Antioxidant Properties of *Antrodia cinnamomea*: An Extremely Rare and Coveted Medicinal Mushroom Endemic to Taiwan

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K.J. Senthil Kumar and Sheng-Yang Wang

Abstract

Antrodia cinnamomea is an extremely rare and endemic fungal species native to forested regions of Taiwan. In modern Taiwanese culture, *A. cinnamomea* is believed to be a valuable gift from the heaven. Thereby, it is claimed as the "National Treasure of Taiwan" and "Ruby" among mushrooms." Traditionally, *A. cinnamomea* was used to prepare Chinese medicine for treating various illness including liver diseases, food and drug intoxication, diarrhea, abdominal pain, hypertension, itchy skin, and tumorigenic diseases. Recent scientific studies strongly support that the pharmacological activities of *A. cinnamomea* go far beyond the original usage, as *A. cinnamomea* has exhibited various pharmacological properties including anticancer, antioxidant, hepatoprotection, antihypertensive, antihyperlipidemic, immunomodulatory, and anti-inflammatory properties. Till date, more than 400 scientific reports have been published on the therapeutic potential of *A. cinnamomea*, or its closely related species *Antrodia salmonea*, and their compounds. In the present review, the taxonomic description of *A. cinnamomea*, ethnomedical value, chemical constituents, and pharmacological effects particularly antioxidant and Nrf2-mediated cytoprotective effects will be discussed.

Keywords

Antioxidant • *Antrodia cinnamomea* • *Antrodia salmonea* • Cytoprotection • Medicinal fungus • Nrf2

K.J.S. Kumar
Department of Forestry, National Chung Hsing University, Taichung, Taiwan

S-Y. Wang (✉)
Department of Forestry, National Chung Hsing University, Taichung, Taiwan

Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan
e-mail: taiwanfir@dragon.nchu.edu.tw

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Abbreviations

AAPH	2,2-Azobis(2-amidinopropane)dehydrochloride
ALT	Alanine aminotransferase
ARE	Antioxidant responsible element
AST	Aspartate aminotransferase
COX-2	Cyclooxygenase-2
ERK	Extracellular signal-regulated kinase
GSH	Glutathione
HO-1	Heme oxygenase-1
IKK	Iκ-B kinase
iNOS	Inducible nitric oxide synthase
Iκ-B	Inhibitor of nuclear factor kappa-B
JNK	c-JUN N-terminal kinase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde
NF-κB	Nuclear factor kappa-B
NO	Nitric oxide
Nrf2	Nuclear factor E2-related factor-2
ROS	Reactive oxygen species
SAPK	Stress-activated protein kinase

6.1 Introduction

Free radicals and oxidants are recognized as having both toxic and beneficial components since they can be either harmful or helpful to the body. They are produced either from normal cell metabolism in situ or external sources such as physical

stress, pollution, cigarette smoke, alcohol, radiation, and medication (Pham-Huy et al. 2008). Oxygen is an indispensable element for life forms. Cells utilize oxygen to generate energy, and free radicals are generated as a consequence of adenosine triphosphate (ATP) production by the mitochondria (Pham-Huy et al. 2008). These by-products are reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that result from the cellular redox processes. However, not all reactive oxygen species are harmful to the body. Some of them are useful in killing and invading pathogens or microbes. The imbalance between the production of ROS and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants triggers oxidative stress (Bhattacharyya et al. 2014). Particularly, intracellular accumulation of relatively high concentrations of ROS induces oxidative damage in DNA and plays a major part in the development of chronic and degenerative ailments such as inflammation, cancer, aging, arthritis, autoimmune disorders, and neurodegenerative and cardiovascular diseases (Pham-Huy et al. 2008). The human body has been architected with several defense mechanisms to counteract oxidative stress by producing antioxidants. Some of the examples are superoxide dismutase (SOD), heme oxygenase-1 (HO-1), glutathione peroxidase (GPx), catalase, and γ-glutamylcysteine ligase (GCL; also known as glutamylcysteine synthetase), whereas nonenzymatic antioxidants are reduced by glutathione (GSH), α-tocopherol, ascorbic acid, ubiquinone, etc., or detoxifying enzymes including NAD(P)H:quinone oxidoreductase-1 (NQO1), glutathione-S-transferase (GST), epoxide hydrolase, and uridine-5-diphosphoglucuronyltransferase (UGT). These are either endogenously produced in situ or externally supplied through antioxidant-rich foods or nutraceutical supplements (Mikhed et al. 2015; Surh 2003; Kinnula et al. 1998). These endogenous and exogenous antioxidants act as "free radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents" by preventing and repairing damages caused by ROS and RNS. Therefore, antioxidants can enhance the immune defense and lower the risk of oxidative stress-related disorders (Bouayed and Bohn 2010; Pham-Huy et al. 2008).

Synthetic and natural antioxidants are currently used in food and pharmaceutical industries, especially those containing oils and fats to protect the substance against oxidation. A number of synthetic phenolic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are widely used in food, cosmetics, and pharmacological industries. In view of increasing risk factors of various deadly diseases to human beings, there has been a global trend toward the use of natural antioxidants derived from dietary vegetables or medicinal plants (Lobo et al. 2010; Sindhi et al. 2013). Also, there have been increasing evidences suggesting that intake of antioxidant-rich food or medicinal plants decreases the incidence of diseases in human. The use of naturally occurring antioxidants in pharmaceutical, nutraceutical, and cosmeceutical industries would be a promising alternative for synthetic antioxidants with respect to low cost, high compatibility with minimal side effects (Lobo et al. 2010). A number of naturally occurring antioxidant compounds from the plant sources have been identified as free radical scavengers or

electron donors. Several attempts have been made to study the antioxidant potential of a wide variety of vegetables like potato, spinach, tomatoes, and legumes and fruits such as olives, citrus, prunes, berries, and cherries. In recent years, the antioxidant properties of green and black teas have been extensively studied and reported to contain up to 30% of the dry weight as phenolic compounds (Lobo et al. 2010). Apart from the dietary sources, medicinal plants used in traditional Chinese medicine and Indian Ayurveda system also provide antioxidants, which protect the body from oxidative injury and boost the immune system (Shukla et al. 2012).

Mushrooms have been valued throughout the world as both food and medicine for thousands of years. Particularly, the Chinese and Egyptians were among the first people to appreciate the value of the mushroom as a specialty in the diet of the royal family. Apart from the diet, many of the world's more than 38,000 species of mushrooms have medicinal uses (Mayell 2001). Among the popular medicinal mushrooms such as *Antrodia cinnamomea*, *Antrodia salmonea*, *Ganoderma lucidum*, *Ophiocordyceps sinensis*, *Phellinus igniarius*, *Trametes versicolor*, *Lentinula edodes*, and *Wolfiporia extensa*, *A. cinnamomea* also known as "niu-chang chih" in Chinese is an extremely rare and endemic species native to forested regions of Taiwan. Taiwanese aborigines utilized this mushroom for treating liver diseases and for protection from food and drug intoxication (Ao et al. 2009; Liu et al. 2012). In modern Taiwanese culture, *A. cinnamomea* is believed to be a valuable gift from the heaven. Thereby, it is claimed as the "National Treasure of Taiwan" and "Ruby among mushrooms" (Geethangili and Tzeng 2011). Traditionally, *A. cinnamomea* was used to prepare Chinese medicine for treating various illness including liver diseases, food and drug intoxication, diarrhea, abdominal pain, hypertension, itchy skin, and tumorigenic diseases (Ao et al. 2009; Geethangili and Tzeng 2011; Liu et al. 2012). Recent scientific studies have strongly supported that the pharmacological activities of *A. cinnamomea* go far beyond the original usage. As evidenced in several reports, *A. cinnamomea* exhibit various biological activities including anticancer, antioxidant, hepatoprotective, antihypertensive, antihyperlipidemic, immunomodulatory, and anti-inflammatory properties (Ao et al. 2009; Levin et al. 2012; Liu et al. 2012; Geethangili and Tzeng 2011; Lu et al. 2013; Yue et al. 2012, 2013). The first report of pharmacological activities of *A. cinnamomea* was published in 1995. New steroid acids were isolated from the fruiting bodies of *A. cinnamomea* exhibiting cytotoxicity to murine leukemia P-388 cells (Chen et al. 1995). After that, *A. cinnamomea* started attracting scientists' attention due to its richness in bioactive compounds such as polysaccharides, flavonoids, triterpenoids, maleic/succinic acid, benzenoids, and benzoquinone derivatives. The particular pharmacological interest in *A. cinnamomea* and its curative properties originated from the realm of traditional practice. Till date, nearly 400 scientific reports have been published regarding the therapeutic potential of *A. cinnamomea* or its compounds.

