggrecan and DKK-1 mRNAs and $\downarrow$ IL-1 β -upregulated MMP-1, MMP-3, MMP-13, β -catenin, GSK-3 β , Ihh, Shh and Gli-2 mRNAs in a concentration-dependent manner $\downarrow$ β -catenin, Ihh & Shh mRNAs compared to NC $\uparrow$ IL-1 β -downregulated TIMP protein expression and $\downarrow$ IL-1 β -upregulated MMP-3, β -catenin, Ihh, Shh and Gli-2 protein levels in a concentration-dependent manner
Zhou et al 2016 ⁷⁸	Animals: 21 New Zealand rabbits weighing (4-week-old, 2.0–2.5 kg) Mode of disease induction: bilateral ACLTs-induced OA Treatment: 0.3 mL articular injection of 100 mg/L/week of palmatine for 5 weeks NC: 0.3 mL of D-Hanks Balanced Salt Solutions DC: OA by ACLTs on knee joints PC: n.a.	$\uparrow$ TIMP-1 mRNA compared to DC and NC groups $\downarrow$ OA-upregulated MMP-3, β -catenin, Ihh, Shh, Gli-2, and TNF- α mRNAs $\downarrow$ OA-upregulated MMP-3, β -catenin, Ihh, Shh, Gli-2, and TNF- α mRNAs $\downarrow$ OA-upregulated β -catenin, Shh and Gli-2 protein expression in articular cartilage via immunohistochemical staining $\downarrow$ erosion and osteophyte formation compared to DC $\downarrow$ cartilage degradation compared to DC
Antiosteoporotic activity		
Lee et al 2010 ²⁷	Cell lines: RAW264.7, BMCs, ST2 cells and BMMs	NS for RANKL-induced osteoclast formation in mouse BMMs
Lee et al 2010 ⁷¹	Mode of disease induction: 1 α ,25-dihydroxyvitamin D3-induced osteoclastogenesis Treatment: 1, 10, 20 and 40 μ M of palmatine for 1 hr NC: Untreated cells DC: RANKL or 1 α ,25-dihydroxyvitamin D3-treated cells PC: n.a.	$\downarrow$ RANKL (NS for OPG & M-CSF) mRNA expression in ST2 cells compared to DC Inhibit pit formation and actin ring formation by BMCs and ST2 co-culture compared to DC

(Continued)

Table 2 (Continued).

Researcher	Study Design	Findings
Ishikawa et al 2015 ⁷⁰	Cell line: MC3T3-E1 cells Mode of disease induction: LPS induction Treatment: 1, 5, 10, 40, 100, and 200 μ M of palmatine for 3 days Negative: no treatment NC: Untreated cells DC: 1 μ g/mL LPS for 24 hrs PC: n.a.	↓ LPS-upregulated RANKL and OPG levels in culture supernatants and their mRNAs expression
Ishikawa et al 2016 ⁶⁹	Cell lines: RAW 264.7 and MC3T3-E1 cells Treatment: 1, 5, 10, 40 and 100 μ M of palmatine for 5 days NC: Untreated co-culture DC: no PC: n.a.	↓ osteoclast number compared to NC ↓ bone resorption activity with the reduced total pit formation and fluorescent intensity of conditioned media compared to NC
Ishikawa et al 2015 ⁷⁰	Animals: Pathogen-free ICR female mice (7-week-old) Mode of disease induction: OVX-induced OP model Treatment: Daily gavage of 1 and 10 mg/kg/day of palmatine for 13 weeks NC: Sham operation mice DC: Bilaterally ovariectomised mice PC: n.a.	↓ OVX-mediated upregulation of osteoclast number, serum RANKL, OPG and RANKL/OPG ratio in a dose-dependent manner

Abbreviations: ↓, decrease or downregulate; ↑, increase or upregulate; $\pm$ dp/dt, first derivative; ACLTs, anterior cruciate ligament transections; BMMs, bone marrow macrophages; CAT, catalase; CK, creatine phosphokinase; COX-2, cyclooxygenase-2; DC, disease control/model; DKK-1, Dickkopf-related protein 1; DMSO, dimethyl sulfoxide; GSK-3 β , glycogen synthase kinase-3 β ; HEAC, human aortic endothelial cells; HMGB1, high mobility group box 1; HO-1, heme oxygenase-1; Ihh, Indian hedgehog; IL-1 β , interleukin-1 β ; iNOS, inducible NO synthase; I/R, ischemia/reperfusion; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MDA, malonaldehyde; MMP-3, matrix metalloproteinase-3; n.a., not available; NC, negative control; NS, not significant; OA, osteoarthritis; OP, osteoporosis; OPG, osteoprotegerin; OVX, ovariectomy; PC, positive control; RANKL, receptor activator of nuclear factor- κ B ligand; Shh, Sonic hedgehog; SOD, superoxide dismutase; ST2, mouse stromal cells from bone marrow; TIMP-1, tissue inhibitors of metalloproteinases; TNF- α , tumor necrosis factor- α .

