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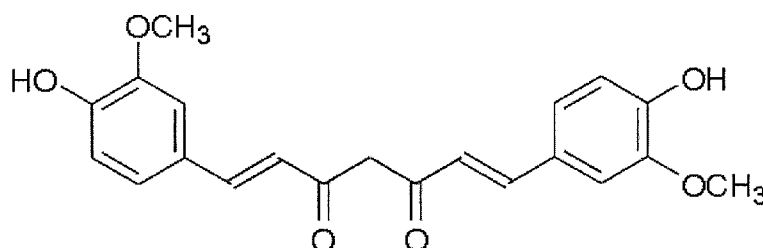


Figure 1

(57) Abstract: Compositions and methods for enhancing heavy metal detoxification are described. The compositions and methods described provide enhanced activity of key detoxification systems including that the induction of phase II detoxification enzymes, such as glutathione s-transferases (GSTs), and NADPH quinone reductase (NQO1) activity.

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UNITED STATES PATENT APPLICATION

for

METHODS AND COMPOSITIONS FOR HEAVY METAL DETOXIFICATION

METHODS AND COMPOSITIONS FOR HEAVY METAL DETOXIFICATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This patent application claims priority to U.S. provisional application Ser. No. 60/917,425 filed on May 11, 2007.

BACKGROUND OF THE INVENTION

Field of the Invention

[002] The present invention relates generally to compositions and methods to promote heavy metal detoxification in mammals in need. More specifically, the invention relates to the use of spent hops, zinc, (1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione), and (3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one) or combinations thereof for heavy metal detoxification in the non-acute state.

Description of the Related Art

[003] There are numerous metals which may pose health concerns due to residential or occupational exposure. Of these; antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc are considered the most problematic. Many of these elements are common to our diet and environment and are actually necessary for maintaining good health. However, exposure to larger amounts may result in acute or chronic toxicity.

[004] Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood components, lungs, kidneys, liver, and other vital organs. Furthermore, long term chronic exposure has been attributed to physical, neurological or muscular degenerative processes which appear to mimic muscular dystrophy,

multiple sclerosis or Parkinson's or Alzheimer's diseases. Additionally, some heavy metals have been identified as potent mutagens and/or carcinogens.

[005] Heavy metal toxicity symptomology is not difficult to recognize. The symptoms, usually severe, are commonly associated with a known exposure or ingestion of the metal. Onset of symptoms is usually rapid and can include cramping, nausea, and vomiting; pain; sweating; headaches; difficulty breathing; impaired cognitive, motor, and language skills; mania; and convulsions.

[006] Symptoms of chronic exposure are very similar to symptoms of other health conditions and often develop slowly over months or even years. However, the symptoms of toxicity resulting from chronic exposure (impaired cognitive, motor, and language skills; learning difficulties; nervousness and emotional instability; and insomnia, nausea, lethargy, and feeling ill) while easily recognized are much more difficult to associate with their cause. A further problem in identifying chronic exposure occurs because the symptoms of chronic exposure may abate from time to time, leading the afflicted individual to postpone seeking treatment, believing the symptoms are related to something other than metal toxicity.

[007] The most commonly encountered toxic metals include aluminum, arsenic, cadmium, iron, lead, and mercury. Arsenic and lead poisonings tend to be the most common due to their wide use in smelting processes, chemical and glass manufacture, or pesticide use (arsenic) while lead exposure can occur from pipes, paint, batteries, and PVC plastics. Target organs for arsenic toxicity include blood, kidneys and digestive, skin and central nervous systems while lead most commonly targets bones, brain, blood, kidneys and the thyroid.

[008] Cadmium toxicity usually targets the brain and kidney resulting from environmental exposures from PVC pipes, batteries and paint pigments.

[009] Aluminum, although not a "heavy" metal per se, has been associated with neurotoxicity (Halatek T, et al., J Environ Sci Health A Tox Hazard Subst Environ Eng. 43(2):118-24, 2008), Alzheimer's disease (Prolo P., et al., Bioinformation. 2007 2(1):24-7, 2007),; Molloy, DW., et al., J Toxicol Environ Health A. 70(23):2011-9, 2007), and cell death (Sato E., et al., Biol Pharm Bull. 30(8):1390-4, 2007).

[0010] Some heavy metals have been identified as potent mutagens and/or carcinogens. These metals have been implicated in apoptosis and cell growth regulation, nuclear transcription regulation and effecting various signal transduction pathways. These metals have also been identified as possessing activity effecting gene expression, carcinogenesis, mutagenesis, and cytotoxicity, as well as in free radical generation. For a review, see Wang S. and Shi X., *Mol Cell Biochem* 222; 3 – 9, 2001.

[0011] Xenobiotic metabolizing enzymes play a major role in regulating the toxic, oxidative damaging, mutagenic, and neoplastic effects of chemical carcinogens. Mounting evidence has indicated that the induction of phase II detoxification enzymes, such as glutathione *S*-transferases (GSTs), and NADPH quinone reductase (NQO1) activity result in protection against toxicity and chemical carcinogenesis, especially during the initiation phase. NQO1 is a flavoprotein that catalyzes two electron reduction of quinones and nitrogen oxides (Riley, R.J. and P. Workman, *Biochem Pharmacol*, 43(8): 1657-69, 1992 and Ross, D., et al., *Cancer Metastasis Rev*, 12(2): 83-101, 1993). Although the major function of this protein may be to reduce the formation of reactive oxygen species by decreasing one electron reduction and the associated redox cycling, it also plays a role in activation of some anticancer drugs and cancer prevention (Begleiter, A., et al., *Cancer Lett*, 45(3): 173-6, 1989 and Begleiter, A., et al., *Oncol Res*, 9(6-7): 371-82, 1997). Recent studies suggest that NQO1 may also be involved in regulation of the transcription factor p53 and apoptosis (Asher, G., et al., *Proc Natl Acad Sci U S A*, 98(3): 1188-93, 2001 and Long, D.J., 2nd, et al., *Cancer Res*, 62(11): 3030-6, 2002).

[0012] The transcriptional activation of the phase II enzymes has been traced to a *cis*-acting transcriptional enhancer called ARE (Rushmore, T.H., et al., *Proc Natl Acad Sci U S A*, 87(10): 3826-30, 1990), or alternatively, the electrophile response element (Friling, R.S., et al., *Proc Natl Acad Sci U S A*, 87(16): 6258-62, 1990). It has been shown that the transcription factor Nrf-2 positively regulates the ARE-mediated expression of the phase II detoxification enzyme genes. Itoh *et al.* (*Biochem Biophys Res Commun*, 236(2): 313-22, 1997) have recently established by gene-targeted disruption in mice that Nrf-2 is a general regulator of the phase II enzyme genes in response to electrophiles and reactive oxygens. More recently, the general regulatory mechanism underlying the electrophile counterattack response has been demonstrated in which electrophilic agents alter the interaction of Nrf-2 with its repressor protein

(Keap-1), thereby liberating Nrf-2 activity from repression by Keap-1, culminating in the induction of the phase II enzyme genes and antioxidative stress protein genes *via* AREs (Itoh, K., et al., *Genes Dev*, 13(1): 76-86, 1999).

[0013] It has been suggested that the dissociation of Nrf-2 from Keap-1 may involve modification of either one of these proteins and could be achieved by direct or indirect mechanisms. For example, Nrf-2 can be phosphorylated by components of the MAP kinase cascade (Yu, R., et al., *J Biol Chem*, 274(39): 27545-52, 1999), which could result in its dissociation. On the other hand, Dinkova-Kostova *et al.* (Dinkova-Kostova, A.T., et al., *Proc Natl Acad Sci U S A*, 98(6): 3404-9, 2001) have provided an alternative possibility that the dissociation of this complex may be potentiated by the direct interaction of electrophilic agents with reactive thiol residues in either of the two proteins. This hypothesis is supported by the strong relationship between the potency of the agents as inducers of the gene expression through the ARE and their rate of reaction with sulfhydryl groups. This mechanism implies that the inducing agent will become covalently bound either to Keap-1 or Nrf-2.

[0014] Heme oxygenase-1 (HO-1) an essential enzyme in heme catabolism, and metallothionein IIA (MT-2A), a small metal-binding protein with clusters of cysteins, are induced in HeLa cells following the treatment with cadmium or zinc. Both proteins are considered to be involved in the defense system against metal toxicity. Heme oxygenase is regulated by both Nrf-2 and MTF-1 transcriptional factors through the activation of ARE and MRE binding sites on heme oxygenase gene.

[0015] By and large, medical research has been directed to acute instances of metal toxin exposure where detoxification and removal of the toxic substance must be accomplished rapidly insofar as the continued presence of the metal toxin places the patient in a true life or death situation. The most common detoxification treatments for metal toxicity include chemical inactivation, metabolic detoxification, or, for example, chelation.

[0016] Currently, these treatments are considered neither appropriate nor indicated for low level toxin exposures, thereby creating a pressing need for safe and effective methods to detoxify individuals with sub-acute metal toxin exposures before toxin build up reaches the requisite level necessitating the more extreme measures described above. The inventors have

identified a number of compounds having a history of safety which modulate the activity of key detoxification enzymes and promote toxin removal from the body. The invention described herein teaches enhancement or inducement of detoxification enzyme systems for sub-acute toxin exposures through modulation of key detoxification enzymes and concomitant administration of additional detoxifying and nutritional agents

SUMMARY OF THE INVENTION

[0017] The present invention relates to compositions and methods to promote heavy metal detoxification under non-acute conditions in mammals. More specifically, the invention relates to the use of spent hops, zinc, (1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione), and (3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one) or combinations thereof for heavy metal detoxification in the non-acute state.

[0018] A first embodiment of the invention describes compositions for promoting heavy metal detoxification in a mammal in need. Here the compositions comprise a therapeutically effective amount of at least two members selected from the group consisting of spent hops; 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione; zinc; and 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one.

[0019] Methods for promoting heavy metal detoxification in a mammal in need are described in a second embodiment. In this embodiment the methods entail administering to the mammal a composition comprising a therapeutically effective amount of at least two members selected from the group consisting of spent hops; 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione; zinc; and 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one.

[0020] A further embodiment of the invention describes a composition comprising: from about 0.10 to about 10.00 grams of spent hops; from about 5 to about 1200 mg of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione; from about 0.10 to about 24 mg of zinc;

and from about 5 to about 1200 mg of 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one.

[0021] Another embodiment describes methods for promoting heavy metal detoxification in a mammal in need. In this embodiment the method utilizes a composition comprising: from about 0.10 to about 10.00 grams of spent hops; from about 5 to about 1200 mg of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione; from about 0.10 to about 24 mg of zinc; and from about 5 to about 1200 mg of 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one.