6.2 Taxonomic Description of *A. cinnamomea* and *A. salmonea*

Both *A. cinnamomea* and *A. salmonea* are parasitic fungi growing in the inner cavity of the endemic tree species *Cinnamomum kanehirae* (Bull camphor tree) Hayata belonging to the family Lauraceae (Geethangili and Tzeng 2011; Lu et al. 2013). Taxonomical description of *A. salmonea* is limited, while *A. cinnamomea* is well studied. The fruiting bodies of *A. cinnamomea* have various forms such as bell-like, hooflike, towerlike, or platelike. They are flat on the surface of wood at the beginning of growth (Fig. 6.1). Then the brim of the front edge rises to roll into plate shaped or stalactites (Geethangili and Tzeng 2011).

A. cinnamomea possesses a unique flavor resulting from a mixture of distinctive aromatic components. The odor is mainly because of the host tree *Cinnamomum kanehirae*. However, the pure mushroom does not possess a similar odor. *A. cinnamomea* also possesses a strong bitter taste due to its high triterpenoid content (Lu et al. 2013). The mycelia isolated from the fruiting bodies of *A. cinnamomea* form orange-red and orange-brown to light cinnamon-colored colonies. The hyphae of *A. cinnamomea* possess generative hyphae 2–3.5 μm. The host species, *C. kanehirae*, possess a high taxonomical importance since the species is endemic to Taiwan (Chang and Chou 1995).

After a long tradition usage, this species was rediscovered by Zang and Su (1990) and placed under genus *Ganoderma* due to the similarity in morphological features with *Ganoderma* species. Five years later, Chang and Chou described the species as *Antrodia cinnamomea*. The specific epithet alludes to the host tree. They properly

Fig. 6.1 Fruiting bodies of *A. cinnamomea* (*orange colored*) growing in the inner cavity of the endemic tree species

placed their species in *Antrodia* because of its dimitic hyphae system with clamped generative hyphae and brown rot-causing ability (Chang and Chou 1995). However, the types of both *Ganoderma camphoratum* and *Antrodia cinnamomea* were found to be conspecific, while *A. cinnamomea* is reduced to a taxonomic synonym. A new nomenclature *Antrodia camphorata* was proposed by Wu et al. (1997), because the name *Ganoderma camphoratum* originally was based on a polypore with contaminating *Ganoderma* spores and hence *Antrodia cinnamomea* was reduced to a taxonomic synonym of *A. camphorata*. In 2004, a phylogenetic analysis based on sequence data obtained from large subunit (LSU) rDNA indicated that *A. camphorata* is not closely related to the genus *Antrodia* or *Antrodiella*. Therefore, this fungus was moved to the new genus *Taiwanofungus* and named as *Taiwanofungus camphoratus* (Wu et al. 2004). Further polymorphism analysis of international transcribed spacer (ITS) regions of rDNA of 11 *A. cinnamomea* strains revealed that *A. cinnamomea* belongs to the genus *Antrodia* (Chiu 2007). The present taxonomic status of *A. cinnamomea* is as follows *Fungi*, *Basidiomycota*, *Agaricomycetes*, *Polyporales*, *Fomitopsidaceae*, *Antrodia*, and *Antrodia cinnamomea* (Chang and Chou 1995). However, the nomenclature and exact taxonomy (genus and species) of *A. cinnamomea* is still the subject of debate and needs further research. In this article, we have chosen the name as *A. cinnamomea* to describe this unique medicinal mushroom.

6.3 Ethnomedical Value

Although *Antrodia cinnamomea* was used by Taiwanese aborigines for several centuries, however most of the anthropological studies did not clearly explain the historical origin of this mushroom as tribal folk medicine in Taiwan or elsewhere. It was originally used by the local tribes to treat food and drug intoxication and abdominal pain and to enhance liver function (Ao et al. 2009). However, its use in urban areas was limited owing to the lack of access and knowledge. In 1773 (38th year of Chien-Lung Years), during the Ching Dynasty, Dr. Wu-Sha was one of the famous physicians in Traditional Chinese medicinal system. Dr. Wu-Sha and his followers moved from Fujian province of China to the Yi-Lan, a northeast province of Taiwan. It was Dr. Wu-Sha who observed that the aborigines were suffering from a headache, hepatitis, and liver cirrhosis due to the frequent alcohol consumption. The locals often chewed the fruiting bodies of *A. cinnamomea* and used to drink its decoction to get relief from the alcoholic hangover. Dr. Wu-Sha adopted this traditional usage and applied to cure a number of illnesses like diarrhea, abdominal pain, hypertension, itchy skin, viral infection, stomatitis, diabetes mellitus, nephritis, proteinuria, hepatitis, liver cirrhosis, hepatoma, influenza, car sickness, calenture, and motion sickness (Levin et al. 2012). After its use for several centuries, the mushroom is now believed to be one of the most potent liver-protecting remedy in Taiwan (Geethangili and Tzeng 2011; Levin et al. 2012). Although primary ethnomedical data describing its liver-protecting ability was recorded in the ancient literature,

however, recently several studies have demonstrated that its pharmacological applications go far beyond the original usage.

6.4 Nrf2: A Key Regulator of Cytoprotection

The cellular defense system against oxidative stress can be achieved either by reducing the formation of reactive oxygen species (ROS) or stimulating their detoxification. Many xenobiotic-metabolizing enzymes are involved in both phase I (oxidation and reduction) and phase II biotransformation (conjugation) reactions (Rahman 2007; Khan et al. 2016). In general, by utilizing cytochrome p450 monooxygenases, the oxidative stress activation takes place primarily during phase I metabolism. The phase II reactions eliminate the harmful actions of phase I enzymes by reducing the electrophilicity of ROS through enzymatic conjugation with endogenous ligands such as glutathione and glucuronic acid (Lee and Surh 2005). A wide variety of phase II enzymes including heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO-1), glutathione synthetase, glutathione-S-transferase (GST), γ-glutamate cysteine ligase (γ-GCLC), γ-glutamyltranspeptidase, UDP-glucuronosyltransferase (UGT), aldo-keto reductase, microsomal epoxide hydrolase, leukotriene B_4 12-hydroxydehydrogenase, and aldehyde dehydrogenase were induced by a number of antioxidants (Lee and Surh 2005; Surh 2003). The induction of the phase II enzyme system is an important event of the cellular stress response during which a diverse array of electrophilic and oxidative toxicants can be eliminated or inactivated before they can cause damage to critical cellular macromolecules (Lee and Lee 2011). Both basal and inducible expression of many of these antioxidant enzymes are regulated by the CNC-bZIP (cap'n'collar family of basic leucine zipper) transcription factor Nrf2 through the antioxidant response element (ARE).

Genomic analysis has revealed that the cis-acting ARE [5′-(G/A)TGA(G/C)nnnGC(G/A)-3′], a specific DNA-promoter-binding region, exists in the 5′ flanking region of genes encoding NQO-1, multiple GST and UGT isozymes, and epoxide hydrolase. It can be transcriptionally activated by numerous antioxidants and/or electrophiles (Lee and Surh 2005; Na and Surh 2014). Nioi et al. have reported that the ARE consensus sequences in the mouse NQO-1 promoter are necessary for its function. Moreover, recent gene array analyses have shown that a series of genes in mammalian cells can be regulated by ARE (Nioi et al. 2003).