chondrocytes co-treated with IL-1 β and DKK-1 (the Wnt/ β -catenin signalling inhibitor).⁷⁸ The co-incubation of cyclopamine (a Hedgehog signalling inhibitor) further enhanced the palmatine-mediated downregulation of GSK-3 β , Ihh, Shh and Gli-2, suggestive the possibly inhibition of both Wnt/ β -catenin and Hedgehog signalling pathways upon palmatine treatment.⁷⁸ These changes were validated by Zhou et al in an in vivo model, whereby decreased expressions of MMP-3, β -catenin, Ihh, Shh, Gli-2 and TNF- α with concomitant increased of TIMP-1 expression were observed in New Zealand rabbits with bilateral anterior cruciate ligament transections (ACLTs)-induced osteoarthritis and treated with palmatine (0.3 mL articular injection of 100 mg/L/week for five weeks).⁷⁸ Histologically, palmatine decreased erosion, osteophyte formation and cartilage degradation in the joints of the treated osteoarthritic rabbits.⁷⁸ These results suggest that palmatine may possess antiarthritic effects through reduction/inhibition of inflammatory mediators with the inhibition of Wnt/ β -catenin and Hedgehog signalling

pathways.⁷⁸ The effectiveness of palmatine on MetS-induced osteoarthritis model remains hypothetical due to the absence of animal models mimicking this process.

The detailed health benefits of palmatine have been summarised in Table 2. Figure 1 summarises the protective effects of palmatine on MetS and its associated diseases.

Safety Evaluations of Palmatine

Similar to other isoquinoline alkaloids, palmatine can bind to DNA through groove-binding mechanism by forming hydrogen and van der Waals bonds.⁷⁹ Other studies demonstrated that palmatine binds to poly (A) and tRNA,^{80,81} adenine-thymine homo and hetero polymers through the intercalation.⁸² It also binds preferentially to DNAs that have a long sequence of AT base pairs.⁸³ Besides, it exerts phototoxic effects by generating singlet molecular oxygen after forming complexes with DNA in water solution after photosensitization. The singlet molecular oxygen causes guanine photooxidation, and eventually cleavage of DNA structure.⁸⁴ These phenomena contribute to the anticancer

Figure 1 The protective effects of palmatine on MetS and the associated diseases.

Abbreviations: ↓, decrease or downregulate; ↑, increase or upregulate; ⊣, inhibit or suppress; AGEs, advanced glycosylation end products; ASBT, apical sodium-dependent bile acid transporter; CAT, catalase; COX, cyclooxygenase; C/EBP-α, CCAAT/enhancer-binding protein-α; HDL-c, high-density lipoprotein cholesterol; HFD, high fat diet; HMGBl, high mobility group box 1; HMGCR, 3-hydroxy-3-methyl glutaryl coenzyme A reductase; HO-1, heme oxygenase -1; iNOS, inducible NO synthase; Ihh, Indian hedgehog; LDL-c, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; MAPKs, mitogen-activated protein kinases; MI, myocardial infarction; MMP, matrix metalloproteinases; NfκB, nuclear factor kappa B; O₂, oxygen; OA, osteoarthritis; OP, osteoporosis; OPG, osteoprotegerin; PAL, palmatine; PPAR-γ, proliferator-activated receptor-γ; RANKL, receptor activator of nuclear factor-κB ligand; Shh, Sonic hedgehog; SOD, superoxide dismutase; TG, triglyceride; TIMP-1, tissue inhibitors of metalloproteinases 1; TNF-α, tumor necrosis factor-α; UCP-2, uncoupling protein-2.

activity of palmatine. As evidence, palmatine is reported to cause singlet oxygen generation and DNA fragmentation in human skin epithelial carcinoma cells in a dose- and time-dependent manner.⁸⁵ Similar to cisplatin, a DNA-intercalating agent,⁸⁶ the mutagenic potential of palmatine can cause cellular damage in normal cells and induce secondary malignancy. However, this safety aspect of palmatine has not been explored using normal cells/animal models.