[0022] A method for promoting heavy metal detoxification in a mammal in need is described in another embodiment. Here the method comprises administering to the mammal a composition comprising a therapeutically effective amount of spent hops.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] Figure 1 is a graphic representation of the chemical structure of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione.

[0024] Figure 2 is a graphic representation of the chemical structure of 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one.

[0025] Figure 3 depicts a Western blot analysis of Nrf-2 in the nuclear fraction of HepG2 cells treated for 4 hours with the indicated test compound. Formula DF-SH is the combination of spent hops, 7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin), 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one (andrographis), Rho dyhydro-isoalpa acids (RIAA) mixture and Zn (525:30:30:4:1).

[0026] Figure 4 depicts a Western blot analysis of Nrf-2 in the nuclear fraction of HepG2 cells treated for 4 hours with the indicated test compound. Formula 3 Components is the combination of Isoalpa acid (IAA), 7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-

dione (curcumin), and 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one (andrographis), (1:1:1).

[0027] Figure 5 graphically depicts the increased induction of metallothionein mRNA levels in volunteers following administration of a test formulation comprising (per tablet): zinc (1.667 mg); 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one (50 mg); 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (50 mg); spent hops (875 mg) and a Rho dihydro-isoalpha acid mixture (6.667 mg).

[0028] Figure 6 graphically depicts the increased induction of metallothionein mRNA levels measured using alternative primers in volunteers following administration of a test formulation comprising (per tablet): zinc (1.667 mg); 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one (50 mg); 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (50 mg); spent hops (875 mg) and a Rho dihydro-isoalpha acid mixture (6.667 mg).

[0029] Figure 7 graphically depicts the effects of test formulation on urinary excretion of clinically important metals. * denotes a detectable increase in excretion.

[0030] Figure 8 graphically depicts the effects of test formulation on urinary excretion of non-clinically important metals.

[0031] Figure 9 graphically depicts the effects of test formulation on urinary excretion of nutrients. * denotes a detectable increase in excretion.

DETAILED DESCRIPTION OF THE INVENTION

[0032] The present invention relates to compositions and methods to promote heavy metal detoxification under non-acute conditions in mammals. More specifically, the invention relates to the use of spent hops, zinc, (1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione), and (3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-

methylenenaphthyl)ethylidene) dihydro-4-hydroxyfuran-2(3H)-one) or combinations thereof for heavy metal detoxification in the non-acute state.

[0033] The patents, published applications, and scientific literature referred to herein establish the knowledge of those with skill in the art and are hereby incorporated by reference in their entirety to the same extent as if each was specifically and individually indicated to be incorporated by reference. Any conflict between any reference cited herein and the specific teachings of this specification shall be resolved in favor of the latter. Likewise, any conflict between an art-understood definition of a word or phrase and a definition of the word or phrase as specifically taught in this specification shall be resolved in favor of the latter.

[0034] Technical and scientific terms used herein have the meaning commonly understood by one of skill in the art to which the present invention pertains, unless otherwise defined. Reference is made herein to various methodologies and materials known to those of skill in the art. Standard reference works setting forth the general principles of recombinant DNA technology include Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, New York (1989); Kaufman *et al.*, Eds., Handbook of Molecular and Cellular Methods in Biology in Medicine, CRC Press, Boca Raton (1995); McPherson, Ed., Directed Mutagenesis: A Practical Approach, IRL Press, Oxford (1991). Standard reference works setting forth the general principles of pharmacology include Goodman and Gilman's The Pharmacological Basis of Therapeutics, 11th Ed., McGraw Hill Companies Inc., New York (2006).

[0035] In the specification and the appended claims, the singular forms include plural referents unless the context clearly dictates otherwise. As used in this specification, the singular forms "a," "an" and "the" specifically also encompass the plural forms of the terms to which they refer, unless the content clearly dictates otherwise. Additionally, as used herein, unless specifically indicated otherwise, the word "or" is used in the "inclusive" sense of "and/or" and not the "exclusive" sense of "either/or." The term "about" is used herein to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the

numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 20%.

[0036] As used herein, the recitation of a numerical range for a variable is intended to convey that the invention may be practiced with the variable equal to any of the values within that range. Thus, for a variable which is inherently discrete, the variable can be equal to any integer value of the numerical range, including the end-points of the range. Similarly, for a variable which is inherently continuous, the variable can be equal to any real value of the numerical range, including the end-points of the range. As an example, a variable which is described as having values between 0 and 2, can be 0, 1 or 2 for variables which are inherently discrete, and can be 0.0, 0.1, 0.01, 0.001, or any other real value for variables which are inherently continuous.

[0037] Reference is made hereinafter in detail to specific embodiments of the invention. While the invention will be described in conjunction with these specific embodiments, it will be understood that it is not intended to limit the invention to such specific embodiments. On the contrary, it is intended to cover alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims. In the following description, numerous specific details are set forth in order to provide a thorough understanding of the present invention. The present invention may be practiced without some or all of these specific details. In other instances, well known process operations have not been described in detail, in order not to unnecessarily obscure the present invention.

[0038] Any suitable materials and/or methods known to those of skill can be utilized in carrying out the present invention. However, preferred materials and methods are described. Materials, reagents and the like to which reference are made in the following description and examples are obtainable from commercial sources, unless otherwise noted.

[0039] A first embodiment of the invention describes compositions for promoting heavy metal detoxification in a mammal in need, where the compositions comprise a therapeutically effective amount of at least two members selected from the group consisting of spent hops; 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione; zinc; and 3-(2-(Decahydro-6-

hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene) dihydro-4-hydroxyfuran-2(3H)-one.

[0040] In one aspect of this embodiment the compositions further comprise a compound selected from the group consisting of (*4S*,*5S*)-3,4-dihydroxy-4-[(*1S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (*4R*,*5R*)-3,4-dihydroxy-4-[(*1R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (*4R*,*5R*)-3,4-dihydroxy-4-[(*1R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (*4R*,*5R*)-3,4-dihydroxy-4-[(*1R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (*4R*,*5R*)-3,4-dihydroxy-4-[(*1S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (*4R*,*5R*)-3,4-dihydroxy-4-[(*1S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (*4R*,*5R*)-3,4-dihydroxy-4-[(*1S*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (*4R*,*5S*)-3,4-dihydroxy-4-[(*1R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (*4R*,*5S*)-3,4-dihydroxy-4-[(*1R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (*4R*,*5S*)-3,4-dihydroxy-4-[(*1R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (*4S*,*5R*)-3,4-dihydroxy-4-[(*1R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (*4S*,*5R*)-3,4-dihydroxy-4-[(*1R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (*4S*,*5R*)-3,4-dihydroxy-4-[(*1R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (*4S*,*5R*)-3,4-dihydroxy-4-[(*1S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (*4S*,*5R*)-3,4-dihydroxy-4-[(*1S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (*4S*,*5R*)-3,4-dihydroxy-4-[(*1S*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one.

2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; and (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one.

[0041] In a further aspect of the embodiment, the compositions further comprise a compound selected from the group consisting of 4*R*,5*S*-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; and (4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one.

[illegible]

(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one; and (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one are described in yet another aspect of this embodiment.

[0043] In some aspects of this embodiment the compositions further comprise a pharmaceutically acceptable excipient selected from the group consisting of coatings, isotonic and absorption delaying agents, binders, adhesives, lubricants, disintegrants, coloring agents, flavoring agents, sweetening agents, absorbants, detergents, and emulsifying agents. In other aspects, the compositions further comprise one or more members selected from the group consisting of antioxidants, vitamins, minerals, proteins, fats, and carbohydrates.

[0044] As used herein, "promoting heavy metal detoxification" refers to inducing detoxification enzyme production, augmenting the enzymatic activity of existing detoxification enzymes, or inhibiting the inhibitors of the detoxification enzymes. Representative, non-limiting examples of detoxification enzymes include alcohol sulphotransferase, amine N-methyltransferase, amine O-sulphotransferase, arylamine N-acetyltransferase (NAT2), catechol O-methyltransferase, cysteine conjugate β -lyase, cysteine N-acetyltransferase, glycine acyltransferase, glutamate acyltransferase, glutathione S-transferases (GST), heme oxygenase-1, histamine N-methyltransferase, microsomal epoxide hydrolase (mEH), metallothioneins, NAD(P)H: quinone oxidoreductase (NQO1), phenol O-methyltransferase, phenol sulphotransferase, rhodanese, thiol S-methyltransferase, thioltransferase, and UDP-glucuronosyl transferase (UDP-GT).

[0045] As used in this specification, whether in a transitional phrase or in the body of the claim, the terms "comprise(s)" and "comprising" are to be interpreted as having an open-ended meaning. That is, the terms are to be interpreted synonymously with the phrases "having at least" or "including at least". When used in the context of a process, the term "comprising" means that the process includes at least the recited steps, but may include additional steps. When

used in the context of a compound or composition, the term "comprising" means that the compound or composition includes at least the recited features or components, but may also include additional features or components.

[0046] As used herein, "compounds" may be identified either by their chemical structure, chemical name, or common name. When the chemical structure and chemical or common name conflict, the chemical structure is determinative of the identity of the compound. The compounds described herein may contain one or more chiral centers and/or double bonds and therefore, may exist as stereoisomers, such as double-bond isomers (*i.e.*, geometric isomers), enantiomers or diastereomers. Accordingly, the chemical structures depicted herein encompass all possible enantiomers and stereoisomers of the illustrated or identified compounds including the stereoisomerically pure form (*e.g.*, geometrically pure, enantiomerically pure or diastereomerically pure) and enantiomeric and stereoisomeric mixtures. Enantiomeric and stereoisomeric mixtures can be resolved into their component enantiomers or stereoisomers using separation techniques or chiral synthesis techniques well known to the skilled artisan. The compounds may also exist in several tautomeric forms including the enol form, the keto form and mixtures thereof. Accordingly, the chemical structures depicted herein encompass all possible tautomeric forms of the illustrated or identified compounds. The compounds described also encompass isotopically labeled compounds where one or more atoms have an atomic mass different from the atomic mass conventionally found in nature. Examples of isotopes that may be incorporated into the compounds of the invention include, but are not limited to, ^2H , ^3H , ^{13}C , ^{14}C , ^{15}N , ^{18}O , ^{17}O , *etc.* Compounds may exist in unsolvated forms as well as solvated forms, including hydrated forms and as N-oxides. In general, compounds may be hydrated, solvated or N-oxides. Certain compounds may exist in multiple crystalline or amorphous forms. Also contemplated within the scope of the invention are congeners, analogs, hydrolysis products, metabolites and precursor or prodrugs of the compound. In general, unless otherwise indicated, all physical forms are equivalent for the uses contemplated herein and are intended to be within the scope of the present invention.