Under basal conditions, Nrf2 is anchored in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap-1), which in turn mediates proteasomal degradation of Nrf2 by acting as an adaptor protein of the Cul3-based E3 ubiquitin ligase complex (Kang et al. 2004; Padmanabhan et al. 2008). Mild oxidative and electrophilic stresses disrupt the binding of Nrf2 and Keap-1 by modifying several cysteine residues of Keap-1, resulting in the accumulation of Nrf2 within the nucleus and further transactivation of ARE-bearing genes (Kansanen et al. 2013; Cho et al. 2002). Numerous comparative studies of the phenotypes of wild-type and Nrf2-disrupted mice have revealed the pivotal role of Nrf2 in protection against oxidant injuries.

Nrf2-disrupted mice have been much more susceptible to toxicities mediated by environmental chemicals and stresses than the wild-type mice (Cho and Kleeberger 2010; Johnson et al. 2008; Osburn and Kensler 2008).

One important Nrf2-target gene, HO-1, is a ubiquitous and redox-sensitive inducible stress protein that degrades heme to CO, iron, and biliverdin (Kobayashi and Yamamoto 2005). The importance of this protein in physiological and pathological states is underlined by the versatility of HO-1 inducers and the protective effects attributed to heme oxygenase products in conditions that are associated with moderate or severe cellular stress. HO-1 is recognized as a protective gene in the kidney involved in degradation of pro-oxidant heme, resulting in the production of anti-inflammatory, antioxidant, and anti-apoptotic metabolites (Abraham and Kappas 2008). The transcriptional activation of ARE-mediated Nrf2 is induced by various chemical compounds, including curcumin, resveratrol, genistein, capsaicin, caffeic acid, sulforaphane, quercetin, silymarin, lucidone, antroquinonol, etc. (Sung et al. 2011).

6.5 Pharmacological Activities of *A. cinnamomea* and *A. salmonea*

In the past two decades, extensive research has been carried on *A. cinnamomea* and *A. salmonea* extracts and its active components in vitro and in vivo. Several review articles have been published on the pharmacological applications and recent research and development on *A. cinnamomea* (Ao et al. 2009; Geethangili and Tzeng 2011; Levin et al. 2012; Liu et al. 2012; Lu et al. 2013; Yue et al. 2012, 2013). The current review presents the antioxidant and cytoprotective effects of the *A. cinnamomea* and its active components in different models of in vitro and in vivo studies. In particular, the Nrf2-mediated cytoprotective effects of *A. cinnamomea* and *A. salmonea* are emphasized.

Several researchers have reported the antioxidant and cytoprotective effects of *A. cinnamomea* and *A. salmonea* in various in vitro and in vivo test models. As summarized in Table 6.1, different extracts and culture conditions of these mushrooms have been found to exhibit antioxidant, hepatoprotective, neuroprotective, and anti-inflammatory activities. The protective effects of *A. cinnamomea* and *A. salmonea* on oxidative stress-induced pathological development of various diseases will be dealt in the following sections. For the preparations of *A. cinnamomea* and *A. salmonea* extracts, we need fruiting bodies and mycelium of *A. cinnamomea* and *A. salmonea*; fermented culture broth (FCBAC); aqueous, ethyl acetate, methanol, ethanol, $CHCl_3$/MeOH, and $CHCl_3$ extracts; submerged cultivation filtrate; and wild and solid-state cultures of *A. cinnamomea* and *A. salmonea.* Due to the lack of evidence of standard designed clinical studies, only some case reports from the medical conference will be described here.

A series of publications have appeared on the structural characterization of the secondary metabolites of *A. cinnamomea* and *A. salmonea*. Most of the investigators have studied the fruiting bodies, though there are a few publications on the

Table 6.1 A summary of the studies conducted on the antioxidant potential of *A. cinnamomea* and *A. salmonea*

Sample/s	Dosage and route	Study model	Observation/s	Reference
Culture filter extract of C in submerged culture	0.2 mg/mL	Cell-free	Free radical scavenging	Song and Yen (2002)
Aqueous extract from AC	12.5–50 μL	Erythrocytes, HUVECs, and HL-60 (in vitro)	AC inhibits AAPH-induced erythrocyte hemolysis, lipid peroxidation, and cell damage	Hseu et al. (2002)
Aqueous extracts of AC	250, 750, and 1,250 mg/kg/day, 4 days/week (p.o.)	Male ICR mice (in vivo)	AC exhibits protection against chronic CCl4-induced hepatic injury through the antioxidant and free radical scavenging activities	Hsiao et al. (2003)
Dry matter of fermented filtrate from AC (DFAC)	205 and 500 mg/kg/day (p.o.)	Male Sprague-Dawley rats (in vivo)	DFAC protects rats from CCl4-induced hepatotoxicity through the upregulation of hepatic phase II detoxifying enzymes and free radical scavenging	Song and Yen (2003)
Mycelia and fruiting bodies of AC	500 and 1,000 mg/kg/day (p.o.)	Male Sprague-Dawley rats (in vivo)	AC protects rats from alcohol-induced acute liver injury through the induction of antioxidant enzymes	Dai (2003)
Methanolic extracts of mycelia of AC	0.5–10 mg/mL	Cell-free	Free radical scavenging, reducing power and metal-chelating activities	Mau et al. (2003)
Methanolic extracts of mycelia of AC	0.5–10 mg/mL	Cell-free	Free radical scavenging, reducing power and metal-chelating activities	Mau et al. (2004)

(continued)

Table 6.1 (continued)

Sample/s	Dosage and route	Study model	Observation/s	Reference
Filtrate of fermented mycelia from AC (FMAC)	500 and 1000 mg/kg/day (p.o.)	Male Wister rats (in vivo)	FMAC prevents CCL4-induced liver fibrosis *via* scavenging free radicals	Lin et al. (2006)
Fermented filtrate of AC (FFAC)		Mice (in vivo)	FFAC inhibits CCL4-induced serum GTP levels in mice	Huang et al. (2006)
Fermented culture broth of AC and aqueous extract of mycelia of AC	25–100 μg/mL and 50–100 μg/mL	HUVECs (in vitro)	HUVECs were protected from CuSO4 or AAPH-induced LDL oxidation	Yang et al. (2006)
Mycelia extract of AC	500 and 1000 mg/kg/day (i.g.)	Male Sprague-Dawley rats (in vivo)	AC prevents alcohol-induced elevation of serum ALT, AST, ALP, and bilirubin	Lu et al. (2007)
Methanolic extract of AC irradiated with γ-rays	0.5–10 mg/mL	Cell-free	Free radical scavenging, reducing power and metal-chelating activities	Huang and Mau (2007)
Water-soluble polysaccharides from AC in submerged culture	200 μg/mL	Chang liver cells (in vitro)	Polysaccharides protects Chang liver cells from H2O2-induced oxidative injury through the upregulation of GST activity and free radical scavenging	Tsai et al. (2007)
Fermented culture broth of AC and aqueous extract of mycelia from AC	25–100 μg/mL and 50–200 μg/mL	HUVECs (in vitro)	AC products prevents AAPH-induced apoptosis in HUVECS	Hseu et al. (2008)
Ethanolic extract of mycelia of AC (EMAC)	250, 500, and 1000 mg/kg/day (p.o.)	Male ICR mice (in vivo)	EMAC protects mice from ethanol-induced liver injury through the induction of antioxidant and phase II enzymes vi Nrf2 signaling pathway	Kumar et al. (2011)

(continued)

Table 6.1 (continued)