In a study by Yi et al, half-maximal inhibitory concentrations of palmatine on 3T3-L1 and HepG2 cells were 84.32 and 112.80 μg/mL, respectively.⁸⁷ An acute toxicity study showed a median lethal dose (LD₅₀) of 1533.68 mg/kg in mice.⁸⁷ In the subchronic toxicity study, no irregularities in clinical signs, body and organ weight, haematological parameters, gross necropsy and histopathology were observed

following oral administration of palmatine in rats (156 mg/kg; 5 female/5 male for 90 days).⁸⁷ All pharmacological studies reviewed in previous sections did not report mortality following palmatine supplementation.⁸⁷ Thus, palmatine is safe within the therapeutic range but this speculation needs to be validated in human studies. One of the highest doses used for MetS prevention in the reviewed studies is 100 mg/kg in mice.^{51,52} After translation using Reagan-Shaw formula,⁸⁸ this dose is equivalent to 486.5 mg for a 60-kg human, which is feasible for supplementation in humans.

Berberine, an isoquinoline alkaloid belonging to the same group as palmatine, has been demonstrated to have the potential to treat MetS and osteoporosis.^{89–91} Comparison of the therapeutic potential between berberine and palmatine against MetS and its complications is

limited, thus it is unsure which compound is more efficacious. In term of toxicity, the LD₅₀ of berberine (oral administration) in mice is 329 mg/kg,⁹² and a dose as low as 10 mg/kg (intraperitoneal administration) suppressed cellular and humoral immune functions in BALB/c mice.⁹³ Therefore, it could be more toxic than palmatine.

Palmatine is a substrate for P-glycoprotein (P-gp; an excretion transporter/efflux pump), but not for multidrug resistance-associated protein-2, which may justify its relative poor bioavailability.⁹⁴ Consequently, combining palmatine with P-gp substrates at this particular concentration range did not affect its intestinal absorption.^{94,95} In diabetic rats, the level of P-gp protein decrease in the small intestine, which could significantly improve palmatine's intestinal permeability.^{96,97} This effect can help patients with insulin resistance in absorbing palmatine. However, delivering this drug in sufficient concentration to exert its systemic effects remains a challenge.

Conclusion

The evidence reviewed suggests that palmatine possesses therapeutic effect against MetS and its related disorders like cardiovascular disease, osteoporosis and osteoarthritis. Despite the generally positive effects derived from preclinical studies, there is a lack of human clinical trial to validate these findings. For this reason, well-planned human clinical trials should be conducted to bridge this research gap.

Acknowledgments

We thank Universiti Kebangsaan Malaysia for funding the researchers through grant GUP-2020-021. Sophia Ogechi Ekeuku is a postdoctoral researcher funded by Faculty of Medicine, Universiti Kebangsaan Malaysia through FPR-1.

Disclosure

The authors report no conflicts of interest in this work.