[0047] Compounds according to the invention may be present as salts. In particular, pharmaceutically acceptable salts of the compounds are contemplated. A "pharmaceutically acceptable salt" of the invention is a combination of a compound of the invention and either an

acid or a base that forms a salt (such as, for example, the magnesium salt, denoted herein as "Mg" or "Mag") with the compound and is tolerated by a subject under therapeutic conditions. In general, a pharmaceutically acceptable salt of a compound of the invention will have a therapeutic index (the ratio of the lowest toxic dose to the lowest therapeutically effective dose) of 1 or greater. The person skilled in the art will recognize that the lowest therapeutically effective dose will vary from subject to subject and from indication to indication, and will thus adjust accordingly.

[0048] The compositions according to the invention are optionally formulated in a pharmaceutically acceptable vehicle with any of the well known pharmaceutically acceptable carriers, including diluents and excipients (see Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, Mack Publishing Co., Easton, PA 1990 and Remington: The Science and Practice of Pharmacy, Lippincott, Williams & Wilkins, 1995). While the type of pharmaceutically acceptable carrier/vehicle employed in generating the compositions of the invention will vary depending upon the mode of administration of the composition to a mammal, generally pharmaceutically acceptable carriers are physiologically inert and non-toxic. Formulations of compositions according to the invention may contain more than one type of compound of the invention), as well any other pharmacologically active ingredient useful for the treatment of the symptom/condition being treated. The term "pharmaceutically acceptable" is used in the sense of being compatible with the other ingredients of the compositions and not deleterious to the recipient thereof.

[0049] As used herein "hop" or "hops" refers to plant cones of the genus *Humulus* which contain a bitter aromatic oil which is used in the brewing industry to prevent bacterial action and add the characteristic bitter taste to beer. More preferably, the hops used are derived from *Humulus lupulus*.

[0050] The term "therapeutically effective amount" is used to denote treatments at amounts of the compositions effective to achieve the therapeutic result sought. Furthermore, one of skill will appreciate that the therapeutically effective amount may be lowered or increased by fine tuning and/or by administering more than one composition of the invention, or by administering a composition of the invention with another composition or compound. See, for

example, Meiner, C.L., "Clinical Trials: Design, Conduct, and Analysis," Monographs in Epidemiology and Biostatistics, Vol. 8 Oxford University Press, USA (1986). The invention therefore provides a method to tailor the administration/treatment to the particular exigencies specific to a given mammal. As illustrated in the following examples, therapeutically effective amounts may be easily determined for example empirically by starting at relatively low amounts and by step-wise increments with concurrent evaluation of beneficial effect.

[0051] As used herein, the term "spent hops" refers to the solid and hydrophilic residue resulting from (1) exposing a hops plant product to a solvent, (2) separating the solvent from the hops plant products, and (3) eliminating the solvent. Representative solvents, without limitation, can include CO₂, water, organic solvents (e.g., alcohols) or mixtures thereof.

[0052] 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione is presented graphically in Figure 1. As used herein, "1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione" refers to curcumin, a yellow pigmented fraction isolated from the rhizomes of *Curcuma longa*. The curcuminoids are considered the important active ingredients and are believed to be responsible for the biological activity of *Curcuma longa*. Though their major activity is anti-inflammatory, curcuminoids have been reported to possess antioxidant, antiallergic, wound healing, antispasmodic, antibacterial, antifungal and antitumor activity as well.

[0053] 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one is presented graphically in Figure 2. As used herein, "3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one" refers to andrographolide / andrographis, a diterpene lactone. Diterpene lactone species, such as andrographolide are commonly found in plants and are used for their anti-inflammatory properties. Their mechanism of action is believed to be due (i) to the inhibition of histamine release from mast cells or (ii) to the inhibition of lipoxygenase and cyclooxygenase activity thereby reducing the synthesis of inflammatory factors produced during the arachidonic acid cascade.

[0054] As used herein, the term "reduced isoalpha acid" refers to alpha acids isolated from hops plant product and subsequently have been isomerized and reduced, including cis and

trans forms. Examples of reduced isoalpa acids (RIAA) include without limitation dihydro-isoalpa acids, more specifically Rho dihydro-isoalpa acids (Table 1), tetra-hydroisoalpa acid (Table 2), and hexa-hydroisoalpa acids (Table 3), and their derivatives. "Rho" refers to those reduced isoalpa acids wherein the reduction is a reduction of the carbonyl group in the 4-methyl-3-pentenoyl side chain.

Table 1

Rho dihydro-isoalpa acids

Chemical Name	Synonym	Structure
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>S</i>) <i>cis</i> n iso-alpha acid	
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>R</i>) <i>cis</i> n iso-alpha acid	
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>R</i>) <i>trans</i> n iso-alpha acid	
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>S</i>) <i>trans</i> n iso-alpha acid	
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>R</i>) <i>cis</i> rho n iso-alpha acid	

Chemical Name	Synonym	Structure
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>S</i>) <i>cis</i> n iso-alpha acid	
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	(6 <i>S</i>) <i>trans</i> rho n iso-alpha acid	
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>R</i>) <i>trans</i> n iso-alpha acid	
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	rho (6 <i>S</i>) <i>cis</i> co iso-alpha acid	
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	rho (6 <i>R</i>) <i>cis</i> co iso-alpha acid	
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	rho (6 <i>R</i>) <i>trans</i> co iso-alpha acid	
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	rho (6 <i>S</i>) <i>trans</i> co iso-alpha acid	
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	rho (6 <i>R</i>) <i>cis</i> co iso-alpha acid	

Chemical Name	Synonym	Structure
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	rho (6 <i>S</i>) <i>cis</i> co iso-alpha acid	
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylpropanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>S</i>) <i>trans</i> co iso-alpha acid	
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	rho (6 <i>R</i>) <i>trans</i> co iso-alpha acid	
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>S</i>) <i>cis</i> ad iso-alpha acid	
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>R</i>) <i>cis</i> ad iso-alpha acid	
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>R</i>) <i>trans</i> ad iso-alpha acid	
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>S</i>) <i>trans</i> ad iso-alpha acid	
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>R</i>) <i>cis</i> ad iso-alpha acid	

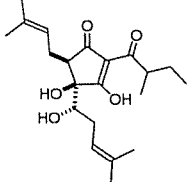
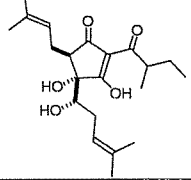
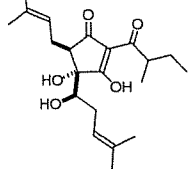
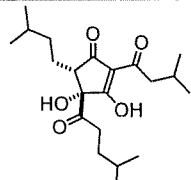
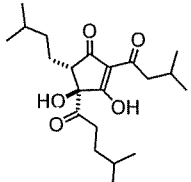
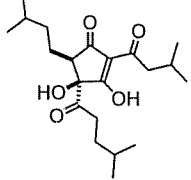
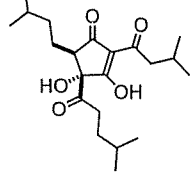
Chemical Name	Synonym	Structure
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>S</i>) <i>cis</i> ad iso-alpha acid	
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>S</i>) <i>trans</i> ad iso-alpha acid	
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	rho (6 <i>R</i>) <i>trans</i> ad iso-alpha acid	

Table 2

Tetrahydro-isoalpha acids

Chemical Name	Synonym	Structure
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one	tetrahydro <i>cis</i> n iso-alpha acid	
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one	tetrahydro <i>trans</i> n iso-alpha acid	
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one	tetrahydro <i>cis</i> n iso-alpha acid	
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one	tetrahydro <i>trans</i> n iso-alpha acid	

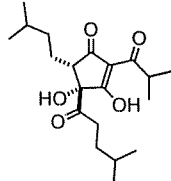
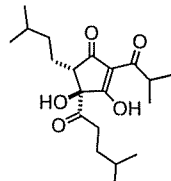
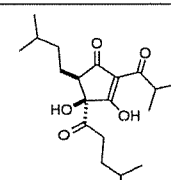
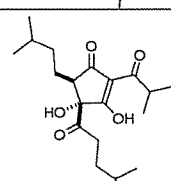
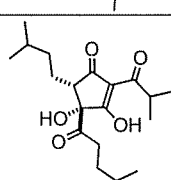
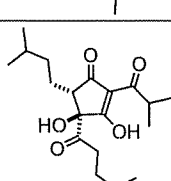
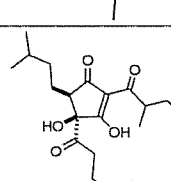
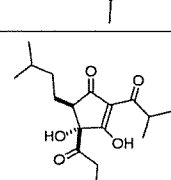
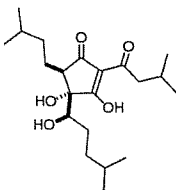
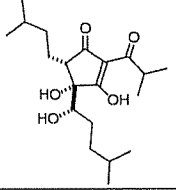
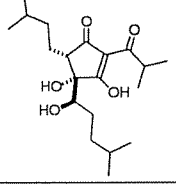
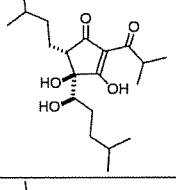
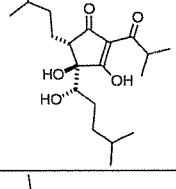
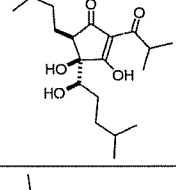
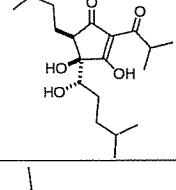
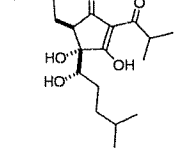
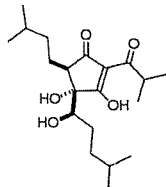
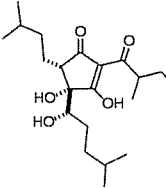
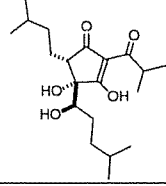
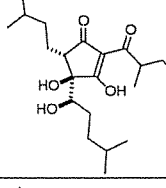
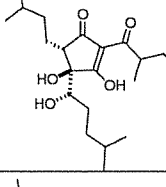
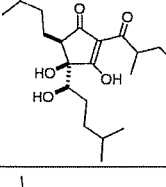
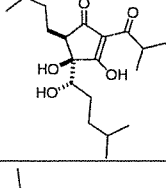
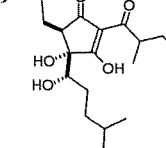
Chemical Name	Synonym	Structure
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-5-(3-methylbutyl)-4-(4-methylpentanoyl)-2-(3-methylpropanoyl)cyclopent-2-en-1-one	tetrahydro <i>cis</i> co iso-alpha acid	
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-5-(3-methylbutyl)-4-(4-methylpentanoyl)-2-(3-methylpropanoyl)cyclopent-2-en-1-one	tetrahydro <i>trans</i> co iso-alpha acid	
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-5-(3-methylbutyl)-4-(4-methylpentanoyl)-2-(3-methylpropanoyl)cyclopent-2-en-1-one	tetrahydro <i>cis</i> co iso-alpha acid	
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-5-(3-methylbutyl)-4-(4-methylpentanoyl)-2-(3-methylpropanoyl)cyclopent-2-en-1-one	tetrahydro <i>trans</i> co iso-alpha acid	
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-2-(2-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one	tetrahydro <i>cis</i> ad iso-alpha acid	
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-2-(2-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one	tetrahydro <i>trans</i> ad iso-alpha acid	
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-2-(2-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one	tetrahydro <i>cis</i> ad iso-alpha acid	
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-2-(2-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one	tetrahydro <i>trans</i> ad iso-alpha acid	

