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Therapeutic Potential of Traditional Chinese Medicine on Inflammatory Diseases

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ABSTRACT

Increased oxidative stress induces inflammation to several tissues/organs leading to cell death and long-term injury. Traditional Chinese Medicine (TCM) with antioxidant, anti-inflammatory, anti-apoptotic, and autophagic regulatory functions has been widely used as preventive or therapeutic strategy in modern medicine. Oxidative stress and inflammation have been widely reported to contribute to cigarette smoke–induced lung inflammation, hepatotoxicity, or sympathetic activation–induced liver inflammation, lipopolysaccharide-induced renal inflammation, and substance P–mediated neurogenic hyperactive bladder based on clinical findings. In this review, we introduce several evidences for TCM treatment including Monascus adlay (MA) produced by inoculating adlay (*Cois lachrymal-jobi* L. var. *ma-yuen* Stapf) with *Monascus purpureus* on lung injury, Amla (*Emblica officinalis* Gaertn. of Euphorbiaceae family) on hepatotoxin-induced liver inflammation, Virgate Wormwood Decoction (茵陳蒿湯Yīn Chén Hāo tāng) and its active component genipin on sympathetic activation–induced liver inflammation, and green tea extract and its active components, catechins, or a modified TCM formula Five Stranguries Powder (五淋散Wǔ Lín Sǎn) plus Crataegi Fructus (山楂Shān Zhā) on hyperactive bladder. The pathophysiologic and molecular mechanisms of TCM on ameliorating inflammatory diseases are discussed in the review.

Key words: Apoptosis, Autophagy, Endoplasmic reticulum stress, Inflammation, Oxidative stress, Traditional Chinese Medicine

INTRODUCTION

Traditional chinese medicine

Many Traditional Chinese Medicine (TCM) treatments have been applied in modern medicine to promote health and prevent or cure diseases. Biological activity and physicochemical properties are the two key factors in screening active compounds of traditional Chinese herbs during early discovery phases. As a great number of the compounds have become available today, new strategies are required in order to search active compounds from the natural substances more effectively and economically. Bioinformatics and structural biology, which enabled us to analyze structural–functional relationships in natural compounds and their relatives,

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pushed our understanding while the importance of clinical and epidemiological studies remains unchanged.

Oxidative stress and inflammation

Oxidative stress and inflammation are constant features and major mediators in the development of acute and chronic diseases. Oxidative stress, resulting from a disruption of the natural balance between pro- and antioxidant systems, may contribute to inflammation. Increased reactive oxygen species (ROS) has been recognized as the major factor leading to inflammation. In response to injury, a massive ROS production can cause lipid peroxidation of cellular membranes and protein and DNA oxidation, which result in cellular injury.[1] ROS may be derived from the mitochondria of hepatocytes, the activated macrophages (Kupffer cells), and the infiltrating neutrophils.[1-3] Induction of cytochrome P4502E1 (CYP2E1) and inducible nitric oxide synthase (iNOS) also enhances further oxidative stress in the damaged tissues.[4] These ROS can trigger the translocation of nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1) to nucleus[3] and activation of inflammatory cytokines, chemokines, and adhesion molecules [intercellular adhesion molecule-1 (ICAM-1)] that, in turn, can contribute to further production of ROS[1,2,5] and consecutively activate the cascade of Bax and cytochrome *c* translocation and caspases (apoptosis).[2] Also, the enhanced ROS evokes other types of cell death like autophagic cell death, necrotic cell death, and pyroptotic cell death.[2] For example, in organs subjected to septic shock, hemorrhage, and ischemia/reperfusion, the overproduced ROS triggered early cellular signal transduction pathways responsible for the activation of NF-κB and AP-1, resulting in upregulation of the ICAM-1 gene in the vascular endothelium and subsequent tissue accumulation of activated neutrophil accumulation.[3,6] Catechins with antioxidant, anti-apoptotic, and anti-inflammatory activity can inhibit redox-sensitive transcription factors in cancer cells, in hepatic stellate cells in hepatic fibrosis, and in insults to cells or tissues mediated by ROS.[3] Effective scavenging of ROS or maintenance of the cellular redox state may represent a useful therapeutic approach for limiting inflammation- and apoptosis-mediated oxidative injury.

The activated monocyte/macrophage cells, infiltrated leukocytes, and/or resident cells are the sources of ROS generated in the tissue/organ and are subject to oxidative injury.[2] Attachment of activated leukocytes to the vascular endothelium and the subsequent production of ROS are the key events in the inflammatory course.[2,3] Leukocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-derived ROS signaling also plays a role in the induction of inflammation.[7] The use of TCM to treat inflammatory diseases has been widely investigated.[8] We discuss several inflammatory diseases induced in the animal models and the application of several efficient TCM in reducing inflammatory diseases in the subsequent sections.

CIGARETTE SMOKE INDUCED LUNG INFLAMMATION

Cigarette smoke (CS) is a complex mixture of more than 4700 chemical compounds including free radicals and oxidants. CS exposure causes an oxidant/antioxidant imbalance, leading to increased oxidative stress and decreased antioxidant defense mechanisms.[9] Toxicity in CS may be due to the combined action of these compounds inducing many cellular processes mediated through ROS. The major player is probably nicotine as it is present in tobacco in higher concentrations. Nicotine may induce intracellular oxidative stress, recognized as the important agent involved in the damage of biological molecules. Moderately higher concentrations of some forms of ROS like NO and H_2O_2 can act as signal transducing agents. NF-kB, an inducible transcription factor detected in lung epithelial cells or neurons, is found to be involved in many biological processes such as inflammation, innate immunity, development, apoptosis, and anti-apoptosis.[10] Electromobility shift analysis showed that nicotine activates inducible NF-kB by binding to consensus sequence of DNA. Further activation of c-Jun terminal kinase indicates that nicotine induces oxidative stress leading to activation of stress-dependent NF-kB pathway in the damaged cells or tissues.[10]