References

- Chan KL, Cathomas F, Russo SJ. Central and peripheral inflammation link metabolic syndrome and major depressive disorder. *Physiology (Bethesda)*. 2019;34(2):123–133.
- Wang HH, Lee DK, Liu M, Portincasa P, Wang DQ. Novel insights into the pathogenesis and management of the metabolic syndrome. *Pediatr Gastroenterol Hepatol Nutr*. 2020;23(3):189–230. doi:10.5223/pghn.2020.23.3.189
- Chin K-Y, Wong SK, Ekeuku SO, Pang K-L. Relationship between metabolic syndrome and bone health – an evaluation of epidemiological studies and mechanisms involved. *Diabetes Metab Syndr Obes*. 2020;13:3667–3690.
- Alberti KGMM, Zimmet P, Shaw J. Metabolic syndrome—a new world-wide definition. A consensus statement from the international diabetes federation. *Diabet Med*. 2006;23(5):469–480. doi:10.1111/j.1464-5491.2006.01858.x
- Aguilar M, Bhuket T, Torres S, Liu B, Wong RJ. Prevalence of the metabolic syndrome in the United States, 2003–2012. *JAMA*. 2015;313(19):1973–1974. doi:10.1001/jama.2015.4260
- Ranasinghe P, Mathangasinghe Y, Jayawardena R, Hills AP, Misra A. Prevalence and trends of metabolic syndrome among adults in the asia-pacific region: a systematic review. *BMC Public Health*. 2017;17(1):101. doi:10.1186/s12889-017-4041-1
- Roberts CK, Sindhu KK. Oxidative stress and metabolic syndrome. *Life Sci*. 2009;84(21–22):705–712. doi:10.1016/j.lfs.2009.02.026
- Vona R, Gambardella L, Cittadini C, Straface E, Pietraforte D. Biomarkers of oxidative stress in metabolic syndrome and associated diseases. *Oxid Med Cell Longev*. 2019;2019:8267234. doi:10.1155/2019/8267234
- Spahis S, Borys JM, Levy E. Metabolic syndrome as a multifaceted risk factor for oxidative stress. *Antioxid Redox Signal*. 2017;26(9):445–461. doi:10.1089/ars.2016.6756
- Manna P, Jain SK. Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: causes and therapeutic strategies. *Metab Syndr Relat Disord*. 2015;13(10):423–444. doi:10.1089/met.2015.0095
- Ye J. Emerging role of adipose tissue hypoxia in obesity and insulin resistance. *Int J Obes*. 2009;33(1):54–66. doi:10.1038/ijo.2008.229
- Bhattacharya I, Dominguez AP, Dragert K, Humar R, Haas E, Battagay EJ. Hypoxia potentiates tumor necrosis factor- α induced expression of inducible nitric oxide synthase and cyclooxygenase-2 in white and brown adipocytes. *Biochem Biophys Res Commun*. 2015;461(2):287–292. doi:10.1016/j.bbrc.2015.04.020
- Kosacka J, Kern M, Kloting N, et al. Autophagy in adipose tissue of patients with obesity and type 2 diabetes. *Mol Cell Endocrinol*. 2015;409:21–32. doi:10.1016/j.mce.2015.03.015
- Netzer N, Gatterer H, Faulhaber M, Bartscher M, Pramsohler S, Pesta D. Hypoxia, oxidative stress and fat. *Biomolecules*. 2015;5(2):1143–1150. doi:10.3390/biom5021143
- Montezano AC, Dulak-Lis M, Tsiropoulou S, Harvey A, Briones AM, Touyz RM. Oxidative stress and human hypertension: vascular mechanisms, biomarkers, and novel therapies. *Can J Cardiol*. 2015;31(5):631–641. doi:10.1016/j.cjca.2015.02.008
- Wong SK, Chin K-Y, Suhaimi FH, Ahmad F, Jamil NA, Ima-Nirwana S. Osteoporosis is associated with metabolic syndrome induced by high-carbohydrate high-fat diet in a rat model. *Biomed Pharmacother*. 2018;98:191–200. doi:10.1016/j.biopha.2017.12.042
- Bennett JM, Reeves G, Billman GE, Sturmberg JP. Inflammation—nature's way to efficiently respond to all types of challenges: implications for understanding and managing “the epidemic” of chronic diseases. *Front Med (Lausanne)*. 2018;5:316.
- Wong SK, Chin KY, Suhaimi FH, Ahmad F, Ima-Nirwana S. Effects of metabolic syndrome on bone mineral density, histomorphometry and remodelling markers in male rats. *PLoS One*. 2018;13(2):e0192416. doi:10.1371/journal.pone.0192416
- de la Iglesia R, Loria-Kohen V, Zulet MA, Martinez JA, Reglero G, Ramirez de Molina A. Dietary strategies implicated in the prevention and treatment of metabolic syndrome. *Int J Mol Sci*. 2016;17(11):1877. doi:10.3390/ijms17111877
- Wong SK, Chin KY, Suhaimi FH, Ahmad F, Ima-Nirwana S. The effects of Vitamin E from *Elaeis guineensis* (Oil Palm) in a rat model of bone loss due to metabolic syndrome. *Int J Environ Res Public Health*. 2018;15(9):1828. doi:10.3390/ijerph15091828
- Pang KL, Chin KY. The role of tocotrienol in protecting against metabolic diseases. *Molecules*. 2019;24(5):923. doi:10.3390/molecules24050923
- Long J, Song J, Zhong L, Liao Y, Liu L, Li X. Palmatine: a review of its pharmacology, toxicity and pharmacokinetics. *Biochimie*. 2019;162:176–184. doi:10.1016/j.biochi.2019.04.008