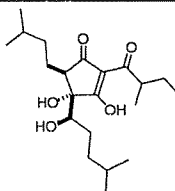
Table 3

Hexahydro-isoalpha acids

Chemical Name	Synonym	Structure
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one	hexahydro (6 <i>S</i>) <i>cis</i> n iso-alpha acid	
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one	hexahydro (6 <i>R</i>) <i>cis</i> n iso-alpha acid	
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one	hexahydro (6 <i>R</i>) <i>trans</i> n iso-alpha acid	
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one	hexahydro (6 <i>S</i>) <i>trans</i> n iso-alpha acid	
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one	hexahydro (6 <i>R</i>) <i>cis</i> n iso-alpha acid	
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one	hexahydro (6 <i>S</i>) <i>cis</i> n iso-alpha acid	
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one	hexahydro (6 <i>S</i>) <i>trans</i> n iso-alpha acid	

Chemical Name	Synonym	Structure
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one	hexahydro (6 <i>R</i>) <i>trans</i> n iso-alpha acid	
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	hexahydro (6 <i>S</i>) <i>cis</i> co iso-alpha acid	
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	hexahydro (6 <i>R</i>) <i>cis</i> co iso-alpha acid	
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	hexahydro (6 <i>R</i>) <i>trans</i> co iso-alpha acid	
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	hexahydro (6 <i>S</i>) <i>trans</i> co iso-alpha acid	
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	hexahydro (6 <i>R</i>) <i>cis</i> co iso-alpha acid	
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	hexahydro (6 <i>S</i>) <i>cis</i> co iso-alpha acid	
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylpropanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	hexahydro (6 <i>S</i>) <i>trans</i> co iso-alpha acid	

Chemical Name	Synonym	Structure
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one	hexahydro (6 <i>R</i>) <i>trans</i> co iso-alpha acid	
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	hexahydro (6 <i>S</i>) <i>cis</i> ad iso-alpha acid	
(4 <i>S</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	hexahydro (6 <i>R</i>) <i>cis</i> ad iso-alpha acid	
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	hexahydro (6 <i>R</i>) <i>trans</i> ad iso-alpha acid	
(4 <i>R</i> ,5 <i>S</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	hexahydro (6 <i>S</i>) <i>trans</i> ad iso-alpha acid	
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	hexahydro (6 <i>R</i>) <i>cis</i> ad iso-alpha acid	
(4 <i>R</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	hexahydro (6 <i>S</i>) <i>cis</i> ad iso-alpha acid	
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>S</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	hexahydro (6 <i>S</i>) <i>trans</i> ad iso-alpha acid	

Chemical Name	Synonym	Structure
(4 <i>S</i> ,5 <i>R</i>)-3,4-dihydroxy-4-[(1 <i>R</i>)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one	hexahydro (6 <i>R</i>) <i>trans</i> ad iso-alpha acid	

[0055] As used herein, the terms “derivatives” or a matter “derived from” refer to a chemical substance related structurally to another substance and theoretically obtainable from it, *i.e.* a substance that can be made from another substance. Derivatives can include compounds obtained via a chemical reaction or *de novo* chemical syntheses. See Verzele, M. and De Keukeleire, D., Developments in Food Science 27: Chemistry and Analysis of Hop and Beer Bitter Acids, Elsevier Science Pub. Co., 1991, New York, USA, herein incorporated by reference in its entirety, for a detailed discussion of hops chemistry.

[0056] A second embodiment of the invention describes methods for promoting heavy metal detoxification in a mammal, where the methods utilize compositions which comprise a therapeutically effective amount of at least two members selected from the group consisting of spent hops; 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione; zinc; and 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one.

[0057] In one aspect of this embodiment the compositions of the method further comprise a compound selected from the group consisting of (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-

methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylpropanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; and (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one.

[0058] In a further aspect of the embodiment, the compositions of the method further comprise a compound selected from the group consisting of 4*R*,5*S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-

[illegible][illegible]

[illegible]

[0060] In some aspects of this embodiment the compositions used further comprise a pharmaceutically acceptable excipient selected from the group consisting of coatings, isotonic and absorption delaying agents, binders, adhesives, lubricants, disintergrants, coloring agents, flavoring agents, sweetening agents, absorbants, detergents, and emulsifying agents. In other aspects the compositions of the methods further comprise one or more members selected from the group consisting of antioxidants, vitamins, minerals, proteins, fats, and carbohydrates.

[0061] The methods of the present invention are intended for use with any mammal that may experience the benefits of the methods of the invention. Foremost among such mammals are humans, although the invention is not intended to be so limited, and is applicable to veterinary uses. Thus, in accordance with the invention, "mammals" or "mammals in need" include humans as well as non-human mammals, particularly domesticated animals including, without limitation, cats, dogs, and horses.

[0062] A composition comprising: from about 0.10 to about 10.00 grams of spent hops; from about 5 to about 1200 mg of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione; from about 0.10 to about 24 mg of zinc; and from about 5 to about 1200 mg of 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one is described in a third embodiment of the invention while a further embodiment of the invention describes methods for promoting heavy metal detoxification in a mammal, where the methods utilize compositions comprising from about 0.10 to about 10.00 grams of spent hops; from about 5 to about 1200 mg of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione; from about 0.10 to about 24 mg of zinc; and from about 5 to about 1200 mg of 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one.

[0063] A further embodiment of the invention describes methods for promoting heavy metal detoxification in a mammal utilizing a composition comprising a therapeutically effective amount of spent hops.

[0064] The following examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature. Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein.

EXAMPLES

Example 1

Effects of test compounds on detoxification enzyme activity

[0065] *Test Materials* – All test compounds were supplied by Metagenics (San Clemente, CA., USA). Test compounds were prepared in dimethyl sulfoxide (DMSO) and stored at -20°C. Sulforaphane, glutathione and 1-Chloro 2, 4-dinitro benzene (CDNB) were purchased from Sigma Chemicals (St.Louis, MO). All other general chemicals were analytical grade.

[0066] *Cell culture and treatment* –The rat liver cell line RL-34 was purchased from the Japan Health Science Foundation (Tokyo, Japan). Cells were maintained in log phase in DMEM media supplemented with 5% heat inactivated FBS, 100 IU penicillin, and 100 µg/ml streptomycin. The human liver cell line HepG2 was purchased from ATCC (Manassas, VA). Cells were maintained in log phase in MEM media supplemented with 10% heat inactivated FBS, 100 IU penicillin, and 100 µg/ml streptomycin.

[0067] *Enzymatic activity in RL-34 cells* – Cells were subcultured in 6-well plates at a density of 3×10^5 cells per well. The following day test compounds in DMSO were added at a final 0.1% DMSO concentration. The cells were incubated with test compound for 2 days in a humidified incubator at 37°C after which the cells were lysed and assayed for enzyme activity. Following treatment with test compounds, cells were washed twice with 0.5 ml PBS and all residual liquid was removed. To lyse the cells, 150 µL Buffer D (0.8% digitonin in 2 mM EDTA) was added to each well and incubated for 15 minutes at room temperature. The cell lysate was centrifuged for 10 minutes at 10,000 rpm. The supernatant containing the cell lysate was transferred into a 96-well plate. Protein determination was performed using the Bicinchoninic Acid Kit (Sigma, St. Louis, MO). Equal protein concentrations were used in all enzyme activity assays.

[0068] *GST activity assay* - Cell lysate (20 µL) containing the GST enzyme was added to each well of a UV flat bottom 96 well microtiter plate. Reaction buffer was added (300 µL) to each well to achieve a final concentration of 8 mM glutathione and 3.2 mM CDNB in 100 mM KPO₄. The enzymatic conversion of CDNB was measured at 340 nm over three minutes to

determine a rate of GST activity (Apati, P., et al., J Pharm Pharmacol, 58(2): 251-6, 2006, and Habig, W.H., M.J. Pabst, and W.B. Jakoby, J Biol Chem., 249: 7130-9, 1974).

[0069] *NADPH quinone oxido reductase assay* (NQO1) - To assay NQO1 activity, 20 μ L of cell lysate containing the NQO1 enzyme was added to each well of a flat bottom 96 well microtiter plate. Reaction buffer was added (200 μ L) to each well to achieve a final concentration of 25 mM Tris-HCl, pH 7.4, 5 μ M FAD, 1 mM G6P, 30 μ M NADP, 2 U/ml G6PDH, 0.06% BSA, 725 μ M MTT and 50 μ M menadione. The reduction of MTT at 610 nm was measured after three minutes to determine the induction of NQO1 activity (Feng, R., et al., J Biol Chem, 280(30): 27888-95, 2005. and Prochaska, H.J. and A.B. Santamaria, Anal Biochem., 169: 328-36, 1988). The induction of NQO1 activity was determined as the ratio of treated over vehicle control.

[0070] *Co-transfection of Reporter and Expression Plasmids* – Construction of the heme oxygenase reporter was described previously (Gong, P., et al., Arch Biochem Biophys, 405(2): 265-74, 2002). The Lipofectamine 2000 transfection reagent from Invitrogen was used to perform the transfections by procedures as described in the manufacturer's protocol. HepG2 cells were subcultured in 96-well white plates at a density of 5×10^4 cells per well in MEM medium without serum and antibiotics. The following day dilute HO-1 reporter construct with pRL-TK (0.2 μ g total DNA in a ratio of 2:1) in 25 μ L of *opti-MEM* and mix gently. Dilute 0.5 μ L of Lipofectamine 2000 in 25 μ L of *opti-MEM* and incubate for 5 minutes at room temperature. Then combine the diluted DNA with diluted Lipofectamine 2000, mix gently and incubate for 20 min at room temperature. The cells were incubated with 50 μ L of above complex for 6 hours in a humidified incubator at 37°C. After 6 hours of incubation, 100 μ L MEM medium without serum and antibiotics were added and incubated for 18 hours. The medium was discarded and test compounds in DMSO were added at a final 0.1% DMSO concentration for 24 hours. The plasmid pRL-TK encoding Renilla luciferase was used as the internal control of each transfection.