Production of ROS is greatly induced by CS and evokes abnormal signal transduction and cellular dysfunction, initiating an oxidative stress cascade that leads to endoplasmic reticulum (ER) stress and autophagy/apoptosis/necrosis in the bronchial epithelial cells or alveolar cells.[5,11] Increases in ROS promote mitochondrial dysfunction-induced caspase-dependent apoptosis and Beclin-1/LC-3–dependent autophagy, as well as ER stress-mediated apoptosis.[12] Overexpression of placenta growth factor (PlGF) by PlGF transgenes[13,14] contributes to pulmonary emphysema pathogenesis.

Monascus adlay and cigarette smoking induced lung inflammation

Monascus adlay (MA) products are made from fungi, such as *Monascus* spp., and grass crops, such as adlay (*Cois lachrymal-jobi* L. var. *ma-yuen* Stapf; also known as Chinese pearl barley and soft-shelled Job's tears). Products of fermented *Monascus* spp. (e.g., anka and red koji) were first mentioned in the ancient Chinese pharmacopoeia, Pen-Chow-Kang-Mu (Systematic Pharmacopoeia) by Li, SC in 1596, and are widely used as a Chinese cuisine.[15,16] The major metabolic component in fermented *Monascus* species is lovastatin (also known as monacolin K), which possesses hypocholesterolemic, anti-fibrosis, anti-inflammatory, antioxidant, and anti-apoptosis properties.[17] A recent report indicated that red mold rice can be applied to reduce hepatic inflammatory damage in Zn-deficient rats.[18] On the other hand, adlay is widely planted in Taiwan, China, and Japan. It not only has a high nutritional value but also is effective in the treatment of warts, chapped skin, rheumatism, and neuralgia, as well as has more general anti-inflammatory, antioxidant, and antitumor properties.[19,20] Evidence shows that MA extracts display higher antioxidant activity, reducing power, scavenging and chelating abilities, and higher total phenol content than uninoculated adlay products.[15] We recently (in 2013) found that components of dietary MA, namely lovastatin and phenolic compounds, synergistically enhance antioxidant and anti-inflammatory defense mechanisms, suggesting their counteracting effect on oxidative stress-induced diseases in MA consumers.

Lovastatin and adlay have previously been used in the treatment of pulmonary disorders, and their effectiveness has been linked to

their antioxidative stress properties. Lovastatin can reduce tissue myeloperoxidase content, bronchoalveolar lavage leukocyte accumulation, proinflammatory cytokine release, and NADPH oxidase expression in the ischemia/reperfusion lung.[21] Lovastatin can improve endothelial function, blunt oxidative stress and inflammation, and attenuate endothelial progenitor cell apoptosis.[17] Furthermore, lovastatin can efficiently restore catalase and glutathione peroxidase activities and nitric oxide levels and improve structural alterations in the diabetic lung.[9] Among rats, consumption of adlay extracts has been shown to suppress microsomal cytochrome P4501A1 enzyme activities and protein expression, increase glutathione content, and glutathione peroxidase, glutathione reductase, and glutathione *S*-transferase activities in rat lungs in a tissue-specific manner.[20] Phenolic components from adlay can inhibit the release and secretion of inflammatory mediators/cytokines[22,23] and decrease O_2^- production/generation.[24]

MA contains higher levels of crude ash, fat, fiber, and protein than uninoculated adlay,[14] indicating its nutritional potential. Previous work on methanolic extracts implicated MA is more effective than adlay at scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and chelating ferrous ions.[15] Our study[5] further shows MA to be more effective than lovastatin in reducing O_2^- and H_2O_2 counts. Trans-coniferyl aldehyde, a phenolic compound in adlay, was recently found to efficiently scavenge DPPH radicals and inhibit O_2^- generation.[24] MA is also characterized by a high content of lovastatin and total phenolic compounds and stronger anti-O_2^- and anti-H_2O_2 activities than either of its source materials (*Monascus purpureus* Went and adlay) alone. Cumulatively, daily intake of MA or lovastatin can significantly suppress CS-induced oxidative stress, ER stress, autophagy, and apoptosis in the rat lung.[5]

CS-induced lung injury is possibly due to an increase in oxidants that may be generated either by CS itself or by inflammatory cells such as neutrophils and macrophages. Clinical findings show that CS increases airway oxidative stress and recruits inflammatory cells into smokers' lungs. Evidence also showed CS exposure is associated with higher levels of neutrophils and infiltrated leukocytes and accumulation of nitroblue tetrazolium (NBT) deposits and 4-hydroxynoneal (4-HNE) in the bronchiole epithelium, the walls of alveolar septal cells, vascular cells, and leukocytes in the lung.[5] NADPH oxidase contains five components: p40phox, p47phox, and p67phox in the cytosol and p22phox and gp91phox in the membrane for producing O_2^-. Chronic alcohol ingestion increases O_2^- production by enhanced gp91phox expression in the lung.[25] CS also enhances O_2^- production by activating lung gp91phox expression.[5]