23. Vrba J, Papouskova B, Pyszkova M, et al. Metabolism of palmatine by human hepatocytes and recombinant cytochromes P450. *J Pharm Biomed Anal.* **2015**;102:193–198. doi:10.1016/j.jpba.2014.09.015
24. Tarabasz D, Kukula-Koch W. Palmatine: a review of pharmacological properties and pharmacokinetics. *Phytother Res.* **2020**;34(1):33–50. doi:10.1002/ptr.6504
25. Meng FC, Wu ZF, Yin ZQ, Lin LG, Wang R, Zhang QW. Coptidis rhizoma and its main bioactive components: recent advances in chemical investigation, quality evaluation and pharmacological activity. *Chin Med.* **2018**;13:13. doi:10.1186/s13020-018-0171-3
26. Rios JL, Francini F, Schinella GR. Natural products for the treatment of type 2 diabetes mellitus. *Planta Med.* **2015**;81(12–13):975–994. doi:10.1055/s-0035-1546131
27. Lee J-W, Mase N, Yonezawa T, et al. Palmatine attenuates osteoclast differentiation and function through inhibition of receptor activator of nuclear factor- κ B ligand expression in osteoblast cells. *Biol Pharm Bull.* **2010**;33(10):1733–1739. doi:10.1248/bpb.33.1733
28. Wang L, Wang X, Zhang SL, et al. Gastroprotective effect of palmatine against acetic acid-induced gastric ulcers in rats. *J Nat Med.* **2017**;71(1):257–264. doi:10.1007/s11418-016-1057-2
29. Yan B, Wang D, Dong S, et al. Palmatine inhibits TRIF-dependent NF- κ B pathway against inflammation induced by LPS in goat endometrial epithelial cells. *Int Immunopharmacol.* **2017**;45:194–200. doi:10.1016/j.intimp.2017.02.004
30. Khaksari M, Esmaili S, Abedloo R, Khastar H. Palmatine ameliorates nephrotoxicity and hepatotoxicity induced by gentamicin in rats. *Arch Physiol Biochem.* **2019**:1–6.
31. Kim YM, Ha YM, Jin YC, et al. Palmatine from Coptidis rhizoma reduces ischemia-reperfusion-mediated acute myocardial injury in the rat. *Food Chem Toxicol.* **2009**;47(8):2097–2102. doi:10.1016/j.fct.2009.05.031
32. Pakseresht Z, Norouzi P, Hojati V, Moghaddam KH. Effect of palmatine hydrochloride on oxidative stress in streptozotocin-induced diabetic rats. *J Adv Med Biomed Res.* **2016**;24(107):119–129.
33. Ramli NZ, Chin KY, Zarkasi KA, Ahmad F. The beneficial effects of stingless bee honey from *Heterotrigona itama* against metabolic changes in rats fed with high-carbohydrate and high-fat diet. *Int J Environ Res Public Health.* **2019**;16(24).
34. Li Y, Lee S, Langleite T, et al. Subsarcolemmal lipid droplet responses to a combined endurance and strength exercise intervention. *Physiol Rep.* **2014**;2(11):e12187. doi:10.14814/phy2.12187
35. Misra A, Gupta A, Tank N, Kaklotar D, Singh S, Sharma P. Pharmacotherapy in metabolic syndrome; **2017**:3.
36. Choi JS, Kim JH, Ali MY, Min BS, Kim GD, Jung HA. Coptis chinensis alkaloids exert anti-adipogenic activity on 3T3-L1 adipocytes by downregulating C/EBP- α and PPAR- γ . *Cytoterapia.* **2014**;98:199–208. doi:10.1016/j.fitote.2014.08.006
37. Ning N, He K, Wang Y, et al. Hypolipidemic effect and mechanism of palmatine from *Coptis chinensis* in hamsters fed high-fat diet. *Phytother Res.* **2015**;29(5):668–673. doi:10.1002/ptr.5295
38. Chen HY, Ye XL, Cui XL, et al. Cytotoxicity and antihyperglycemic effect of minor constituents from Rhizoma Coptis in HepG2 cells. *Fitoterapia.* **2012**;83(1):67–73. doi:10.1016/j.fitote.2011.09.014
39. Chiang JY. Bile acids: regulation of synthesis. *J Lipid Res.* **2009**;50(10):1955–1966. doi:10.1194/jlr.R900010-JLR200
40. Han H, Xin P, Zhao L, et al. Excess iodine and high-fat diet combination modulates lipid profile, thyroid hormone, and hepatic LDLr expression values in mice. *Biol Trace Elem Res.* **2012**;147(1–3):233–239. doi:10.1007/s12011-011-9300-x
41. Park WH, Pak YK. Insulin-dependent suppression of cholesterol 7 α -hydroxylase is a possible link between glucose and cholesterol metabolisms. *Exp Mol Med.* **2011**;43(10):571–579. doi:10.3858/emmm.2011.43.10.064