[0071] *Luciferase activity* – Two days after the transfection, cells were washed with phosphate-buffered saline and lysed in passive lysis buffer from the Dual Luciferase reporter assay system per the manufacturer's protocol (Promega). First, the cell lysate was assayed for

the firefly luciferase activity using 100 µl of the substrate LARII. Then 100 µl of the STOP & GLO reagent was added to quench the firefly luciferase activity and activate the *Renilla* luciferase, which was also measured. The assays were carried out in a PerkinElmer luminometer, and the relative luciferase activity was calculated as follows: 100,000/activity of *Renilla* luciferase (in units) ÷ activity of firefly luciferase (in units). Each set of transfections was repeated three times.

[0072] *Quantitative PCR* – HepG2 cells were subcultured in 6-well plates at a density of 3×10^5 cells per well. The following day test compounds in DMSO were added at a final 0.1% DMSO concentration. The cells were incubated with test compound for 8 hours in a humidified incubator at 37°C. The mRNA was then purified for gene expression analysis using TRI reagent (Sigma Chemicals) according to the manufacture's instructions. Following mRNA purification, the cDNA template was generated for use in the QPCR analysis. This was performed with the Omniscript RT kit (Qiagen, Valencia, CA) at 37°C for 60 min in a Techgene thermocycler (Techne, Burlington, NJ) to yield a 20 µL reaction with 2 µg starting mRNA. The blinded samples were sent to Dr. John Tine at the Center for Functional Genomics, University at Albany (Rensselaer, NY). Quantitative PCR was performed utilizing Taqman[®] chemistry and $\Delta\Delta C_t$ quantification. Assays were performed in triplicate with 20 ng total RNA per well and GAPDH as the internal control. The remaining five genes analyzed were GSTA1, NQO1, HMOX1, and two groupings of metallothioneins, hMT1&2 and hMT1&2alt. The protocol for metallothionein gene expression was taken from the work by Aydemir et al (Proc Natl Acad Sci U S A, 103(6): 1699-704, 2006), where a combination of two forward primers, two reverse primers, and two Taqman[®] probes gave matches for MT1H, -1H-like, -1G, -1L, -1E, -1A, and MT2.

[0073] *Western blot detection* – The HepG2 cell line was cultured as above and test compounds were added for four hours, after which the cells were lysed and the nuclear fraction was isolated (Dignam, J.D., R.M. Lebovitz, and R.G. Roeder, Nucleic Acids Res, 11(5): 1475-89, 1983). Total protein concentration was determined using the Bicinchoninic Acid Kit (Sigma). Total cell lysates (60 µg total protein) were electrophoresed and detection of Nrf-2 was performed using primary antibody incubation overnight at 4°C (Santa Cruz Biotechnology, Inc, Santa Cruz, CA). Secondary antibody linked to horseradish peroxidase was incubated for one h (GE Healthcare, Piscataway, NJ), after which proteins were visualized using the enhanced

chemiluminescence (ECL) system. Densitometry was performed using Kodak Molecular Imaging Software v.4.0 (Eastman Kodak Company, Rochester, NY) measuring the net intensity of the band.

[0074] *Statistical analysis* – The induction of GST and NADPH quinone reductase assay activity were determined as the ratio of treated with test compounds over vehicle (DMSO) control. GST and NQO1 activity was normalized for equal protein. A minimum of two wells were used for each condition. The induction of heme oxygenase promoter activity was determined as the ratio of treated with test compounds over vehicle control. Luciferase activity was normalized with Renilla luciferase activity as a control. A minimum of 4 wells were used for each condition. The data represents the average of three independent experiments (SEM).

RESULTS

[0075] *Enzymatic activity* – Sulforaphane is a known activator of Nrf-2 and was used as a control in the enzymatic activity assays. According to the convention of the scientific literature, 20% induction of activity by test compounds over DMSO control is considered active. GST activity (Table 4) was induced $\geq 20\%$ by Sulforaphane (3 $\mu\text{g/ml}$), Andrographis (25 $\mu\text{g/ml}$), Xanthohumol (1 $\mu\text{g/ml}$), Prune (20 $\mu\text{g/ml}$), Withania (20 $\mu\text{g/ml}$), Osteosine (20 $\mu\text{g/ml}$), MCHA (20 $\mu\text{g/ml}$), and Arthred Porcine (20 $\mu\text{g/ml}$) in RL-34 cell line. NQO1 enzymatic activity (Table 5) was induced $\geq 20\%$ by (1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin) (5 $\mu\text{g/ml}$), (3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one) (andrographis) (25 $\mu\text{g/ml}$), Xanthohumol (1 $\mu\text{g/ml}$), Sulforaphane (3 $\mu\text{g/ml}$), Withania (20 $\mu\text{g/ml}$), I3C (25 $\mu\text{g/ml}$), Resveratrol (20 $\mu\text{g/ml}$), Milk Thistle (25 $\mu\text{g/ml}$), IAA (5 $\mu\text{g/ml}$), Pomegranite (25 $\mu\text{g/ml}$), Parthenolide (20 $\mu\text{g/ml}$), Amla (25 $\mu\text{g/ml}$), Oleanolic acid (25 $\mu\text{g/ml}$), DIM (25 $\mu\text{g/ml}$), Quercetin (20 $\mu\text{g/ml}$), Berberine (20 $\mu\text{g/ml}$), Cinnamon (25 $\mu\text{g/ml}$), Puerariae (20 $\mu\text{g/ml}$), Wasabia Rhizome (25 $\mu\text{g/ml}$), Watercress (25 $\mu\text{g/ml}$), Epimedium (20 $\mu\text{g/ml}$), chlorogenic acid (25 $\mu\text{g/ml}$), Abelmoschus (20 $\mu\text{g/ml}$), Bonistein (20 $\mu\text{g/ml}$), Peppermint (25 $\mu\text{g/ml}$), Blueberry (25 $\mu\text{g/ml}$), Picrorhiza (25 $\mu\text{g/ml}$), Wasabia Powder (25 $\mu\text{g/ml}$), HHIAA (5 $\mu\text{g/ml}$), Osteosine (20 $\mu\text{g/ml}$), Dandelion (25 $\mu\text{g/ml}$), Green Coffee (25 $\mu\text{g/ml}$) and THIAA (5 $\mu\text{g/ml}$).

Table 4

Screening of test compounds for GST enzyme activity in the RL-34 cell line

Test Compound (ug/mL)	Fold Induction of GST activity	SEM	n	Test Compound (ug/mL)	Fold Induction of GST activity	SEM	n
Sulforaphane (3 ug/mL)	1.8	0.12	5	Blueberry (25 ug/mL)	1.0	0.05	2
Andrographis (25 ug/mL)	1.4	0.03	4	Irish Moss (25 ug/mL)	1.0	0.08	2
Xanthohumol (1 ug/mL)	1.4	0.05	3	Fructus LL (20 ug/mL)	1.0	0.08	2
Prune (20 ug/mL)	1.4		1	FOS (20 ug/mL)	1.0	0.05	2
Formula DF-SH (50 ug/mL)	1.3	0.10	3	DIM (25 ug/mL)	1.0	0.00	2
Withania (20 ug/mL)	1.3	0.02	2	Broc 13 (25 ug/mL)	1.0		1
Osteosine (20 ug/mL)	1.2		1	Puerariae (20 ug/mL)	1.0	0.07	2
MCHA (20 ug/mL)	1.2		1	THIAA (5 ug/mL)	1.0	0.02	3
Arthred Porcine (20 ug/mL)	1.2		1	Peppermint (25 ug/mL)	1.0	0.05	2
Glucosamine (20 ug/mL)	1.1		1	Alma (25 ug/mL)	1.0	0.02	4
Resveratrol (20 ug/mL)	1.1	0.08	2	Milk Basic Protein (20 ug/mL)	1.0		1
CLA (20 ug/mL)	1.1	0.03	2	Policosanol (20 ug/mL)	1.0	0.05	2
Dandelion (25 ug/mL)	1.1	0.19	4	Milk Thistle (25 ug/mL)	1.0	0.04	4
I3C (25 ug/mL)	1.1	0.08	2	Glabridin (20 ug/mL)	1.0		1
Black Cohosh (20 ug/mL)	1.1	0.04	2	Wasabia Powder (25 ug/mL)	1.0	0.04	2
Spent Hops (50 ug/mL)	1.1	0.07	3	Phloridzin (20 ug/mL)	1.0	0.01	2
IAA (5 ug/mL)	1.1	0.05	4	Green Coffee (25 ug/mL)	1.0	0.03	2
Vitamin C (20 ug/mL)	1.1	0.01	2	Broc 12 (25 ug/mL)	1.0		1
Ipriflavone (20 ug/mL)	1.1	0.00	2	Picrorhiza (25 ug/mL)	1.0	0.03	3
chlorogenic acid (25 ug/mL)	1.1		1	Green Tea (25 ug/mL)	1.0		1
K-Citrate (20 ug/mL)	1.1	0.04	2	Berberine (20 ug/mL)	1.0		1
Hyaluronic acid (20 ug/mL)	1.1		1	DHEA (20 ug/mL)	1.0	0.01	2
Arthred Bovine (20 ug/mL)	1.0	0.01	2	Broc 10 (25 ug/mL)	1.0		1
Cinnamon (25 ug/mL)	1.0	0.01	2	Bonepep (20 ug/mL)	1.0	0.01	2
RIAA (5 ug/mL)	1.0	0.00	2	Broc 14 (25 ug/mL)	1.0		1
Astragalus (20 ug/mL)	1.0		1	Oleuropein (20 ug/mL)	1.0	0.04	2
Broc 19 (25 ug/mL)	1.0		1	Vitamin K2 (20 ug/mL)	1.0		1
Broc 49 (25 ug/mL)	1.0		1	Garlic (25 ug/mL)	1.0	0.06	2
Bonistein (20 ug/mL)	1.0		1	Devils Claw (20 ug/mL)	1.0	0.12	2
Broc 11 (25 ug/mL)	1.0		1	Kolla 2 (20 ug/mL)	1.0	0.04	2
Rutin (20 ug/mL)	1.0	0.04	2	Wasabia Rhizome (25 ug/mL)	1.0	0.00	2
Perilla (20 ug/mL)	1.0	0.03	3	Hesperidin (20 ug/mL)	1.0	0.07	2
Olive Oil (20 ug/mL)	1.0	0.05	2	Watercress (25 ug/mL)	1.0	0.05	2
Parthenolide (20 ug/mL)	1.0	0.06	2	Dioscorea (20 ug/mL)	1.0	0.05	3
HHIAA (5 ug/mL)	1.0	0.01	3	DHA (20 ug/mL)	1.0	0.03	2
Quercitin (20 ug/mL)	1.0	0.02	2	Black Tea (20 ug/mL)	1.0	0.13	2
Terminalin (25 ug/mL)	1.0	0.03	2	Salvia MB (20 ug/mL)	0.9	0.04	2
Pomegranite (25 ug/mL)	1.0	0.04	3	Abelmoschus (20 ug/mL)	0.9	0.10	2
Oleanolic acid (25 ug/mL)	1.0	0.01	4	Soy Isoflavones (20 ug/mL)	0.9	0.04	2
Flaxseed (20 ug/mL)	1.0	0.07	2	Broc 09 (25 ug/mL)	0.9		1
Curcumin (5 ug/mL)	1.0	0.04	2	Black Rice (20 ug/mL)	0.8	0.14	2
Inulin (20 ug/mL)	1.0	0.04	2	Red Yeast Rice (20 ug/mL)	0.8	0.09	2
Epimedium (20 ug/mL)	1.0	0.09	2				