Sample/s	Dosage and route	Study model	Observation/s	Reference
Methanol extract of mycelia of AC (MEMAC)	25, 50, and 75 μg/mL	RAW264.7 cells (in vitro)	MEMAC protects macrophage cells from immunogen-induced lipid peroxidation	Wen et al. (2011)
Methanol extract of mycelia of AC (MEMAC)	5, 25, and 50 mg/kg (i.p.)	Male ICR mice (in vivo)	MEMAC inhibits λ-carrageenan-induced decrease on CAT, SOD, and GPx in mice	Wen et al. (2011)
Ethyl acetate extract of culture broth of AC		Cell-free	Free radical scavenging, reducing power and metal-chelating activities	Wu et al. (2011)
Ethanolic extract of mycelia of AC (EMAC)	250, 500, and 1000 mg/kg/day (p.o.)	Male ICR mice (in vivo)	Increased serum antioxidant capacity in alcohol-treated mice	Wang et al. (2013)
AC extracts	250, 500, and 1000 mg/kg/day (p.o.)	Male Sprague-Dawley rats (in vivo)	AC alleviates endothelial lipid injury by inhibiting lipid peroxidation of ox-LDL and increase of HDL and SOD levels in high-fat diet rats	Qi et al. (2014)
Fermented culture broth of AS	25, 50, and 100 μg/mL	HUVECs	Protects HUVECS against TNF-α-induced atherogenesis through the upregulation of Nrf2 signaling pathway	Yang et al. (2014)
Aqueous extracts of AC	1–10 mg/mL	Cell-free	Free radical scavenging, reducing power and metal-chelating activities	Hsieh et al. (2015)
Crude oil from AC		Cell-free	Free radical scavenging, reducing power and metal-chelating activities	Zhang et al. (2015)

(continued)

Table 6.1 (continued)

Sample/s	Dosage and route	Study model	Observation/s	Reference
Fermented culture broth of AS	25, 50, and 100 μg/mL	RAW264.7 cells	Inhibits LPS-induced inflammatory response through the upregulation of Nrf2 signaling pathway	Yang et al. (2015)

constituents of the mycelia of *A. cinnamomea* in submerged cultures. The compounds identified are predominantly polysaccharides, benzenoids, diterpenes, triterpenoids, steroids, and maleic/succinic acid derivatives.

More than 80 compounds have been identified and structurally elucidated. Terpenoids are predominantly found in the fruiting bodies. Nearly 40 compounds have been reported. A few terpenoids have been found in mycelia from solid-state and submerged cultivation. There are about 30 triterpenoids with similar structures. A common feature of these structures is ergostane or lanostane skeleton (Geethangili and Tzeng 2011). Due to the high amount of terpenoids (63%) in the fruiting bodies of *A. cinnamomea* and *A. salmonea*, this group of natural compounds has been the focus of many phytochemical studies. In addition to polysaccharides, several other constituents such as benzenoids, lignans, quinone derivatives, and maleic/succinic acid derivatives have been described from *A. camphorata.* Also, sterols, nucleotides, and fatty acids have been found in these species. Furthermore, unique ubiquinone derivatives such as antroquinonol and 4-acetylantroquinonol B were isolated only from the cultured mycelia of *A. cinnamomea*. These compounds have never been reported from the fruiting bodies of *A. cinnamomea* or *A. salmonea*. The antioxidant potential of compounds isolated from these mushrooms has been summarized in Table 6.2.

6.5.1 Hepatoprotective Effects of Crude Extracts of *A. cinnamomea*

A number of scientific studies have demonstrated the potential of *A. cinnamomea* in the treatment of liver diseases, biologically active constituents responsible, and their mode of action (Ao et al. 2009; Geethangili and Tzeng 2011; Levin et al. 2012; Liu et al. 2012; Lu et al. 2013; Yue et al. 2012, 2013). In the present review, we have summarized the hepatoprotective effects of *A. cinnamomea* by modulating Nrf2 signaling pathway. A study by Kumar et al. (2011) evaluated the effect of the ethanolic extract of *A. cinnamomea* (EMAC) in ethanol-induced acute hepatotoxicity in mice. Ethanolic extracts of mycelia of *A. cinnamomea* (250, 500, and 1000 mg/kg BW, once a day for 10 days) were orally administered. At the end of the EMAC treatment, hepatotoxicity was induced by administering 3 doses of ethanol (5 g/kg BW) through oral gavage with 12 h interval. Serum biochemical analysis showed

Table 6.2 A summary of the studies conducted on the antioxidant potential of phytocompounds derived from *A. cinnamomea*

Chemical class	Compound name	Dosage and route	Study model	Observation	Reference
Lanostanes	Lanosta-8,24-diene-3β,15α,21-triol	–	PMA-activated leukocytes	Protects leukocytes through the induction of PCK-mediated antioxidant burst	Shen et al. (2006)
Naphthoquinones	2,3-Dimethoxy-5-(2′,5′-dimethoxy-3′,4′-methylenedioxyphenyl)-7-methyl-[1] [4]-naphthoquinone	–	PMA-activated leukocytes	Protects leukocytes through the induction of PCK-mediated antioxidant burst	Shen et al. (2006)
	2,3-Dimethoxy-6-(2′,5′-dimethoxy-3′,4′-methylenedioxyphenyl)-7-methyl-[1] [4]-naphthoquinone	–	PMA-activated leukocytes	Protects leukocytes through the induction of PCK-mediated antioxidant burst	Shen et al. (2006)
Benzenoids	Isobutylphenol	500 μM	Cell-free	Free radical scavenging	Wu et al. (2007)
	Antrocamphin A	100 μM	fMLP-induced neutrophils	Inhibits fMLP-induced production of superoxide anion	Chen et al. (2007)
Benzoquinone	5-Methyl-benzo[1,3] dioxole-4,7-dione	500 μM	Cell-free	Free radical scavenging	Wu et al. (2007)
	2,3-Dimethoxy-5-methyl[1,4] benzoquinone	500 μM	Cell-free	Free radical scavenging	Wu et al. (2007)
Phenylmethanoids	4,7-Dimethoxy-5-methyl-1,3-benzodioxole	62.5, 125, 250, and 500 μM	RAW264.7 cells	Inhibits LPS-induced inflammation in macrophage cells *via* induction of HO-1	Shie et al. (2016)
		100 μM	fMLP-induced neutrophils	Inhibits fMLP-induced production of superoxide anion	Chen et al. (2007)

(continued)

Table 6.2 (continued)

Chemical class	Compound name	Dosage and route	Study model	Observation	Reference
Polysaccharides	Neutral polysaccharides	400 and 800 mg/kg/day (p.o.)	Male ICR mice (in vivo)	AC prevents *Propionibacterium acnes* and lipopolysaccharide-induced elevation of ALT and AST in mice	Han et al. (2006)
	Antrodan	40 and 80 mg/kg (o.p.)	Male Sprague-Dawley rats	Antrodan protected against liver damage by suppressing LPS-stimulated serum ALT, AST, and NO through upregulation of CAT, SOD, and GPx	Ker et al. (2014)
	Polysaccharides	4 μL	Cell-free	Free radical scavenging	Wu et al. (2007)
Steroids	Antcin C	5, 10, and 20 μM	HepG2 cells	Protects liver cells from AAPH-induced oxidative stress through upregulation of antioxidant and detoxifying enzymes *via* Nrf2 signaling pathway	Gokila Vani et al. (2013)
		25, 50, and 100 mg/kg (i.p.)	Male ICR mice	Protects mice liver from AAPH-induced oxidative stress through upregulation of antioxidant and detoxifying enzymes *via* Nrf2 signaling pathway	Gokila Vani et al. (2013)
	Antcin A	100 μM	fMLP-induced neutrophils	Inhibits fMLP-induced production of superoxide anion	Chen et al. (2007)

	Antcin B	100 μM	fMLP-induced neutrophils	Inhibits fMLP-induced production of superoxide anion	Chen et al. (2007)
	Antcin M		Neutrophils and microglial cells	Inhibits NADPH oxidase activity	Shen et al. (2007)
Ergostanes	Methyl antcinate L	–	Neutrophils and microglial cells	Inhibits NADPH oxidase activity	Shen et al. (2007)
	Methyl antcinate K	–	Neutrophils and microglial cells	Inhibits NADPH oxidase activity	Shen et al. (2007)
Triterpenoids	Eburicoic acid	1, 5, and 10 mg/kg (i.p.)	Male ICR mice	Inhibits λ-carrageenan-induced decrease on CAT, SOD, and GPx in mice	Deng et al. (2013)
		5, 10, and 20 mg/kg (i.p.)	Male ICR mice	Inhibits CCl_4-induced decrease on CAT, SOD, and GPx and increase GSH level in in mice liver tissues	Huang et al. (2013)
	Dehydroeburicoic acid	1, 5, and 10 mg/kg (i.p.)	Male ICR mice	Inhibits λ-carrageenan-induced decrease on CAT, SOD, and GPx in mice	Deng et al. (2013)
		5, 10, and 20 mg/kg (i.p.)	Male ICR mice	Inhibits CCl_4-induced decrease on CAT, SOD, and GPx and increase GSH level in in mice liver tissues	Huang et al. (2013)
Ubiquinone derivatives	Antroquinonol	5, 10, and 20 μg/mL	HepG2 cells	Protects liver cells from alcohol-induced oxidative stress through upregulation of antioxidant and detoxifying enzymes *via* Nrf2 signaling pathway	Kumar et al. (2011)