42. Lan T, Rao A, Haywood J, Kock ND, Dawson PA. Mouse organic solute transporter alpha deficiency alters FGF15 expression and bile acid metabolism. *J Hepatol.* **2012**;57(2):359–365. doi:10.1016/j.jhep.2012.03.025
43. Walters JR, Tasleem AM, Omer OS, Brydon WG, Dew T, le Roux CW. A new mechanism for bile acid diarrhea: defective feedback inhibition of bile acid biosynthesis. *Clin Gastroenterol Hepatol.* **2009**;7(11):1189–1194. doi:10.1016/j.cgh.2009.04.024
44. Chiang JYL, Ferrell JM. Bile acid biology, pathophysiology, and therapeutics. *Clin Liver Dis (Hoboken).* **2020**;15(3):91–94. doi:10.1002/cld.861
45. He K, Kou S, Zou Z, et al. Hypolipidemic effects of alkaloids from rhizoma coptidis in diet-induced hyperlipidemic hamsters. *Planta Med.* **2016**;82(8):690–697. doi:10.1055/s-0035-1568261
46. Wan Hasan WN, Chin KY, Abd Ghafar N, Soelaiman IN. Annatto-derived tocotrienol promotes mineralization of MC3T3-E1 cells by enhancing BMP-2 protein expression via inhibiting RhoA activation and HMG-CoA reductase gene expression. *Drug Des Devel Ther.* **2020**;14:969–976. doi:10.2147/DDDT.S224941
47. Haerer W, Delbaere K, Bartlett H, Lord SR, Rowland J. Relationships between HMG-CoA reductase inhibitors (statin) use and strength, balance and falls in older people. *Intern Med J.* **2012**;42(12):1329–1334. doi:10.1111/j.1445-5994.2011.02622.x
48. Notarnicola M, Messa C, Refolo MG, Tutino V, Miccolis A, Caruso MG. Synergic effect of eicosapentaenoic acid and lovastatin on gene expression of HMGCoA reductase and LDL receptor in cultured HepG2 cells. *Lipids Health Dis.* **2010**;9:135. doi:10.1186/1476-511X-9-135
49. Brondani LA, Assmann TS, de Souza BM, Boucas AP, Canani LH, Crispim D. Meta-analysis reveals the association of common variants in the uncoupling protein (UCP) 1–3 genes with body mass index variability. *PLoS One.* **2014**;9(5):e96411. doi:10.1371/journal.pone.0096411
50. Oh KS, Kim M, Lee J, et al. Liver PPAR α and UCP2 are involved in the regulation of obesity and lipid metabolism by swim training in genetically obese db/db mice. *Biochem Biophys Res Commun.* **2006**;345(3):1232–1239. doi:10.1016/j.bbrc.2006.04.182
51. Ma H, Hu Y, Zou Z, Feng M, Ye X, Li X. Antihyperglycemia and antihyperlipidemia effect of protoberberine alkaloids from rhizoma coptidis in HepG2 cell and diabetic KK-Ay mice. *Drug Dev Res.* **2016**;77(4):163–170. doi:10.1002/ddr.21302
52. Semwal DK, Rawat U, Semwal R, Singh R, Singh GJ. Anti-hyperglycemic effect of 11-hydroxypalmatine, a palmatine derivative from *Stephania glabra* tubers. *J Asian Nat Prod Res.* **2010**;12(2):99–105. doi:10.1080/10286020903117325
53. Khademvatan K, Alinejad V, Eghtedar S, Rahbar N, Agakhani N. Survey of the relationship between metabolic syndrome and myocardial infarction in hospitals of Urmia University of medical sciences. *Glob J Health Sci.* **2014**;6(7 Spec No):58–65. doi:10.5539/gjhs.v6n7p58
54. Maeda K, Ruel M. Prevention of ischemia-reperfusion injury in cardiac surgery: therapeutic strategies targeting signaling pathways. *J Thorac Cardiovasc Surg.* **2015**;149(3):910–911. doi:10.1016/j.jtcvs.2014.11.067
55. Monserrat-Mesquida M, Quetglas-Llabres M, Capo X, et al. Metabolic syndrome is associated with oxidative stress and proinflammatory state. *Antioxidants (Basel).* **2020**;9(3).
56. Schanze N, Bode C, Duerschmied D. Platelet contributions to myocardial ischemia/reperfusion injury. *Front Immunol.* **2019**;10:1260. doi:10.3389/fimmu.2019.01260
57. Park JS, Arcaroli J, Yum HK, et al. Activation of gene expression in human neutrophils by high mobility group box 1 protein. *Am J Physiol Cell Physiol.* **2003**;284(4):C870–C879. doi:10.1152/ajpcell.00322.2002
58. Amarasekara DS, Yun H, Kim S, Lee N, Kim H, Rho J. Regulation of osteoclast differentiation by cytokine networks. *Immune Netw.* **2018**;18(1):e8. doi:10.4110/in.2018.18.e8
59. Luo G, Li F, Li X, Wang ZG, Zhang B. TNF α and RANKL promote osteoclastogenesis by upregulating RANK via the NF κ B pathway. *Mol Med Rep.* **2018**;17(5):6605–6611.