[0076] Formula DF-SH is the combination of spent hops, (1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) (curcumin), (3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one) (andrographis), Rho Isoalpha acid and Zn (525:30:30:4:1)

Table 5

Screening of test compounds for NQO-1 enzyme activity in the RL-34 cell line

Induction of NQO1				Induction of NQO1			
Test Compound (ug/mL)	activity	SEM	n	Test Compound (ug/mL)	activity	SEM	n
Curcumin (5 ug/mL)	4.6	1.59	3	Broc 09 (25 ug/mL)	1.1		1
Andrographis (25 ug/mL)	4.5	1.00	4	Broc 12 (25 ug/mL)	1.1		1
Formula DF-SH (50 ug/mL)	4.0	0.43	3	Broc 14 (25 ug/mL)	1.1		1
Xanthohumol (1 ug/mL)	3.7	0.78	3	Flaxseed (20 ug/mL)	1.1	0.05	2
Sulforaphane (3 ug/mL)	3.7	0.76	4	Sly Isoflavones (20 ug/mL)	1.1	0.02	2
Withania (20 ug/mL)	2.4	0.72	2	Arthred Porcine (20 ug/mL)	1.1		1
I3C (25 ug/mL)	2.4	0.34	2	Garlic (25 ug/mL)	1.0		1
Resveratrol (20 ug/mL)	2.2	0.44	2	Hesperidin (20 ug/mL)	1.0	0.04	2
Spent Hops (50 ug/mL)	2.0	0.15	3	Terminalin (25 ug/mL)	1.0	0.16	2
Milk Thistle (25 ug/mL)	2.0	0.04	4	Broc 11 (25 ug/mL)	1.0		1
IAA (5 ug/mL)	1.9	0.16	4	Perilla (20 ug/mL)	1.0	0.05	2
Pomegranite (25 ug/mL)	1.8	0.41	3	Prune (20 ug/mL)	1.0		1
Parthenolide (20 ug/mL)	1.7	0.25	2	CLA (20 ug/mL)	1.0	0.05	2
Alma (25 ug/mL)	1.6	0.09	4	Salvia MB (20 ug/mL)	1.0	0.03	2
Oleanolic acid (25 ug/mL)	1.5	0.08	4	MCHA (20 ug/mL)	1.0		1
DIM (25 ug/mL)	1.5	0.22	2	Dioscorea (20 ug/mL)	1.0	0.12	2
Quercitin (20 ug/mL)	1.4	0.07	2	Oleuropein (20 ug/mL)	1.0	0.02	2
Berberine (20 ug/mL)	1.4	0.47	2	Broc 13 (25 ug/mL)	1.0		1
Cinnamon (25 ug/mL)	1.4	0.28	2	Glabridin (20 ug/mL)	1.0	0.02	2
Puerariae (20 ug/mL)	1.4	0.06	2	Arthred Bovine (20 ug/mL)	1.0	0.13	3
Wasabia Rhizome (25 ug/mL)	1.4	0.17	2	Black Cohosh (20 ug/mL)	1.0	0.03	2
Watercress (25 ug/mL)	1.3	0.03	2	Olive Oil (20 ug/mL)	1.0	0.06	2
Epimedium (20 ug/mL)	1.3		1	Phloridzin (20 ug/mL)	1.0	0.02	2
chlorogenic acid (25 ug/mL)	1.2	0.02	2	Kolla 2 (2 0ug/mL)	1.0	0.04	2
Abelmoschus (20 ug/mL)	1.2	0.28	2	Glucosamine (20 ug/mL)	1.0		1
Bonistein (20 ug/mL)	1.2		1	Astragalus (20 ug/mL)	1.0		1
Peppermint (25 ug/mL)	1.2	0.09	4	DHA (20 ug/mL)	0.9	0.03	2
Blueberry (25 ug/mL)	1.2	0.04	2	Hyaluronic acid (20 ug/mL)	0.9		1
Picrorhiza (25 ug/mL)	1.2	0.03	2	Inulin (20 ug/mL)	0.9	0.03	2
Wasabia Powder (25 ug/mL)	1.2	0.03	2	FOS (20 ug/mL)	0.9	0.07	2
HHIAA (5 ug/mL)	1.2	0.07	3	Vitamin C (20 ug/mL)	0.9		1
Osteosine (20 ug/mL)	1.2		1	Bonepep (20 ug/mL)	0.9		1
Dandelion (25 ug/mL)	1.2	0.01	2	K-Citrate (20 ug/mL)	0.9	0.05	2
Green Coffee (25 ug/mL)	1.2	0.01	2	Black Tea (20 ug/mL)	0.9	0.00	2
THIAA (5 ug/mL)	1.2	0.08	3	Green Tea (25 ug/mL)	0.9		1
RIAA (5 ug/mL)	1.1	0.01	2	Vitamin K2 (20 ug/mL)	0.9	0.02	2
Irish Moss (25 ug/mL)	1.1		1	Milk Basic Protein (20 ug/mL)	0.9		1
Broc 10 (25 ug/mL)	1.1		1	Rutin (20 ug/mL)	0.9	0.07	2
Broc 19 (25 ug/mL)	1.1		1	Fructus LL (20 ug/mL)	0.9	0.03	2
Broc 49 (25 ug/mL)	1.1		1	Ipriflavone (20 ug/mL)	0.8	0.02	2
Black Rice (20 ug/mL)	1.1	0.10	2	Red Yeast Rice (20 ug/mL)	0.8	0.04	2
Policosanol (20 ug/mL)	1.1	0.06	2	Devils Claw (20 ug/mL)	0.8	0.14	2
DHEA (20 ug/mL)	1.1	0.11	2				

[0077] Formula DF-SH is the combination of spent hops, (1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) (curcumin), (3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one) (andrographis), Rho Isoalpha acid and Zn (525:30:30:4:1)

[0078] *HO-1 Transcriptional activation* – Heme oxygenase is regulated by both Nrf-2 and MTF-1 transcriptional factors through the activation of ARE and MRE binding sites on heme oxygenase gene. Sulforaphane, a known compound for the activation of Nrf-2 transcriptional factor induces the heme oxygenase promoter activity in the HepG2 cell line (Table 6). Thirty percent induction of the activity by test compounds over DMSO control is considered as active. Test compounds (1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) (curcumin), xanthohumol, (3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one) (andrographis) extract, sulforaphane, IAA, oleanolic acid, green tea catechins, milk thistle, terminalin, ZnCl₂, garlic, amla fruit extract and cinnamon ground induced the expression of heme oxygenase promoter activity.

Table 6

Activation of the heme oxygenase (HO-1) luciferase reporter by test compounds in the HepG2 cell line

Test compounds	Fold Induction of heme oxygenase luciferase activity	
	Activity	+ SEM
Curcumin	6.1	+/-2.55
Xanthohumol	5.4	+/- 0.64
Andrographis Extract	4.4	+/- 0.57
Sulforaphane	4.2	+/- 0.70
IAA	3.6	+/- 0.85
Oleanolic Acid	2.7	+/- 1.20
Green Tea catechins	2.6	+/- 0.49
Milk Thistle	2.0	+/- 0.28
Terminalin	1.7	+/- 0.01
ZnCl₂	1.5	+/- 0.01
Garlic	1.4	+/- 0.01
Amla Fruit extract	1.3	+/- 0.50
Cinnamon ground	1.3	+/- 0.01

[0079] *Gene Expression* – The relative expression (RE) of the genes analyzed was reported relative to GAPDH. To determine the relative expression to the control cells, the ratio of treatment to the DMSO control average RE value was calculated for all samples. The average of hMT1&2 and hMT1&2alt was calculated and expressed as MT 1,2. The average of GSTA1 and NQO1 was calculated and expressed as Phase II gene induction. The average gene expression for metallothionein (MT 1,2), heme oxygenase (HMOX1), and phase II enzymes (GSTA1 and NQO1) is seen in Table 7. As a control, CdCl₂, was shown to induce both MT and HMOX1, however was unable to increase phase II. Alternatively, sulforaphane increased phase II and HMOX1, but not MT. Test compounds that increased $\geq 20\%$ (in rank order) phase II include andrographis, IAA and curcumin; HMOX1 include curcumin, andrographis, fucus mesh, gymnogongrus, IAA, and xanthohumol; MT include oleanolic acid, fucus mesh, xanthohumol and gymnogongrus.