(continued)

Table 6.2 (continued)

Chemical class	Compound name	Dosage and route	Study model	Observation	Reference
		250, 500, and 1,000 mg/kg (i.p.)	Male ICR mice	Protects mice liver from alcohol-induced oxidative stress through upregulation of antioxidant and detoxifying enzymes *via* Nrf2 signaling pathway	Kumar et al. (2011)
		15 mg/kg (p.o.)	B-cell-deficient mice (B6.129S2-Igh-6tm1Cgn/J)	Antroquinonol promoted the Nrf2 antioxidant pathway and inhibited the activation of T cells and NLRP3 inflammasome	Yang et al. (2013)
		10, 30, and 100 mg/kg (o.p.)	APP transgenic mice	Protects against Aβ-induced oxidative stress and neuroinflammation through the activation of Nrf2 signaling pathway	Chang et al. (2015)
		50 mg/kg (p.o.)	BALB/c mice	Prevents focal segmental glomerulosclerosis (FSGS) through the inhibition of TGF-β-induced NF-kB activity *via* activation Nrf2 signaling pathway	Tsai et al. (2011)
	4-Acetylantroquinonol B	5, 10, and 20 μg/mL	HepG2 cells	Inhibits ALT, AST, and MDA levels and enhance GSH level through the upregulation of antioxidant enzyme *via* Nrf2 signaling pathway in alcohol-induced liver cells	Wang et al. (2013)

that the ethanol-induced elevated levels of serum ALT and AST were significantly reduced by EMAC in a dose-dependent manner. Also, ethanol-induced increased levels of MDA and GSH depletion were prevented by the EMAC treatment. Treatment with EMAC (1,000 mg/kg BW) was comparable to those in the silymarin-positive group (200 mg kg BW). The levels of protein expression of heme oxygenase-1 (HO-1) and Nrf2 were found increased in groups orally administered with EMAC. This study suggested that the hepatoprotective effects of EMAC might be through a mechanism that involves Nrf2 activation and upregulation of the expression of the downstream antioxidant gene.

6.5.2 Antioxidant Potential of *A. salmonea*

The fermented culture broth extract of mycelia of *A. salmonea* (FMAS) showed potent antioxidant effects against LPS-induced oxidative stress and inflammation in murine macrophage cells (RAW264.7) *in vitro* (Yang et al. 2015). Incubation of macrophage cells with various doses of FMAS (25, 50, and 100 μg/mL) for 24 h did not show any cytotoxic effect on the macrophage cells. To measure the antioxidative potential of FMAS, the authors measured LPS-induced intracellular ROS accumulation using a $DCFH_2$-DA fluorescence microscopic analysis. Incubation of RAW264.7 cells with LPS (1 μg/mL) caused a significant increase in the intracellular ROS. However co-treatment with AS (25–100 μg/mL) resulted in a significant as well as a dose-dependent reduction in ROS accumulation. This data suggested that FMAS could suppress LPS-induced ROS generation in macrophages. Next, exposure of macrophages to AS (50 μg/mL) found to upregulate antioxidant genes such as HO-1 and NQO-1 in a time-dependent manner. The increase in HO-1 mRNA and protein levels was observed after 3 and 4 h, respectively. However, the increased levels of NQO-1 mRNA and protein levels were found 1 h after the FMAS treatment and then gradually decreased. In addition, total GSH level significantly increased after treatment with FMAS, correlating with the increased protein expression of γ-GCLC. Moreover, the authors found that FMAS treatment significantly increased the total Nrf2 expression in LPS-induced macrophages. Aberrant Nrf2 activation by FMAS was observed within 2 h, whereas the increase in Nrf2 expression gradually decreased when FMAS was applied at later time points. Further studies with immunofluorescence and luciferase reporter assays confirm that treatment with FMAS increased nuclear accumulation of Nrf2, thereby activating ARE-dependent transcription of antioxidant genes including HO-1, NQO-1, and γ-GCLC. These results strongly suggest that FMAS protects macrophages from LPS-induced oxidative stress and inflammation through the induction of Nrf2-mediated antioxidant genes. To further demonstrate the importance of FMAS-induced Nrf2 activation in LPS-induced macrophage cells, Nrf2 activity was determined with a Nrf2 knockdown model using siRNA transfection. The siRNA-induced reduction in Nrf2 was not altered by FMAS treatment even after 18 h. It means that the transfection with siNrf2 abrogated the protective effect of FMAS on LPS-induced production of pro-inflammatory cytokines in RAW264.7 cells

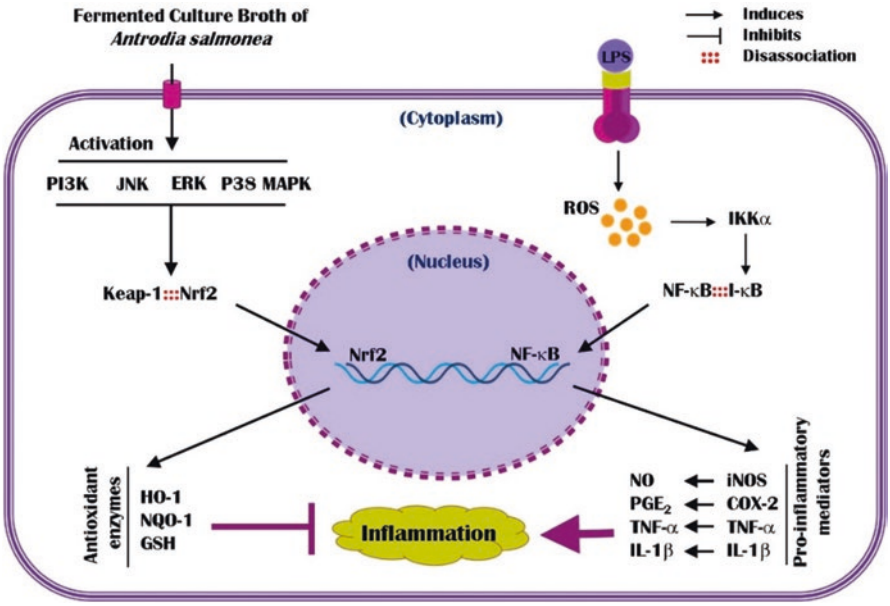

Fig. 6.2 Fermented broth culture extracts of *A. salmonea* inhibits LPS-induced inflammation in murine macrophage cells *via* induction of Nrf2-mediated antioxidant genes (Yang et al. 2015)

(Fig. 6.2). This finding demonstrates that activation of Nrf2 is directly involved in FMAS-mediated anti-inflammatory effects in macrophages.

The same research group also demonstrated that FMAS protects human endothelial cells (EA.hy926) from TNF-α-induced oxidative stress and inflammation (Yang et al. 2014). The initial study showed that pretreatment with FMAS inhibited TNF-α-induced angiogenic and atherogenic factors such as the protein and enzymatic activity of matrix metalloproteinase-9 (MMP-9) and intercellular adhesion molecule-1 (ICAM-1), which are associated with reduced adhesion of U937 leukocytes to endothelial cells. FMAS treatment suppressed the TNF-α-induced transcriptional activation of nuclear factor κB (NF-κB) through the inhibition of nuclear export. Also, the data revealed that FMAS-mediated inhibition of NF-κB activity is associated with reduced IKKα phosphorylation and increased I-κBα degradation. In addition, the protective effect of FMAS was found to be highly correlated with the increased expression of heme oxygenase-1 (HO-1) and γ-glutamylcysteine synthetase (γ-GCLC), which was induced by the transcriptional activation of Nrf2/ARE. Furthermore, HO-1 knockdown by HO-1-specific shRNA diminished the protective effects of FMAS on TNF-α-stimulated invasion, tube formation, and U937 adhesion in EA.hy 926 cells. These data suggest that FMAS prevents TNF-α-induced NF-κB activation through the induction of antioxidant genes *via* a Nrf2 signaling cascade (Fig. 6.3).