60. Soysa NS, Alles N. Positive and negative regulators of osteoclast apoptosis. *Bone Rep.* **2019**;11:100225. doi:10.1016/j.bonr.2019.100225
61. Callaway DA, Jiang JX. Reactive oxygen species and oxidative stress in osteoclastogenesis, skeletal aging and bone diseases. *J Bone Miner Metab.* **2015**;33(4):359–370. doi:10.1007/s00774-015-0656-4
62. Fontani F, Marcucci G, Iantomasi T, Brandi ML, Vincenzini MT. Glutathione, N-acetylcysteine and lipoic acid down-regulate starvation-induced apoptosis, RANKL/OPG ratio and sclerostin in osteocytes: involvement of JNK and ERK1/2 signalling. *Calcif Tissue Int.* **2015**;96(4):335–346. doi:10.1007/s00223-015-9961-0
63. Marathe N, Rangaswami H, Zhuang S, Boss GR, Pilz RB. Pro-survival effects of 17beta-estradiol on osteocytes are mediated by nitric oxide/cGMP via differential actions of cGMP-dependent protein kinases I and II. *J Biol Chem.* **2012**;287(2):978–988. doi:10.1074/jbc.M111.294959
64. Halade GV, Rahman MM, Williams PJ, Fernandes G. High fat diet-induced animal model of age-associated obesity and osteoporosis. *J Nutr Biochem.* **2010**;21(12):1162–1169. doi:10.1016/j.jnutbio.2009.10.002
65. Calder PC, Ahluwalia N, Brouns F, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. *Br J Nutr.* **2011**;106(Suppl 3):S5–S78. doi:10.1017/S0007114511005460
66. Campos RM, de Piano A, da Silva PL, et al. The role of pro/anti-inflammatory adipokines on bone metabolism in NAFLD obese adolescents: effects of long-term interdisciplinary therapy. *Endocrine.* **2012**;42(1):146–156. doi:10.1007/s12020-012-9613-3
67. Chin KY, Ima-Nirwana S, Mohamed IN, et al. Insulin-like growth factor-1 is a mediator of age-related decline of bone health status in men. *Aging Male.* **2014**;17(2):102–106. doi:10.3109/13685538.2014.896895
68. Jackuliak P, Payer J. Osteoporosis, fractures, and diabetes. *Int J Endocrinol.* **2014**;2014:820615.
69. Ishikawa S, Tamaki M, Ogawa Y, et al. Inductive effect of palmatine on apoptosis in RAW 264.7 cells. *Evid Based Complement Alternat Med.* **2016**;2016:7262054. doi:10.1155/2016/7262054
70. Ishikawa S, Ogawa Y, Tamaki M, et al. Influence of palmatine on bone metabolism in ovariectomized mice and cytokine secretion of osteoblasts. *In Vivo.* **2015**;29(6):671–677.
71. Lee WC, Kim JK, Kang JW, et al. Palmatine attenuates D-galactosamine/lipopolysaccharide-induced fulminant hepatic failure in mice. *Food Chem Toxicol.* **2010**;48(1):222–228. doi:10.1016/j.fct.2009.10.004
72. Wang M, Shen J, Jin H, Im HJ, Sandy J, Chen D. Recent progress in understanding molecular mechanisms of cartilage degeneration during osteoarthritis. *Ann N Y Acad Sci.* **2011**;1240:61–69. doi:10.1111/j.1749-6632.2011.06258.x
73. World Health Organization. Chronic rheumatic conditions; **2020**. Available from: <https://www.who.int/chp/topics/rheumatic/en/>. Accessed August 20, 2020.
74. Buchele G, Gunther KP, Brenner H, et al. Osteoarthritis-patterns, cardio-metabolic risk factors and risk of all-cause mortality: 20 years follow-up in patients after hip or knee replacement. *Sci Rep.* **2018**;8(1):5253. doi:10.1038/s41598-018-23573-2
75. Oztas B, Sahin D, Kir H, et al. The effect of leptin, ghrelin, and neuropeptide-Y on serum Tnf-Alpha, Il-1beta, Il-6, Fgf-2, galanin levels and oxidative stress in an experimental generalized convulsive seizure model. *Neuropeptides.* **2017**;61:31–37. doi:10.1016/j.npep.2016.08.002
76. Williams RC, Skelton AJ, Todryk SM, Rowan AD, Preshaw PM, Taylor JJ. Leptin and pro-inflammatory stimuli synergistically up-regulate MMP-1 and MMP-3 secretion in human gingival fibroblasts. *PLoS One.* **2016**;11(2):e0148024. doi:10.1371/journal.pone.0148024
77. Berenbaum F. Osteoarthritis is an inflammatory disease (osteoarthritis is not osteoarthritis!). *Osteoarthritis Cartilage.* **2013**;21(1):16–21. doi:10.1016/j.joca.2012.11.012
78. Zhou X, Lin X, Xiong Y, et al. Chondroprotective effects of palmatine on osteoarthritis in vivo and in vitro: a possible mechanism of inhibiting the Wnt/beta-catenin and Hedgehog signaling pathways. *Int Immunopharmacol.* **2016**;34:129–138. doi:10.1016/j.intimp.2016.02.029