Table 7

Gene expression in HepG2 cells following treatment with test compounds (values shown are $\mu\text{g/ml}$)

	MT 1,2 (ave)		HMOX1		Phase II (ave)	
	Ratio to DMSO	+/- SD	Ratio to DMSO	+/- SD	Ratio to DMSO	+/- SD
DMSO Control	1.00	0.26	1.00	0.15	1.00	0.09
CdCl ₂ (40 μM)	83.31	18.50	21.23	5.28	0.94	0.23
Sulforaphane (3)	0.67	0.16	8.82	1.98	1.84	0.44
Andrographis (25)	0.87	0.12	2.43	0.37	2.29	0.39
Curcumin (5)	0.90	0.48	2.09	1.14	1.15	0.43
ZnCl ₂ (10)	12.43	8.98	0.97	0.16	0.94	0.09
IAA (5)	0.89	0.12	1.26	0.15	1.26	0.18
RIAA (5)	0.83	0.22	2.54	1.82	1.15	0.35
THIAA (5)	0.90	0.17	1.11	0.01	0.99	0.13
Xanthohumol (1)	0.90	0.40	1.11	0.12	1.02	0.19
Spent Hops (20)	1.35	0.31	1.31	0.15	0.69	0.29
Spent Hops (50)	2.12	0.61	1.25	0.08	1.14	0.64
SH formula (10)	0.95	0.13	1.64	0.20	1.06	0.07
SH formula (20)	1.04	0.13	3.55	0.15	1.52	0.07
SH formula (50)	0.79	0.30	1.14	0.12	0.66	0.09
3C formula (1)	1.15	0.14	0.85	0.12	0.84	0.16
3C formula (2.5)	1.05	0.11	1.03	0.05	1.00	0.20
3C formula (5)	2.07	2.00	30.34	49.01	29.93	59.01
3C formula (7.5)	1.12	0.11	2.14	0.33	11.12	21.07
Oleanolic (25)	2.39	0.52	1.88	1.41	0.91	0.27
Withania (20)	1.06	0.18	1.17	0.23	0.98	0.24
As. Nod Mesh (25)	1.05	0.08	0.82	0.02	0.68	0.16
C. Chamissoi (25)	1.01	0.07	1.04	0.13	0.86	0.15
C. Crispus (25)	1.07	0.18	0.90	0.20	0.79	0.16
Fucus Mesh (25)	1.28	0.18	2.12	1.37	6.82	11.77
Green Tea (25)	0.85	0.09	0.77	0.00	0.83	0.11
Gymnogongrus (25)	1.19	0.03	1.63	0.89	0.97	0.02
Irish Moss (25)	2.07	2.26	0.91	0.07	0.91	0.07
Sarcoditheca (25)	0.88	0.09	0.85	0.15	0.77	0.08

[0080] 3C formula is the combination of equal ratios of IAA, (1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) (curcumin), and (3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one) (andrographis). SH formula is the combination of spent hops, Rho Isoalpa acid, (1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) (curcumin), (3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one) (andrographis), and zinc (525:4:30:30:1). The gray shading indicates only one replicate

[0081] *Nrf-2 Translocation* – The translocation of Nrf-2 from the cytoplasm to the nucleus has been shown to occur with treatment of xenobiotics and antioxidants (Chan, J.Y. and

M. Kwong, Biochim Biophys Acta, 1517(1): 19-26, 2000, and Chan, K., et al., Proc Natl Acad Sci U S A, 93(24): 13943-8, 1996). As seen in Figures 3 and 4, Nrf-2 was observed in the nuclear fraction of HepG2 cells treated with test compound after 4 hours. As a control, sulforaphane displayed a dramatic increase in Nrf-2 nuclear translocation. The combination of spent hops, (1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) (curcumin), (3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene) dihydro-4-hydroxyfuran-2(3H)-one) (andrographis), Rho Isoalpha acid and Zn (525:30:30:4:1) and IAA, (1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) (curcumin), (3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene) dihydro-4-hydroxyfuran-2(3H)-one) (andrographis) (1:1:1) also showed a marked translocation of Nrf-2 to the nucleus.

Example 2

Effects of test formulation on metallothionein mRNA production

[0082] This experiment was a study investigating the effects of a unique nutraceutical combination upon biomarkers of metal detoxification and elimination.

[0083] Nine volunteers were recruited and followed for 14 days. Baseline evaluations were obtained at Visit 1. Treatment with a test formulation comprising (per tablet): zinc (1.667 mg); 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene) dihydro-4-hydroxyfuran-2(3H)-one (50 mg); 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (50 mg); spent hops (875 mg) and a Rho dihydro-isoalpha acid mixture (6.667 mg) was begun immediately thereafter and continued for 10 days.

[0084] Clinical evaluations were made at Visit 2 (Day 3), Visit 3 (Day 6), Visit 4 (Day 8), Visit 5 (Day 10), and Visit 6 (Day 14). Visit 6 occurred 4 days after discontinuation of treatment. Basic safety labs at visits 1 and 5 were obtained including complete blood counts, comprehensive metabolic panels and gamma glutamyl transferase levels. Efficacy endpoints included messenger RNA levels of Metallothionein 1 and 2 (reflecting gene up-regulation) and unprovoked urinary excretion of toxic and nutrient trace elements.

[0085] Metallothionein mRNA measurements estimations in human blood samples were performed as follows:

[0086] *RNA Extraction* - After collection, 2ml whole blood was mixed with 5 ml of RNAlater (Ambion, Austin, TX). RNA extractions were performed with the RiboPure Blood RNA Isolation Kit (Ambion, Austin, TX) according to the manufacturers specifications. RNA was quantified with a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE).

[0087] *Reverse Transcriptase Assays* - RNA was converted to first strand cDNA by use of the RETROscript First Strand Synthesis Kit (Ambion, Austin, TX) and primed with oligo-dT according to the manufacturers specifications.

[0088] *Primers and Probes* - A Taqman gene expression assay to quantify GAPDH expression, which was used as an endogenous control, was obtained from Applied Biosystems (Foster City, CA; assay Hs99999905_m1). Primers and probes for Taqman-based assays targeting human metallothioneins have been previously described (Aydemir TB, Blanchard RK, and Cousins RJ, 2006. Zinc supplementation of young men alters metallothionein, zinc transporter, and cytokine gene expression in leukocyte populations. Proc. Natl. Acad. Sci. USA 103, 1699-1704). Forward and reverse primers, and HPLC-purified Taqman probes labeled with 5'-FAM and 3'-TAMRA, were obtained from Operon Biotechnologies, Inc (Huntsville, AL).

[0089] The sequences of these reagents are shown below.

hMT1,2-F	5'- GCACCTCCTGCAAGAAAAGCT
hMT1,2-R	5'- GCAGCCTTGGGCACACTT
hMT1,2-T	5'- FAM CACAGCCCACAGGGCAGCAGG TAMRA

hMT1,2Alt-F	5'- GCACCTCCTGCAAGAAGAGCT
hMT1,2Alt-R	5'- GCAGCCCTGGGCACACTT
hMT1,2Alt-T	5'- FAM ACAGCCCACAGGACAGCAGG

[0090] *Quantitative PCR Assays* - Two-step Taqman-based RT-qPCR was performed. First strand cDNA was synthesized as described above. In the first experiment, the cDNA equivalent of 20 ng starting RNA was then included in qPCR reactions. Reactions to detect metallothionein expression contained 1X Taqman Master Mix (Applied Biosystems, Foster City,

CA), forward and reverse primers at 400 nM, and Taqman probe at 200 nM. Reactions to detect GAPDH expression contained 1X Taqman Master Mix (Applied Biosystems) and 1X gene-specific assay reagents as recommended by the manufacturer (Applied Biosystems). All reactions were run in triplicate. Reactions that did not contain template cDNA were included as negative controls. In a second experiment with a subset of the samples, the cDNA equivalent of 100 ng starting RNA was included in qPCRs with the other conditions as described above. A reference sample was included in both assays to allow cross-assay comparison.

[0091] Reaction plates were processed on an Applied Biosystems 7900HT Sequence Detection System. The AmpliTaq Gold polymerase was activated at 95°C for 10 min followed by 40 cycles consisting of denaturation for 15 seconds at 95°C and annealing and extension for 60 seconds at 60°C.

[0092] Amplification data was analyzed with the ABI Prism SDS 2.1 software (Applied Biosystems). Relative quantification of gene expression was performed by the $\Delta\Delta C_t$ method (Winer J, Jung CKS, Shackel I, Williams, PM. 1999. Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes *in vitro*. Anal Biochem. 270, 41-49), with GAPDH expression serving as an endogenous control to normalize expression within each sample.

[0093] Results are depicted graphically in Figures 5 and 6 demonstrating the efficacy of the intervention in increasing measures of hepatic detoxification metallothionein mRNA levels.

Example 3

Effects of test formulation on urinary excretion of heavy metals

[0094] This study was conducted to determine the effects of a test formulation on urinary excretion of heavy metals in human volunteers.

[0095] *Study Design* – The study group and treatment regimen were as described in Example 2 above. Urine samples were collected and heavy metal determination performed by Genova Diagnostics, North Carolina, USA according to their in house procedures.

[0096] *Results* – Table 8 reports on the elimination of clinically relevant trace elements in test volunteers. Figures 7 and 8 graphically display the results of a test formulation on the excretion of clinically important (Figure 7) and non- clinically important metals (Figure 8). As can be seen in Figure 7, the test formulation significantly promoted excretion of a number of important metals. Figure 9 graphically displays the results of the test formulation on nutrient excretion in the urine.

Table 8

Urinary Excretion of Heavy Metals

Metal	V1 avg [*]	V5 avg	% Change		Metal	V1 avg	V5 avg	% Change
Aluminum	10.41	11.29	8.4		Manganese	0.51	2.17	327.0
Antimony	0.061	0.063	4.1		Mercury	ND	ND	
Arsenic	17.25	21.36	23.9		Molybdenum	39.5	45.75	15.8
Barium	2.35	2.95	25.5		Nickle	1.3883	1.4867	7.1
Bismuth	ND	ND			Niobium	ND	ND	
Cadmium	0.16	0.30	90.5		Platinum	1.256	0.087	- 93.1 ^{***}
Calcium	93.13	93.38	- 1.9		Potassium	2307.13	2594.38	12.5
Cesium	5.33	5.01	- 5.9		Rubidium	1831.38	1758.38	- 4.0
Chromium	2.25	1.38	- 38.9		Selenium	89.75	89.00	- 0.8
Cobalt	0.12	0.30	148.4		Strontium	101.75	96.00	- 5.7
Copper	8.29	9.46	14.2		Sulfur	519.38	466.38	- 10.2
Creatinine	43.12 ^{**}	58.86	36.5		Thallium	0.31	0.29	- 6.9
Gadolinium	ND	ND			Thorium	ND	ND	
Gallium	0.033	0.059	80.0		Tin	2.19	1.68	- 23.3
Iron	ND	ND			Tungsten	ND	ND	
Lead	0.35	0.617	76.2		Uranium	ND	ND	
Lithium	22.50	24.50	8.9		Vanadium	1.10	1.08	- 2.3
Magnesium	54.13	51.00	- 5.8		Zinc	330.5	326.0	- 1.4

* = amounts reported are in µg/g creatinine unless otherwise noted.

** = reported as mg/dL

*** = data presented only for subjects with detectable levels of metal

ND = below detectable limits.

CLAIMS

1. A composition for promoting heavy metal detoxification in a mammal in need thereof, said composition comprising a therapeutically effective amount of at least two members selected from the group consisting of spent hops; 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione; zinc; and 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one.
2. The composition according to Claim 1, wherein the composition further comprises a compound selected from the group consisting of (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-

4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylpropanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; and (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one.