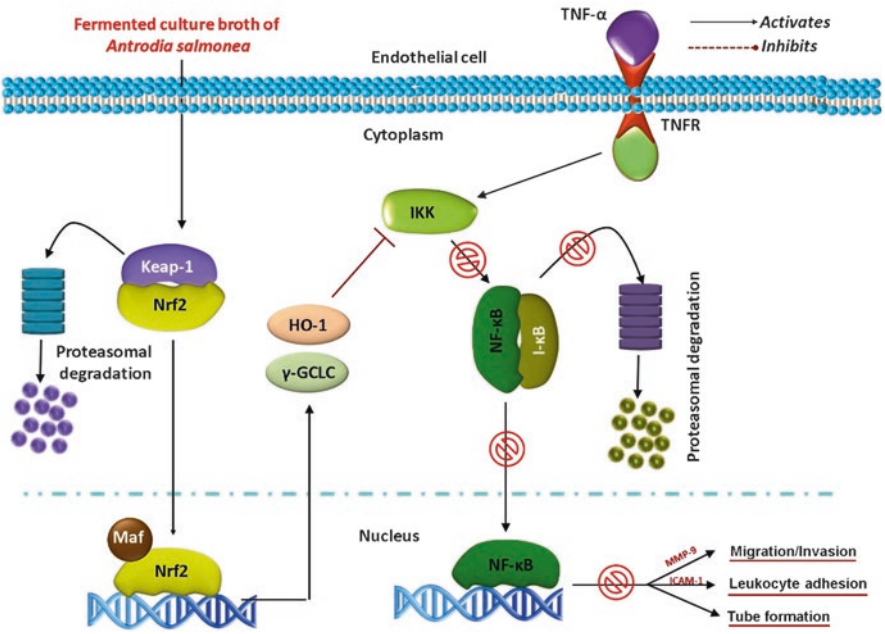

Fig. 6.3 Fermented broth culture extracts of *A. salmonea* upregulate antioxidant gene expression *via* activation of Nrf2/ARE signaling pathway and suppression TNF-α-induced angiogenesis and atherogenesis in human endothelial cells (Yang et al. 2014)

6.5.3 Cytoprotective Effects of Antroquinonol

Antroquinonol (Fig. 6.4) is a ubiquinone derivative isolated from the mycelia of *A. cinnamomea*. Recent studies have indicated that ubiquinone derivatives are potent antioxidant agents and also have shown protection against cancer, male infertility, periodontal diseases, Parkinsonism, and cardiovascular diseases (Lu et al. 2013). Ubiquinone derivatives belong to a larger class of lipophilic benzoquinones. These are structurally correlated with vitamin K and are involved in cellular respiration (Saupe et al. 1994). One of the most extensively studied ubiquinone derivative is coenzyme Q_{10}, which is an important component for cell survival because it is a key intermediate in the electron transport system of mitochondria (Jimenez-Santos et al. 2014; Kumar et al. 2016). Recently, antroquinonol has attracted much attention due to its potent antioxidant and hepatoprotective effects.

Kumar and co-workers have reported that antroquinonol exhibits a potent liver protective effect against alcohol-induced oxidative stress *via* induction of phase II detoxifying enzymes and their corresponding regulatory factors which stabilize the hepatotoxic effect of alcohol. The potential liver protective efficacy of antroquinonol was evaluated in both in vitro and in vivo models to understand its mechanism of action (Kumar et al. 2011). In in vitro studies, it was found that pretreatment of human hepatic cells HepG2 with antroquinonol (5, 10, and 20 μM) eliminates

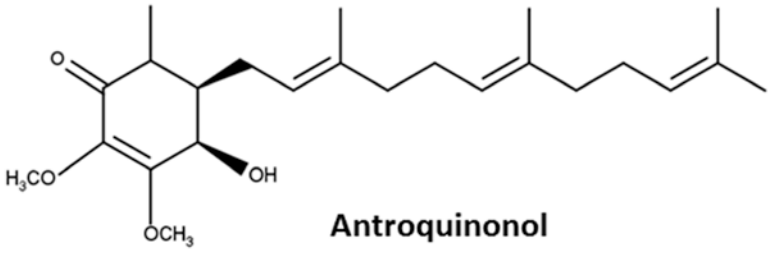

Fig. 6.4 Chemical structure of antroquinonol

alcohol-induced alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as MDA elevation in a dose-dependent manner. This protective effect was highly comparable with silymarin (100 μM), a known hepatoprotective agent. Also, antroquinonol treatment rendered alcohol-induced ROS and nitric oxide (NO) production in a dose-dependent manner (5, 10, and 20 μM). A significant induction in HO-1 protein and mRNA levels was observed in antroquinonol-treated cells under ethanol treatment condition. It is well known that HO-1 expression was regulated by Nrf2-dependent ARE transcriptional activation (Morse and Choi 2002). Antroquinonol increased the nuclear translocation of Nrf2 as evidenced by accumulation of Nrf2 protein level in the nucleus. This study also revealed that Nrf2 activation was regulated by its upstream kinases including p38 MAPK and JNK/SAPK. Further an in vivo study with male ICR mice strongly supports the *in vitro* data that antroquinonol protects liver cells from alcohol-induced liver damage confirmed by reduced levels of ALT, AST, and MDA as well as increased level of GSH in the bloodstream or hepatic tissues (Fig. 6.5). Tsai et al. (2011) demonstrated that antroquinonol is a potent agent against focal segmental glomerulosclerosis (FSGS). A sequential event of ROS overproduction, inflammation, and fibrosis causes the formation of glomerulosclerosis (Assaily et al. 2011). Antroquinonol, isolated from the solid-state fermented mycelia of *A. cinnamomea*, exhibited a protective effect against FSGS-induced inflammation in experimental mice. Also, the FSGS-induced increase in urine protein and serum creatinine levels was inhibited by antroquinonol. A significant preventive effect against FSGS-mediated deposition of hyaline masses in the glomeruli, podocyte injury, sclerotic lesions, and expansion of cellular matrix was showed in antroquinonol treatment group.

Moreover, antroquinonol treatment blocked FSGS-induced ROS accumulation in the kidneys, glomeruli, and renal tubules. Furthermore, antroquinonol exhibited a significant increase of Nrf2 nuclear export and GPx secretion in mice kidney tissues. These findings suggest that antroquinonol protects kidney through Nrf2 activation thereby inhibiting NF-κB-dependent inflammatory pathway as well as suppressing TGF-β1-mediated fibrosis pathway in FSGS-induced mice.