The increased oxidative stress may evoke ER stress,[26] autophagy, and apoptosis in the airway, alveolar epithelial cells, and even the endothelial cells, leading to structural damage in the lung. Crowley-Weber *et al*,[27] found that nicotine can activate GRP78 in human hepatoma cells, leading to apoptosis. Prolonged ER stress in endothelial cells subjected to CS stimuli is followed by induction of autophagy, which is characterized by an increase in LC3 II/I ratio and ATG12 activation.[28] Long-term CS-induced emphysematous alveolar septa are characterized by thin and almost avascular alveoli possibly due to increases in the apoptotic appearance of epithelial and endothelial cells in the lung.[13,29] A previous study indicated that apoptotic septal cells in the emphysematous lung of PlGF transgenic mice were mainly type II pneumocytes and partly endothelial cells.[13] Titanium dioxide nanoparticle inhalation also induced PlGF upregulation and may cause pulmonary emphysema.[30] CS increases ROS levels in a dose-dependent manner, leading to increases in the Bax/Bcl-2 ratio and the number of caspase 3–mediated poly-(ADP-ribose)-polymerase (PARP) fragments (which play a role in the mitochondria-mediated intrinsic apoptosis pathway); this, in turn, promotes apoptosis in the bronchiole epithelium and alveolar septal wall.[5] This report indicates that increased oxidative stress contributes to ER stress, Beclin-1 autophagy, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells, and PlGF overexpression in damaged bronchiole epithelial cells and alveolar parenchyma.[5]

How might MA suppress CS-induced lung injury? There are two possible mechanisms. First, it may act a source of lovastatin and phenolic compounds to prevent oxidative stress and inflammation. This is supported by the finding that CS-exposed rats treated with MA had higher levels of lung Bcl-2, manganese superoxide dismutase (MnSOD), and catalase expression than the rats that were exposed to CS but not given the MA supplement.[5] CS decreased MnSOD expression in the lungs;[31] however, MA preserved lung MnSOD in response to CS. In addition, the MA-treated individuals had lower levels of gp91Phox expression, ROS formation, NBT deposits, and 4-HNE levels than the animals that were exposed to CS but not given MA. Second, both MA and lovastatin decrease autophagy and apoptosis by suppressing gp91Phox-mediated oxidative stress, GRP78-mediated ER stress, Beclin-1–regulated autophagy, and Bax/Bcl-2–mediated intrinsic (mitochondria-dependent) apoptotic pathways. These results are similar to the previous observations from Kim *et al*.[29] They found protection against CS-induced cell death by adenoviral transfection of antioxidant gene hemeoxygenase-1, which subsequently inhibited apoptosis and autophagy-related signaling.

In conclusion, supplementary MA suppresses CS-induced lung injury by preserving antioxidant defense mechanisms and decreasing oxidative stress. It is evident that these anti-CS activities of MA stem from its lovastatin and phenolic components. We also recognize that dietary MA can reduce CS-induced acute lung injury by inhibiting ER stress, autophagy, apoptosis, and PlGF expression and, therefore, may be a beneficial nutritional therapy for oxidative stress–related diseases.

N-NITROSODIETHYLAMINE–INDUCED LIVER INFLAMMATION

Hepatocellular carcinoma (HCC) is the most common type of liver cancer, representing 83% of all cases. It represents the third cause of cancer-related deaths and the first cause of death amongst cirrhotic patients. Hepatitis viral infection, food additives, alcohol, fungal toxins (aflatoxins), toxic industrial chemicals, air and water pollutants are the major risk factors of HCC.[32] *N*-nitrosodiethylamine (DEN) is one potent hepatocarcinogenic nitrosamine present in tobacco smoke, water, cheddar cheese, cured and fried meals, occupational settings, cosmetics, agricultural chemicals, and pharmaceutical agents.[33,34] DEN produces the pro-mutagenic products, O^6-ethyl deoxy guanosine and O^4- and O^6-ethyl deoxy

thymidine in livers, leading to HCC.[35,36] These toxic products produced by DEN may evoke oxidative stress and inflammation in livers, consequently leading to HCC.

Overproduction of ROS leads to DNA damage and mutagenesis associated with various stages of liver injury.[3,37,38] These increased ROS may be derived from the mitochondria of hepatocytes, the activated Kupffer cells, and the infiltrating neutrophils.[3,38] Induction of CYP2E1 and iNOS also enhances further oxidative stress in the damaged liver.[39] These ROS trigger translocation of NF-kB and AP-1 to the nucleus and activation of several inflammatory cytokines and adhesion molecules, contributing to further production of ROS and consecutive cell death.[3]

DEN-induced liver injury is associated with increased neutrophil and Kupper cell infiltration, oxidative stress marker accumulation, hepatocyte apoptosis and autophagy, and plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (γ-GT) activities, with concurrent increased ROS levels in the liver and the secreted bile.[40]

Amla (*Emblica officinalis* Gaertn. of Euphorbiaceae family) and liver inflammation

Recent advances in the use of herbal treatment for non-alcoholic fatty liver disease have been reviewed.[41] Amla (*Emblica officinalis* Gaertn. of Euphorbiaceae family) has been used as the major constituent of various valuable Indian polyherbal formulations for its liver protecting activity.[42] Its fruits being rich sources of polyphenols and ascorbic acid are found to be effective as hypolipidemic and antiathrosclerotic agents to reduce serum and hepatic cholesterol in rabbits.[43] Additionally, Amla possesses anti-mutagenic and anti-carcinogenic properties owing to the combined presence of β-carotene, ascorbic acid, and chlorophyllin and modulates several biochemical events associated with tumor promotion.[44,45]

The active components in Amla possibly are tannoids, vitamin C like materials, and tannins.[46-48] In the aqueous extracts of processed and dried fruit, vitamin C accounts for approximately 45-70% of the antioxidant activity.[48] The effectiveness of the fruits is not only attributed to the high content of ascorbic acid, but also partly attributed to tannins such as emblicanin A and emblicanin B.[48] Components of polyphenol compounds in the amla are as follows: 3.458% gallic acid, 0.635% corilagin, 0.880% geraniin, 0.659% elaeocarpus, 1.240% chebulagic acid, and 0.220% ellagic acid.[49]