79. Mi R, Tu B, Bai XT, Chen J, Ouyang Y, Hu YJ. Binding properties of palmatine to DNA: spectroscopic and molecular modeling investigations. *Luminescence.* **2015**;30(8):1344–1351. doi:10.1002/bio.2904
80. Islam MM, Pandya P, Chowdhury SR, Kumar S, Kumar GS. Binding of DNA-binding alkaloids berberine and palmatine to tRNA and comparison to ethidium: spectroscopic and molecular modeling studies. *J Mol Struct.* **2008**;891(1):498–507. doi:10.1016/j.molstruc.2008.04.043
81. Giri P, Hossain M, Kumar GS. Molecular aspects on the specific interaction of cytotoxic plant alkaloid palmatine to poly(A). *Int J Biol Macromol.* **2006**;39(4):210–221.
82. Bhadra K, Maiti M, Kumar GS. Molecular recognition of DNA by small molecules: AT base pair specific intercalative binding of cytotoxic plant alkaloid palmatine. *Biochim Biophys Acta.* **2007**;1770(7):1071–1080. doi:10.1016/j.bbagen.2007.03.001
83. Bhadra K, Maiti M, Kumar GS. Interaction of isoquinoline alkaloid palmatine with deoxyribonucleic acids: binding heterogeneity, and conformational and thermodynamic aspects. *Chem Biodivers.* **2008**;5(4):575–590.
84. Hirakawa K, Kawanishi S, Hirano T. The mechanism of guanine specific photooxidation in the presence of berberine and palmatine: activation of photosensitized singlet oxygen generation through DNA-binding interaction. *Chem Res Toxicol.* **2005**;18(10):1545–1552. doi:10.1021/tx0501740
85. Ali D, Ali H. Assessment of DNA damage and cytotoxicity of palmatine on human skin epithelial carcinoma cells. *Toxicol Environ Chem.* **2014**;96(6):941–950. doi:10.1080/02772248.2014.987510
86. Fung C, Dinh P, Ardeshir-Rouhani-Fard S, Schaffer K, Fossa SD, Travis LB. Toxicities associated with cisplatin-based chemotherapy and radiotherapy in long-term testicular cancer survivors. *Adv Urol.* **2018**;2018:8671832. doi:10.1155/2018/8671832
87. Yi J, Ye X, Wang D, et al. Safety evaluation of main alkaloids from *Rhizoma Coptidis*. *J Ethnopharmacol.* **2013**;145(1):303–310. doi:10.1016/j.jep.2012.10.062
88. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J.* **2008**;22(3):659–661. doi:10.1096/fj.07-9574LSF
89. Wong SK, Chin K-Y, Ima-Nirwana S. Berberine and musculoskeletal disorders: the therapeutic potential and underlying molecular mechanisms. *Phytomedicine.* **2020**;73:152892.
90. Cao C, Su M. Effects of berberine on glucose-lipid metabolism, inflammatory factors and insulin resistance in patients with metabolic syndrome. *Exp Ther Med.* **2019**;17(4):3009–3014.
91. Hu X, Zhang Y, Xue Y, Zhang Z, Wang J. Berberine is a potential therapeutic agent for metabolic syndrome via brown adipose tissue activation and metabolism regulation. *Am J Transl Res.* **2018**;10(11):3322–3329.
92. Singh N, Sharma B. Toxicological effects of berberine and sanguinarine. *Front Mol Biosci.* **2018**;5:21. doi:10.3389/fmolb.2018.00021
93. Mahmoudi M, Zamani Taghizadeh Rabe S, Balali-Mood M, et al. Immunotoxicity induced in mice by subacute exposure to berberine. *J Immunotoxicol.* **2016**;13(2):255–262. doi:10.3109/1547691X.2015.1058306
94. Zhang X, Qiu F, Jiang J, Gao C, Tan Y. Intestinal absorption mechanisms of berberine, palmatine, jateorhizine, and coptisine: involvement of P-glycoprotein. *Xenobiotica.* **2011**;41(4):290–296. doi:10.3109/00498254.2010.529180

95. Yu CP, Huang CY, Lin SP, Hou YC. Activation of P-glycoprotein and CYP 3A by coptidis rhizoma in vivo: using cyclosporine as a probe substrate in rats. *J Food Drug Anal.* 2018;26(2S):S125–S132. doi:10.1016/j.jfda.2017.11.005
96. Kobori T, Harada S, Nakamoto K, Tokuyama S. Functional alterations of intestinal P-glycoprotein under diabetic conditions. *Biol Pharm Bull.* 2013;36(9):1381–1390. doi:10.1248/bpb.b13-00369
97. Neearti P, Barla R, Bedada SK. Influence of diabetes mellitus on P-glycoprotein function in rat intestine. *Pharmacologia.* 2011;2:293–298. doi:10.5567/pharmacologia.2011.293.298

Drug Design, Development and Therapy

Dovepress

Publish your work in this journal

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which has also

been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/drug-design-development-and-therapy-journal>