Another study reported that antroquinonol protects mice kidney from preventing the development of accelerated and progressive IgAN (AcP-IgAN) through the inhibition of inflammasome and activation of Nrf2 (Yang et al. 2013). Excessive ROS generation, systemic T cell activation, and macrophage infiltration in the

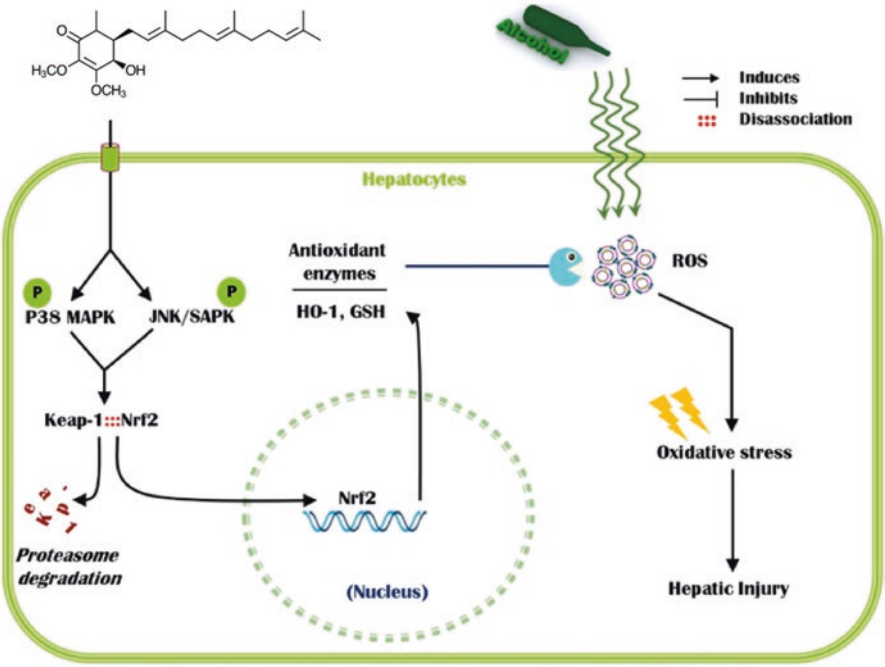

Fig. 6.5 Schematic representation of antroquinonol-induced upregulation of antioxidant genes *via* Nrf2/ARE signaling pathway, which suppressed alcohol-induced oxidative stress and hepatic injury in human hepatic HepG2 cells

kidney implicated in the AcP-IgAN are the most frequent types of primary glomerulonephritis. AcP-IgAN was induced by daily injection of purified IgA antiphosphorylcholine antibodies and pneumococcal C-polysaccharide antigen (PnC) into B-cell-deficient (B6.129S2-Igh-6tm1Cgn/J) mice. Treatment with antroquinonol (15 mg/kg) reduced urine protein, serum blood urea nitrogen (BUN), and serum creatinine levels in AcP-IgAN-induced mice. Administration of antroquinonol administration substantially impeded the development of severe renal lesions, such as intense glomerular proliferation, crescents, sclerosis, and periglomerular interstitial inflammation, in mice with induced AcP-IgAN. In addition, AcP-IgAN mice showed elevated ROS levels in the serum, urine, and renal tissues, compared to normal controls. However, administration of antroquinonol completely inhibited the increase in serum ROS levels on day 3 and substantially inhibited the increase in ROS levels in the serum, urine, and renal tissues on day 28 compared to disease control mice. Moreover, renal cytoplasmic levels of HO-1 protein and GPx activity were increased by antroquinonol in AcP-IgAN-induced mice. Further mechanistic analysis in AcP-IgAN mice revealed that, during the early developmental stage of the AcP-IgAN model, treatment with antroquinonol augmented the transcriptional activity of Nrf2, thereby suppressing activation of T cells and the activity of NLRP3 inflammasome. Furthermore, antroquinonol treatment improved proteinuria/renal

function, and histopathological analysis supports the potential therapeutic effects of antroquinonol against kidney-related disorders.

Chang et al. (2015) demonstrated that antroquinonol ameliorate the Alzheimer's disease (AD)-like phenotype seen in amyloid precursor protein (APP) transgenic mice. Alzheimer's disease (AD) is the most common form among the chronic neurodegenerative diseases. Accumulation of brain amyloid-β peptides (Aβ), a 40–42 amino acid peptide cleaved from amyloid precursor protein (APP), triggers the pathophysiology of AD. Also, oxidative stress and neuroinflammation induced by Aβ play a critical role in the pathogenesis of AD. In earlier studies, antroquinonol has been reported to reduce oxidative stress and inflammatory cytokines *via* activating the Nrf2 signaling pathway, which was found in lower levels in AD. In this study, the authors found that treatment with antroquinonol improved AD-like pathological and behavioral deficits in the *APP* transgenic mouse. Further analysis showed that oral intake of antroquinonol was able to cross the blood-brain barrier without any adverse side effects. Morris water maze test results showed that consumption of antroquinonol for 2 months improved learning and memory process in mice, reduced hippocampal Aβ levels, and abrogates the degree of astrogliosis. These effects had high correlation with decreased levels of histone deacetylase 2 (HDAC2) and increased transcriptional activation of Nrf2. Together these data strongly suggest that antroquinonol could be a suitable candidate for the prevention of AD-like pathological and behavioral deficits.

6.5.4 Hepatoprotective Effect of Antcin C

Drinking alcohol is one of the social behaviors of human since the beginning of civilization. Frequent and high consumption of alcohol results in serious problems in the body including alcohol liver diseases (ALD) (Pari and Karthikesan 2007). ALD is the most common liver disease in Western countries, causing over 20,000 deaths per year in the USA alone. Many cascades were involved in ALD, including oxidative stress and mitochondrial damage (Gilpin et al. 2011). In the human body, ethanol is metabolized to acetaldehyde by a process of enzyme catalysis. The metabolized acetaldehyde is further oxidized into acetate and then converted into carbon dioxide through the citric acid cycle (Das and Vasudevan 2007). Ethanol also affects the immune system *via* modulating cytokine production, in turn decreasing total hepatic glutathione (GSH) and increasing the levels of hepatic triglycerides and lipid peroxidation. GSH is identified as a free radical scavenger and a regenerator of α-tocopherol and plays a significant role in the sustaining of protein sulfhydryl groups (Dey and Cederbaum 2006). Decreased hepatic GSH content results in the increased susceptibility to hepatic injury *via* induction of TNF-α (Fernandez-Checa et al. 2005).

Antcin C (Fig. 6.6), a steroid-like compound isolated from *A. cinnamomea*, protects human hepatic HepG2 cells against 2′-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced oxidative stress and apoptosis (Gokila Vani et al. 2013).

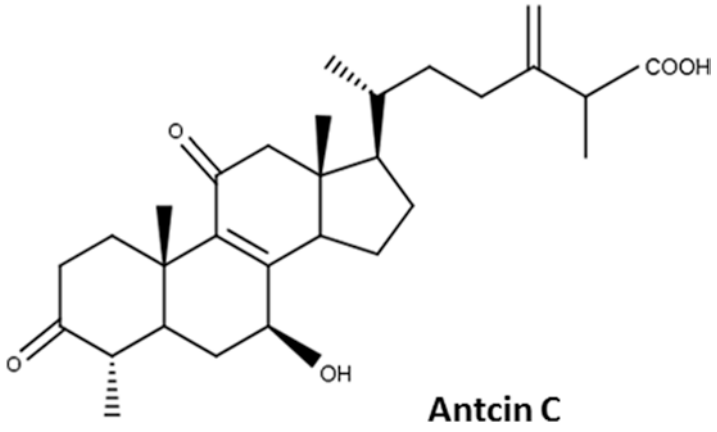

Fig. 6.6 Chemical structure of antcin C

Exposure of HepG2 cells to 10 mM AAPH markedly increased apoptotic cell death followed by accumulation of intracellular ROS.

However, pretreatment with antcin C (5, 10, and 20 μM) protects hepatic cells from AAPH-induced cell death in a dose-dependent manner. The AAPH-induced accumulation of intracellular ROS was eliminated by antcin C. Also, pretreatment with antcin C prevents AAPH-induced lipid peroxidation, ALT, AST secretion, and GSH depletion in HepG2 cells. The antioxidant potential of antcin C was correlated with induction of antioxidant genes including HO-1, NQO-1, γ-GCLC, and SOD *via* transcriptional activation of Nrf2. The Nrf2 activation by antcin C is mediated by JNK1/JNK2 and PI3K activation, which was confirmed by the fact that pharmacologic inhibition of JNK1/JNK2 and PI3K abolished antcin C-induced Nrf2 activity. In addition, AAPH-induced apoptosis was inhibited by antcin C through the downregulation of pro-apoptotic factors including Bax, cytochrome c, caspase-9, caspase-4, caspase-12, caspase-3, and PARP. An in vivo study showed that 80 mg/kg of AAPH elevated serum ALT, AST, and hepatic lipid peroxidation and depletion of total GSH in ICR mice. Antcin C (25, 50, and 100 mg/kg) treatment protected mice liver from AAPH-induced hepatic injury as evidenced by a reduction in hepatic enzymes including ALT, AST, and MDA levels in circulation. Further, immunocytochemical analyses with mice liver tissues showed that antcin C increased HO-1 and Nrf2 protein expression in a dose-dependent manner. The hepatoprotective effect of antcin C was highly comparable with silymarin (200 mg/kg), a well-known hepatoprotective drug. These results strongly suggest that antcin C could protect liver cells from oxidative stress and cell death *via* Nrf2/ARE activation (Fig. 6.7).