ROS are regularly produced *in vivo* as a result of carcinogen treatment causing oxidative stress that leads to damage of nucleic acids, proteins, and lipids, resulting in chromosomal instability, mutations, loss of organelle function, and membrane damage, which play an important mechanistic role in the development of cancer.[37] Autophagy and apoptosis also concomitantly occurred in the DEN-treated liver.[40]

Amla treatment significantly counteracted the DEN-induced leukocyte and Kupffer cell infiltration and significantly depressed the DEN-enhanced oxidative stress and partially restored the DEN-depressed antioxidant protein expression in the livers.[40] Amla treatment decreased DEN-induced apoptosis and autophagy production in the livers and consequently reduced the ALT, AST, and g-GT activities.[40] Polyphenolic compounds found in various TCM downregulated the inflammatory responses through inhibiting iNOS, cyclooxygenase-2 (COX-2), and CYP2E1 via their inhibitory effects on NF-kB or AP-1 in anti-inflammatory and anti-aging mechanisms.[50] Oral administration of berberine (50 mg/kg) inhibited the iNOS expression and CYP2E1 activity in the DEN- plus phenobarbital-treated livers, suggesting that the anti-HCC potential of berberine might be due to inhibition of oxidative metabolic activity of CYP2E1 and decrease in NO production in rats.[39]

In conclusion, Amla suppressed the DEN-induced liver oxidative injury by scavenging ROS and decreasing apoptosis and autophagy production in the liver. Amla can modulate the liver function and significantly and partially preserve the endogenous antioxidant defense mechanisms in DEN-induced hepatocellular toxicity. Amla is promising as an effective drug or healthy food in cancer prevention and cellular protection in the liver.

VIRGATE WORMWOOD DECOCTION (茵陳蒿湯 YĪN CHÉN HĀO TĀNG) AND GENIPIN ON SYMPATHETIC ACTIVATION–INDUCED LIVER INFLAMMATION

According to previous studies,[3,51] exacerbated activation of hepatic sympathetic nerve increases the release of norepinephrine and subsequently reduces portal venous blood flow, oxygenation, and bile flow. The hepatic dysfunction includes ROS generation, proinflammatory response, and liver injury. Genipin with its O_2^-, H_2O_2, and HOCl scavenging activity can reduce norepinephrine-induced ROS in the Kupffer cells and endothelial cells. Hepatic sympathetic denervation, Virgate Wormwood Decoction and genipin treatment exerted hepatoprotective and choloretic activity to ameliorate sympathetic activation–induced liver injury.[51]

Genipin treatment inhibited norepinephrine-enhanced ROS levels in the endothelial and Kupffer cells in *in vitro* culture in a dose-dependent manner.[51] All the impaired responses found *in vivo*, however, can be ameliorated by hepatic sympathetic denervation, Virgate Wormwood Decoction, and genipin treatment. There are several mechanisms for the induction of hepatoprotective effects by Virgate Wormwood Decoction and genipin. First, the improvement of bile flow secretion may be by genipin-enhanced Mrp2 density in the canalicular membrane and microvilli and genipin-stimulated exocytosis and insertion of Mrp2 in the bile canaliculi.[52] Second, a similar formula containing Herba Artemisiae, Fructus Gardeniae, Radix Scutellariae, and Rhizoma Rhei has been successfully used in the treatment of neonatal serum total bilirubin and the incidence of hyperbilirubinemia.[53] Among the Virgate Wormwood Decoction containing components, flavonoid-like component can prevent inflammation and ischemia/reperfusion-induced oxidative injury[54] and crocetin component can improve lipopolysaccharide (LPS)- or prostaglandin E2-induced inflammation.[55] Genipin with anti-inflammatory and antithrombotic effects[56] and geniposide with detoxication, antioxidant, and anticarcinogenesis potential[57] inhibit the production of exudate and NO in the rat air pouch edema.[58] Genipin and geniposide have also been shown to inhibit LPS/interferon-gamma (IFNg)-induced lipid peroxidation, NO production, iNOS expression, and

I-kappaB-beta degradation in a murine macrophage cell line.[59] Genipin inhibits hepatocyte cell death by suppression of Fas-caspase 3 mediated apoptosis.[60] Because antioxidant flavonoids from Crataegi Fructus or catechins downregulate ROS, ICAM-1 expression, and leukocyte adhesion in neurogenic inflammation.[6,7]

Virgate Wormwood Decoction and genipin treatment decreased sympathetic activation–enhanced liver ROS, ICAM-1 expression, and neutrophil NADPH oxidase activity and consequently reduced plasma AST level. Virgate Wormwood DecoctionVirgate Wormwood Decoction and genipin may have anti-vasoconstriction, antioxidant, anti-inflammatory, anti-adhered, and anti-apoptotic cell death activity to prevent hepatotoxin or sympathetic activation–induced oxidative injury in the rat liver.

CORDYCEPS SOBOLIFERA AND LPS-INDUCED ACUTE RENAL FAILURE

Cordyceps cum Larva Cicadae (蟬花 Chán Huā; *Cordyceps sobolifera* (CDS)) is one kind of economic traditional Chinese herb and is famous as *Cordyceps sinesis* (冬蟲夏草 Dōng Chóng xià cǎo). Based on the viewpoint of TCM, the therapeutic effects of *Cordyceps* include being as nutritious materials, enhancement of physical strength, reduction of fatigue syndrome, action as analgesic and antipyretic agent, regulation of immune system, anti-oncogenesis, and improvement of renal function. CDS can improve the clearance of creatinine and blood urea nitrogen (BUN) as well as the decrease in urinary protein.[61] CDS at 30 g has been used to prevent the progression of chronic renal failure in early or intermediate stage in human beings. In addition, CDS may improve the reduction of tubulointerstitial lesion, which may be contributed by the damage of nephron tubular Na^+/K^+ pump, cell-mediated oxidation to the endothelium of vessel and tubular cell, and decreased renal blood flow.[61] The possible mechanism for renal protection has been suggested that CDS pretreatment attenuates glomerulosclerosis by activating urokinase-type plasminogen activator for decreasing the accumulation of extracellular matrix.

In LPS-induced renal failure, renal tubular structure was impaired possibly by the enhanced ER stress, apoptosis, or autophagy.[61] In contrast, CDS treatment caused a significant decrease in the ratio of Bax/Bcl-2 when compared with the LPS group. Basically, the rise in Bcl-2 and drop in Bax would prevent cytochrome *c* to move outside from the mitochondria, reduce the increased levels of ROS, and thus attenuate programmed cell death in the renal tubule.[2] There is limited literature to evaluate the effect of LPS on ER stress. Autophagy and apoptosis concomitantly occurred in the damaged distal tubule and proximal tubular cells of the damaged kidney.[2] The enhanced response of apoptosis and autophagy to LPS injury, primarily found in the renal cortex and outer medulla, was diminished by long-term CDS pretreatment. Two months of CDS supplementation could attenuate LPS-induced apoptosis, autophagy, ER stress, and ED-1 infiltration, possibly by the upregulation of anti-apoptotic protein and downregulation of pro-apoptotic and autophagic proteins' expression.

ER stress and oxidative stress are known to contribute to apoptotic cell death in response to several stresses.[62] LPS increased ER molecular stress chaperone GRP78 and caspase 12 protein expression and also increased autophagy-Beclin-1/LC3 and apoptosis-PARP protein expression. LPS induced ER stress, autophagy, and apoptosis signaling in the damaged rat kidneys.[61] Chronic CDS treatment significantly attenuated ER stress, autophagy, and apoptosis in the renal tubules subjected to LPS insults, leading to the improvement of renal function.

ROS AND HYPERACTIVE BLADDER

Hyperactive bladder is a bladder dysfunction characterized by increased urinary frequency and/or urge incontinence. Many clinical conditions can cause disturbances to normal urination, such as stress urinary incontinence, urinary retention, overactive bladder, interstitial cystitis, prostatitis, benign prostatic hyperplasia, and urinary tract infections. Many patients suffer from hyperactive bladder, but it is not completely controlled with modern western medicine.

It was previously reported that substance P (SP) facilitates bladder afferent signaling through neurokinin type 1 receptor and induces ROS formation.[1,7] The tachykinin SP belongs to a family of neuropeptides that are widely distributed in the mammalian central and peripheral afferent nervous systems and produce their biological actions by activating three distinct receptor types, neurokinin types 1, 2, and 3.[63] The neurotransmitter primarily located in afferent nerves commonly innervates smooth muscle, submucosal layers, and blood vessels of visceral organs including urinary bladder.[1,7,63] Once released from the activated sensory afferents, SP, through neurokinin type 1 and 2 receptors, acts on smooth muscle or blood vessels to regulate visceral motility and blood flow and evokes visceral inflammation, hyperreflexia, and hyperalgesia.[1,63] Furthermore, SP may increase adhesion molecules' expression by endothelial cells, lead to chemotaxis and activation of immune cells, and increase mucus secretion, and water absorption/secretion in the lungs, gastrointestinal tract, and genitourinary tract.[1,7,63] Clinical trial shows that bladder biopsies in some patients diagnosed with interstitial cystitis (one type of hyperactive bladder) have increased density of SP-containing fibers and mRNA of neurokinin type 1 receptors.[64,65] Administration of SP has also been known to cause bladder inflammation and generation of ROS by inflammatory cells.[1,7] Additionally, the release of SP by an exogenous route or electrical stimulation of pelvic afferent nerves to urinary bladder evokes a set of responses (loosely defined as neurogenic inflammation) that includes vasodilatation, plasma protein extravasation, smooth muscle contraction, inflammation, several adhesion molecules' expression, and leukocyte adhesion and infiltration to the tissue.[1,63] SP-induced hyperactive bladder could also be from the central nervous system, because spinal administration of SP enhanced the bladder contractility and spinal administration of neurokinin type 1 receptor antagonist blocked the micturition responses.[1]

In the inflammatory course, neutrophils' activation, adhesion, migration, and subsequent generation of highly ROS including O_2^- and H_2O_2 impair endothelial function and smooth muscle activity.[1,7,66] ROS are known to affect muscle tone and vascular smooth muscle strip contraction, and increase the neural activity/conduction velocity *in vitro* by mechanisms such as alterations in

membrane conductance, calcium homeostasis, calcium-dependent processes, and eicosanoid and nitric oxide metabolism.[67] The neutrophil NADPH oxidase–derived oxidant signaling is an important determinant of the crosstalk between inflammation and the control of endothelial cell activation.[68] SP mediated neurogenic inflammation and activated the neutrophils to release large amount of O_2^- and H_2O_2 by activating and priming the neutrophil NADPH oxidase response.[69-71] These observations indicate SP behaves as mediator of inflammation, which is triggered by NADPH oxidase–derived ROS signaling leading to bladder hyperactivity.