6.5.5 Hepatoprotective Effect of 4-Acetylantroquinonol

A previous study by Kumar et al. (2011) reported that ethanol extract of mycelia of *A. cinnamomea* (EMAC) protected alcohol-induced liver injury in mice. Another

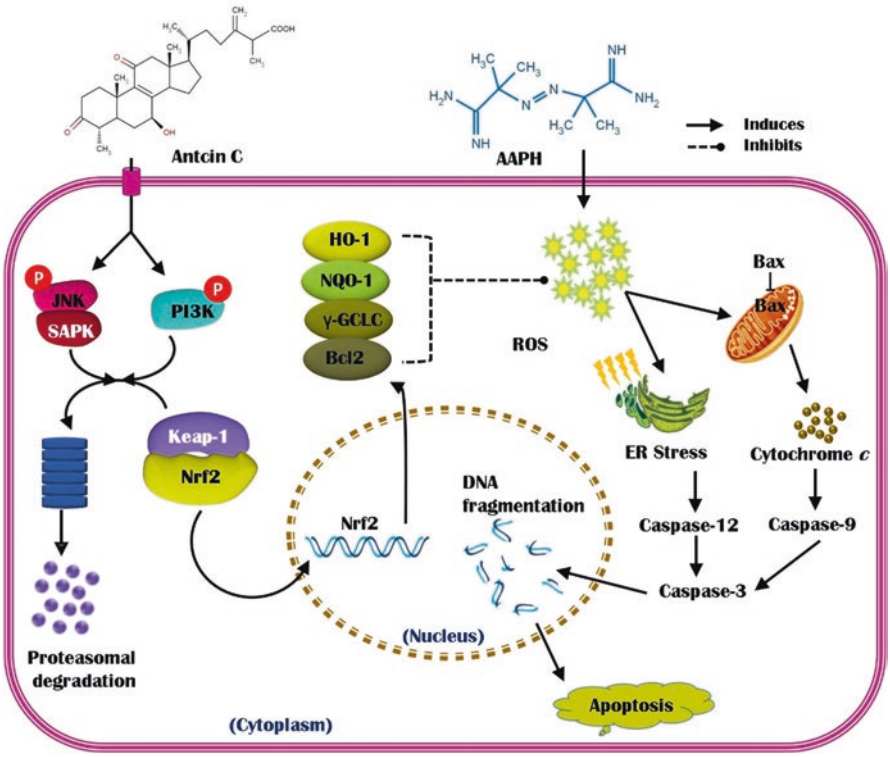

Fig. 6.7 Schematic representation of antcin C-induced upregulation of antioxidative gene expression *via* the Nrf2/ARE signaling pathway and suppression of AAPH-induced apoptosis in human hepatic HepG2 cells

study by Wang et al. (2013) confirmed that EMAC from the solid-state culture increased serum total antioxidant capacity in LPS-induced mice. The metabolite profiling of EMAC revealed that it consists of nine primary metabolites, cytosine, uracil, cytidine, uridine, adenine, inosine, guanosine, adenosine, and deoxyadenosine, and five representative secondary metabolites, such as dehydroeburicoic acid, dehydrosulfurenic acid, 3-isobutyl-4-[4-(3-methyl-2-butenyloxy)-phenyl] furan-2,5-dione, antroquinonol, and 4-acetylantroquinonol B (Fig. 6.8). Quantification of these secondary metabolites showed that antroquinonol was the most abundant compound in the mycelium extract, followed by inosine, 4-acetylantroquinonol B, 3-isobutyl-4-[4-(3-methyl-2-butenyloxy)phenyl]furan-2,5-dione, dehydroeburicoic acid, and dehydrosulfurenic acid. Also, *in vitro* analysis showed that compared to antroquinonol, 4-acetylantroquinonol B exhibited potent anti-inflammatory effects by suppressing NO secretion in LPS-induced murine macrophage cells (RAW264.7).

Also, pretreatment with 4-acetylantroquinonol B downregulated LPS-induced iNOS and COX-2 protein expression in RAW264.7 cells. Next, the authors

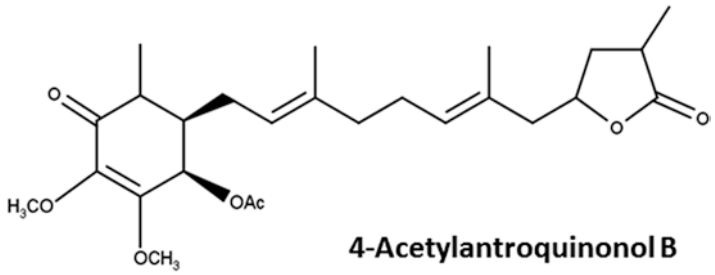

Fig. 6.8 Chemical structures of 4-acetylantroquinonol B

examined the antioxidant potential of 4-acetylantroquinonol B *in vitro*. The noncytotoxic concentrations of 4-acetylantroquinonol B (5, 10, and 20 μg/mL) showed the protective effect on ALT, AST, and MDA production in EtOH-induced HepG2 cells. Also, the GSH levels were reduced in cultured HepG2 cells treated with ethanol, and pretreatment with 4-acetylantroquinonol B protected against hepatic GSH depletion, as evidenced by the restoration or accumulation of GSH above normal levels. The protection against alcohol-induced oxidative stress by 4-acetylantroquinonol B caused the increase in cellular antioxidant genes such as HO-1. It was found that the HO-1 protein levels significantly increased after incubation with 4-antroquinonol B in ethanol-induced HepG2 cells. Furthermore, it has been proved that HO-1 can be activated by Nrf2, a major transcription factor regulating antioxidant response element (ARE)-driven phase II gene expression. Also, 4-acetylantroquinonol B treatment increased Nrf2 accumulation in the nuclear fraction. These results support that 4-acetylantroquinonol B acts against an ethanol-induced hepatic oxidative stress, at least through activation of Nrf2 and induction of HO-1 expression.

6.6 Summary and the Future Perspectives

Recent pharmacological studies on *A. cinnamomea* and *A. salmonea* and their derived components have mostly been performed in vitro and in vivo. *A. cinnamomea* and *A. salmonea* extracts from its fruiting bodies, mycelium, and culture filtrates showed potent antioxidant, anti-inflammatory, and hepatoprotective effects. It took nearly 20 years since the introduction of *A. cinnamomea* to the mainstream research in Taiwan and to shift the research to next levels thus identifying chemical components responsible for its potential biological effects and their targets. However, the new species *A. salmonea* is just the beginning of the exploration. The studies carried out so far clearly demonstrate that compounds isolated from different conditions such as wild harvesting, solid-state cultivation of fruiting bodies, and submerged fermentation culture or parts including fruiting bodies and mycelia showed variation in chemical components. For example, antroquinonol can be found only in mycelia of *A. cinnamomea*, and the compound possesses several

biological activities such as cytotoxicity, anti-inflammation, antioxidant, and hepatoprotective effects. On the other hand, benzenoid and triterpenoids obtained from the fruiting bodies of *A. cinnamomea* showed potent anticancer effects on various cancer types in vitro and in vivo. Particularly, preclinical studies documented a number of antioxidant, and hepatoprotective components from *A. cinnamomea* or *A. salmonea* may provide novel and active medicinal products. Currently, a number of *A. cinnamomea*-based dietary supplements are available. However, the manufacturers cannot claim any specific health benefits, since human clinical trials for studying the effectiveness of *A. cinnamomea* and its active components are still in progress.

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