Green tea extracts and hyperactive bladder

People living in Far East countries including China, Taiwan, and Japan have a tradition of green tea consumption. Green tea is made from the leaf of tea plant *Camellia sinensis*. For more than 4000 years, its use has been recommended to treat headaches, fatigue and depression, general discomfort, insufficient digestion, and sleepiness. It contains many polyphenols that might affect inflammatory process. Green tea extracts containing flavonoids and polyphenols like (+)-catechin (C), (−)-epicatechin (EC), (+)-gallocatechin (GC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epigallocatechin gallate (EGCG) have been reported to exert protective effects against cancer, as well as inflammatory and cardiovascular diseases.[72] The green tea extract containing several catechins can inhibit proinflammatory and pro-apoptotic oxidative injury by reducing the production of ROS, translocation of NF-κB and AP-1, and expression of ICAM-1.[3,73] Recently, TCM are rapidly gaining attention in the West as sources of new drugs, dietary supplements, and functional foods. Green tea now is widely used as a TCM, functional beverage, and dietary supplement.[6] Green tea extracts can scavenge hydroxyl radical and HOCl activity.[3,73] Green tea extracts efficiently scavenge several ROS *in vitro* and *in vivo* and are supposed to be better than vitamins A, E, and C with regard to antioxidant and anti-inflammatory activities.[3,66,73] Flavonoids (including catechins) differentially regulate IFNg-induced ICAM-1 expression in human keratinocytes and catechins are also known to promote anti-inflammatory and antioxidant activities.[3,63,73]

In leukocytes incubated with SP, ROS activity is displayed in a dose-dependent manner and SP-induced ROS release is greatly inhibited by catechins, confirming a downregulating effect on the oxidative stress by catechins. Catechins inhibit histamine release from the mast cells through inhibiting tyrosine phosphorylation of proteins (focal adhesion kinase 125). Flavonoids (including catechins) differentially regulate IFNg-induced ICAM-1 expression in human keratinocytes and catechins promote anti-inflammatory and antioxidant activities.[72,74,75] Attachment of leukocytes to the vascular endothelium by upregulation of ICAM-1 is one potentially early event in the inflammatory course.[72,74] Catechins also downregulate ICAM-1 expression in the SP-induced bladder tissues.[75]

Five stranguries powder (五淋散 Wǔ Lín Sǎn) plus crataegi fructus (山楂 Shān Zhā)and hyperactive bladder

Our clinical experience suggests possible indication of TCM formulae for the treatment of urinary tract disorders including hyperactive bladder as well as chronic urinary tract infections. Among many TCM prescriptions, three famous formulae, namely Five Strangury Powder, Ju Ling Tang (originated from On Cold Damage (傷寒論 Shāng Hán Lùn)), and Lung Tan Hsieh Kan Tang, are frequently employed in Taiwan and China. In the pathophysiology of TCM, Five Stranguries Powder (Gardenia (山栀 Shān Zhī) and Hoelen (茯苓 Fú Líng) Formula or six-ingredient powder for painful urinary dysfunction) is indicated to patients with chronic urinary inflammations because it is believed to remove endogenous heat and promote urination. Oral administration of a Kampo and TCM formula containing Gardeniae Fructus extract and other ingredients found in Five Stranguries Powder plays a therapeutic role in the anxiolytic effect of Kamishoyosan (Japanese traditional Kampo medicine).[76] Five Stranguries Powder has been applied to patients with urinary difficulty, edema, vomiting, and diarrhea in our clinical experience.

The dried fruit of *Crataegus pinnatifida*, used as a local soft drink material and medical herb, had demonstrated its antioxidant effect in previous studies.[77,78] The fruits of the species Crataegus pinnatifida (Chinese Shan Zha) are tart, bright red, and resemble small crabapple fruits. The dried fruits of *Crataegus* pinnatifida (Shan Zha) are used in naturopathic medicine and traditional Chinese medicine. The constituents of *Crataegi* Fructus responsible for its antioxidant properties are identified as flavonoids, hyperoside, isoquercitrin, epicatechin, chlorogenic acid, quercetin, rutin, and protocatechuic acid.[77] The flavonoid content in the dried fruit of Crataegus can exert its anti-inflammatory effect to decrease the release of prostaglandin E2 (PGE_2) and nitric oxide in LPS-treated macrophages.[78] The polyphenolic contents of EC and hyperoside in the Crataegus species enhanced the antioxidant capacity of the extract.[78,79] Pretreatment of Crataegus flavonoids decreased ROS production, thiobarbituric acid reactive substances content, and nitrite/nitrate concentration in tissue and increased the antioxidant level in tissue for reduction of oxidative injury.[80] Furthermore, treatment with *Crataegus* flavonoids can lower the level of tumor necrosis factor-α (TNF-α) and NF-κB and increase iNOS mRNA level.[80] The accumulated data revealed the anti-inflammatory and antioxidant potential of Crataegi Fructus, which may be administered alone or added in the TCM formula like Five Stranguries Powder forming a modified formula to further enhance its clinical therapeutic potential.

In neutrophils incubated with SP, NADPH oxidase activity and ROS production are increased in a dose-dependent manner, and these responses are significantly decreased by Five Stranguries Powder, Crataegi Fructus, or Five Stranguries Powder plus *Crataegi* Fructus, confirming a downregulating effect on oxidative stress by TCM. SP-induced ROS generation in the bladder, as well as bladder hyperreflexia was markedly ameliorated by oral Five Stranguries Powder plus *Crataegi* Fructus. Oral administration of a Kampo and TCM formula containing Gardeniae Fructus extract and other ingredients found in WLS plays a therapeutic role in the anxiolytic effect of Kamishoyosan (Japanese traditional Kampo medicine).[76] The anti-inflammatory potential of flavonoid contents from the dried fruit of *Crataegus* decreased the release of PGE_2 and nitric oxide, induced by LPS in macrophages.[78] *Crataegus* species subjected to drought and

cold stress treatments caused increases in polyphenolic levels of EC and hyperoside and, thus, enhanced the antioxidant capacity of the extracts.[75,79] Pretreatment of Crataegi Fructus (*Crataegus* flavonoids) decreased ROS production, thiobarbituric acid reactive substances content, and nitrite/nitrate concentration in the brain homogenate and increased the brain homogenate–associated antioxidant level in brain ischemic insults.[80] Additionally, pretreatment with *Crataegi* Fructus (Crataegus flavonoids) could decrease the protein level of TNF-α and NF-κB and increase the mRNA and protein level of iNOS, consequently protecting against brain cell death caused by ischemia/reperfusion injury.[80] Furthermore, it was found that pretreatment with flavonoid contents from the dried fruit of *C. pinnatifida* (Crataegi Fructus) decreased the hepatic expression of iNOS and COX-2 induced by LPS in rats.[78] The inhibitory effect of green tea extract supplementation as a sole source of drinking solution leads to scavenging of ROS, inhibition of COX-2, and inactivation of phosphorylated forms of NF-κB.[81] Intragastric administration of ethanol to male Sprague-Dawley rats caused significant gastric mucosal damage, which was accompanied by elevated expression of COX-2 and iNOS as well as transient activation of redox-sensitive transcription factors such as NF-κB and AP-1.[82] Simultaneous administration with the water extract of Five Stranguries Powder, Crataegi Fructus, or Five Stranguries Powder plus Crataegi Fructus all significantly reduced SP-induced neutrophil NADPH oxidase activity.[7] However, the antioxidant activity in reduction of neutrophil NADPH oxidase activity was displayed in the order Five Stranguries Powder plus Crataegi Fructus > Crataegi Fructus > Five Stranguries Powder. In an *in vivo* study of SP-induced bladder hyperactivity, oral pretreatment of Five Stranguries Powder, Crataegi Fructus, or Five Stranguries Powder plus Crataegi Fructus for 1 week potentially ameliorated SP-induced hyperactivity and ROS generation from the bladder surface.[7] Oral Five Stranguries Powder plus Crataegi Fructus exerted a more efficient potential than Crataegi Fructus or Five Stranguries Powder to improve SP-induced bladder hyperactivity and bladder ROS level. Furthermore, the level of neutrophil adhesion to venule endothelium was significantly decreased after oral Five Stranguries Powder plus Crataegi Fructus, Crataegi Fructus, and Five Stranguries Powder. The decreased neutrophil adhesion potential was displayed in the order Five Stranguries Powder plus Crataegi Fructus > Crataegi Fructus > Five Stranguries Powder. Five Stranguries Powder, a commonly used TCM prescription, strongly inhibited SP-induced NADPH oxidase activity, hyperactivity, and bladder ROS in the rats. However, synergistic effect in Five Stranguries Powder plus Crataegi Fructus by addition of Crataegi Fructus to Five Stranguries Powder to ameliorate inflammation and hyperactive bladder was observed.

Because of high availability, safety, and feasibility, TCM of green tea extract and Five Stranguries Powder , and Five Stranguries Powder plus Crataegi Fructus can be directly used in the humans for clinical trials. For prevention of or decrease in occurrence of hyperactive bladder, green tea extract as a popular beverage or supplementary food can be continuously used for at least 2 weeks. As to Five Stranguries Powder plus Crataegi Fructus, this modified formula can be used directly to treat patients suffering from hyperactive bladder symptom. Even more, the combination of green tea extract and Five Stranguries Powder plus Crataegi Fructus may have a better effect to improve hyperactive bladder. However, it requires further investigation to confirm this. In the future, application of green tea extract at a dose of 50 mg/kg and Five Stranguries Powder (100 mg/kg) plus Crataegi Fructus (20 mg/kg) can be referenced. The dosage of TCM of green tea extract or Five Stranguries Powder plus Crataegi Fructus can be decided by the severity of hyperactive bladder, which can be evaluated by the urinary or blood ROS. Other natural polyphenols/flavonoids or modified TCM formula can be further explored to develop new therapeutic drugs.

According to these results, green tea extract and Crataegi Fructus may exert anti-inflammatory and antioxidant activity in

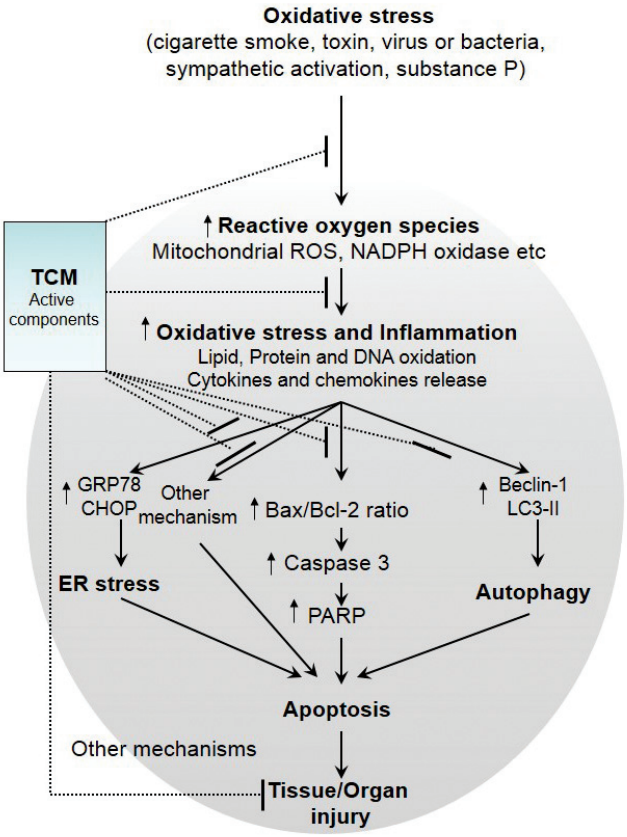

Figure 1. Effects of TCM on oxidative stress induced by several stimuli, ER stress, autophagy, apoptosis, and other mechanisms in the rat models. Cigarette smoke, toxin, virus, bacteria, sympathetic overt activation, and particulate components like substance P increase the production of reactive oxygen species, possibly by causing upregulation of NADPH oxidase subunit gp91Phox expression or mitochondrial ROS production and other sources. The ROS cause lipid, protein, and DNA oxidation and release of several cytokines or chemokines. This leads to upregulation of GRP78, which increases ER stress. ROS also increases the Bax/Bcl-2 ratio and upregulates expression of caspase 3 and PARP, leading to apoptosis. Elevated levels of oxidative stress enhance Beclin-1 expression, which promotes autophagy. ROS also evoke other mechanism, for example, upregulates PlGF, an emphysema risk factor, in the lung. The active components present in the TCM have antioxidant, anti-ER stress, anti-autophagy, and anti-apoptotic activities, leading to decreased inflammation

Table 1. Summary of the efficient TCM on treating several inflammatory diseases from recent studies

TCM	Model	Treatment and dosage	Proposed mechanisms
Monascus adlay	Rats exposed to doses of CS ranging from one cigarette (0.8 mg nicotine) to two cigarettes (1.6 mg nicotine) to three cigarettes (2.4 mg nicotine)	Daily ~40 mg of *Monascus*-fermented products and ~0.4 mg of lovastatin (kg)	Supplementary MA suppresses lung injury by preserving antioxidant defense mechanisms and decreasing oxidative stress through its lovastatin and phenolic components. Dietary MA can reduce acute lung injury by inhibiting ER stress, autophagy, apoptosis, and PlGF expression and, therefore, may be a beneficial nutritional therapy for oxidative stress-related diseases
Amla (*Emblica officinalis* Gaertn. of Euphorbiaceae family)	*N*-nitrosodiethylamine-induced rat liver inflammation		
Virgate Wormwood Decoction (茵陳蒿湯 Yīn Chén Hāo Tāng) and genipin	Acute urine retention was set by infusion of twofold micturition volume of saline into the bladder for induction of liver inflammation	One week duration of oral dose for Virgate Wormwood Decoction (2 g/kg/day) and genipin (100 mg/kg/day)	Virgate Wormwood Decoction with active component, genipin, can ameliorate acute urine retention-induced liver inflammation by amelioration of oxidative stress possibly by the inhibition of sympathetic-induced hypoxia/hypoperfusion and leukocyte NADPH oxidase activity
Cordyceps sobolifera (蟬花 Chán Huā)	Peritoneal injection of lipopolysaccharide (20 mg/kg body weight)-induced acute renal failure	Daily 2 ml of *Cordyceps sobolifera* powder suspension (75 mg/ml H_2O) or water by way of oral feeding in the morning of each day	The multiple beneficial effects of *Cordyceps sobolifera* might be due to its blocking of lipopolysaccharide-triggered ER stress, autophagy, and apoptosis in the renal tubules
Green tea extract and catechins	10 μg substance P was injected to the rat through intra-arterial catheter at a volume of 1 ml/kg for hyperactive bladder induction	Daily oral catechins from green tea extract (50 mg/kg)	Daily pretreatment of catechins may ameliorate substance P-induced bladder hyperactivity by the downregulation of ROS formation and ICAM-1 expression
Five Stranguries Powder (五淋散 Wǔ Lín Sǎn) plus Crataegi Fructus (山楂 Shān Zhā)	10 μg substance P was injected to the rat through intra-arterial catheter at a volume of 1 ml/kg for hyperactive bladder induction	Daily oral Five Stranguries Powder (100 mg/kg), Crataegi Fructus (20 mg/kg), Five Stranguries Powder plus Crataegi Fructus (100 mg/kg WLS+20 mg/kg Crataegi Fructus)	A modified formula (Five Stranguries Powder plus Crataegi Fructus) ameliorates SP-induced hyperactive bladder by inhibition of neutrophil NADPH oxidase activity and ROS production

TCM: Traditional chinese medicine; NADPH: Nicotinamide adenine dinucleotide phosphate; ROS: Reactive oxygen species; ER: Endoplasmic reticulum; PlGF: Placenta growth factor; MA: Monascus adlay

hyperactive bladder through interactions with ROS, COX-2, iNOS, and the nuclear factors NF-κB and AP-1. Daily pretreatment of catechins may ameliorate SP-induced bladder hyperactivity by the downregulation of ROS formation and ICAM-1 expression, indicating a therapeutic potential in the treatment of hyperactive bladder. Besides, a modified formula (Five Stranguries Powder plus Crataegi Fructus) ameliorates SP-induced hyperactive bladder by inhibition of neutrophil NADPH oxidase activity and ROS production. In the future, TCM could be used in the hyperactive bladder for clinical trial.

CONCLUSION

In summary, as shown in Figure 1, we suggest that the therapeutic potential of TCM via its active components suppresses several risk factor–induced tissue/organ injuries by preserving antioxidant defense mechanisms and decreasing oxidative stress; further, we believe that these antioxidant and anti-inflammatory activities of TCM stem from its synergistically active components. We believe that suitable TCM treatment can reduce risk factor–induced oxidative injury by inhibiting ER stress, autophagy, apoptosis, and other mechanisms and, therefore, may be a potentially therapeutic strategy for oxidative stress–related diseases. The dosage of TCM and the protective mechanisms are clearly summarized in Table 1.

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