- [illegible]

methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (*4R*,*5S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (*4R*,*5S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (*4R*,*5S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (*4R*,*5S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (*4R*,*5S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (*4R*,*5S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (*4R*,*5S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (*4R*,*5S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; (*4R*,*5S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one; and (*4R*,*5S*)-3,4-dihydroxy-2-(3-methylbutanoyl)-5-(3-methylbutyl)-4-(4-methylpentanoyl)cyclopent-2-en-1-one.

- [illegible]

[illegible]

5. The composition according to Claim 1, wherein the composition further comprises a pharmaceutically acceptable excipient selected from the group consisting of coatings, isotonic and absorption delaying agents, binders, adhesives, lubricants, disintergrants, coloring agents, flavoring agents, sweetening agents, absorbants, detergents, and emulsifying agents.

6. The composition according to Claim 1, wherein the composition further comprises one or more members selected from the group consisting of antioxidants, vitamins, minerals, proteins, fats, and carbohydrates.
7. A method for promoting heavy metal detoxification in a mammal in need thereof, said method comprising administering to the mammal in need a composition comprising a therapeutically effective amount of at least two members selected from the group consisting of spent hops; 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione; zinc; and 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene)dihydro-4-hydroxyfuran-2(3H)-one.
8. The method according to Claim 7, wherein the composition used further comprises a compound selected from the group consisting of (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-

methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*R*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylpropanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*R*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(3-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*R*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-2-(2-methylbutanoyl)-5-(3-methylbut-2-en-1-yl)cyclopent-2-en-1-one; and (4*S*,5*S*)-3,4-dihydroxy-4-[(1*S*)-hydroxy-4-methylpent-3-en-1-yl]-5-(3-methylbut-2-en-1-yl)-2-(2-methylpropanoyl)cyclopent-2-en-1-one.

- [illegible]

[illegible]

4-[(1S)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one.

- [illegible]

[(1S)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1S)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1S)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1S)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1S)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1S)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1S)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one; (4*S*,5*S*)-3,4-dihydroxy-4-[(1S)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one; and (4*S*,5*S*)-3,4-dihydroxy-4-[(1S)-1-hydroxy-4-methylpentyl]-2-(3-methylbutanoyl)-5-(3-methylbutyl)cyclopent-2-en-1-one.

11. The method according to Claim 7, wherein the composition used further comprises a pharmaceutically acceptable excipient selected from the group consisting of coatings, isotonic and absorption delaying agents, binders, adhesives, lubricants, disintergrants, coloring agents, flavoring agents, sweetening agents, absorbants, detergents, and emulsifying agents.
12. The method according to Claim 7, wherein the composition used further comprises one or more members selected from the group consisting of antioxidants, vitamins, minerals, proteins, fats, and carbohydrates.
13. A composition according to Claim 1, wherein the composition comprises:
 - a. from about 0.10 to about 10.00 grams of spent hops;
 - b. from about 5 to about 1200 mg of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione;
 - c. from about 0.10 to about 24 mg of zinc; and

- d. from about 5 to about 1200 mg of 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene) dihydro-4-hydroxyfuran-2(3H)-one.
14. The method according to Claim 7, wherein the composition used comprises:
- a. from about 0.10 to about 10.00 grams of spent hops;
 - b. from about 5 to about 1200 mg of 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione;
 - c. from about 0.10 to about 24 mg of zinc; and
 - d. from about 5 to about 1200 mg of 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenenaphthyl)ethylidene) dihydro-4-hydroxyfuran-2(3H)-one.
15. A method for promoting heavy metal detoxification in a mammal in need thereof, said method comprising administering to the mammal in need a composition comprising a therapeutically effective amount of spent hops.

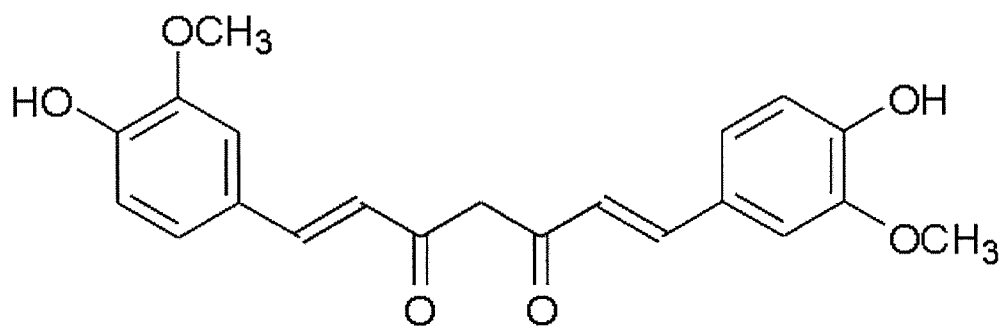


Figure 1

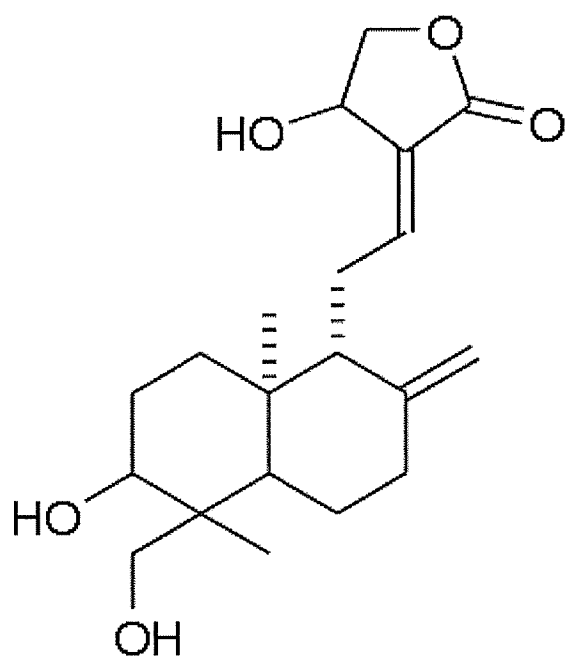


Figure 2

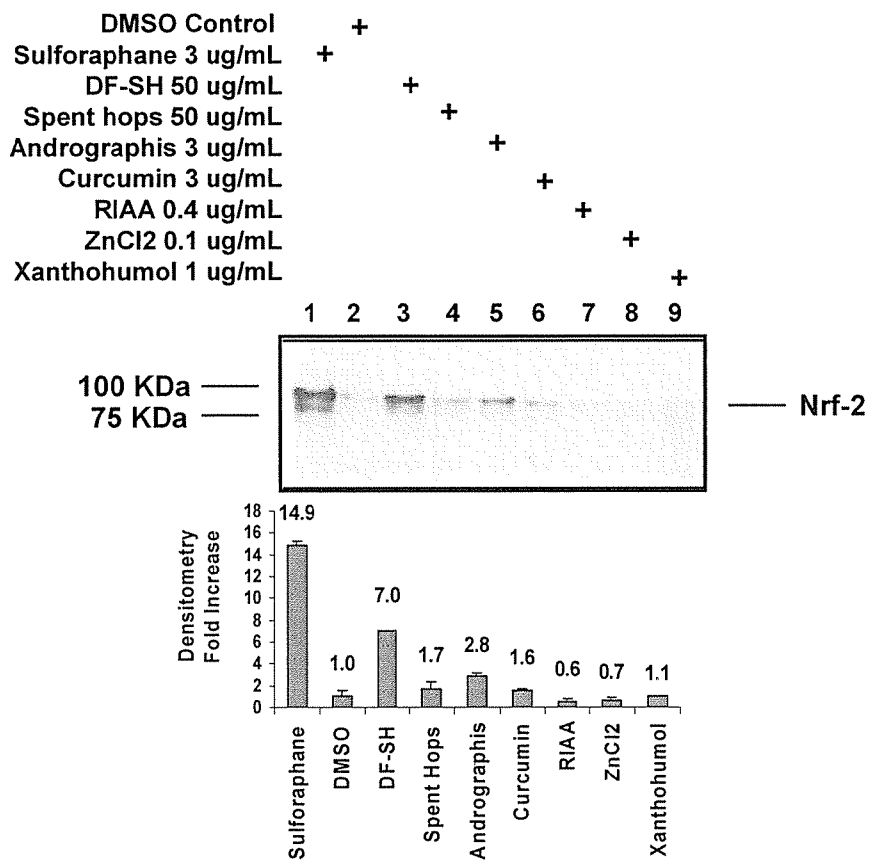


Figure 3

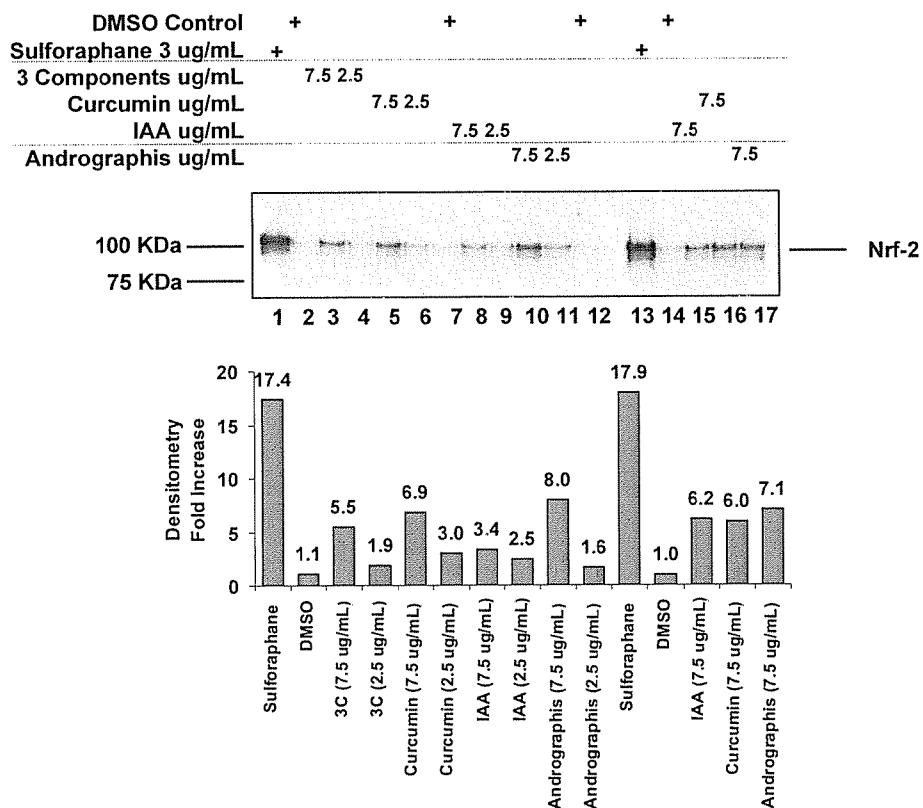


Figure 4

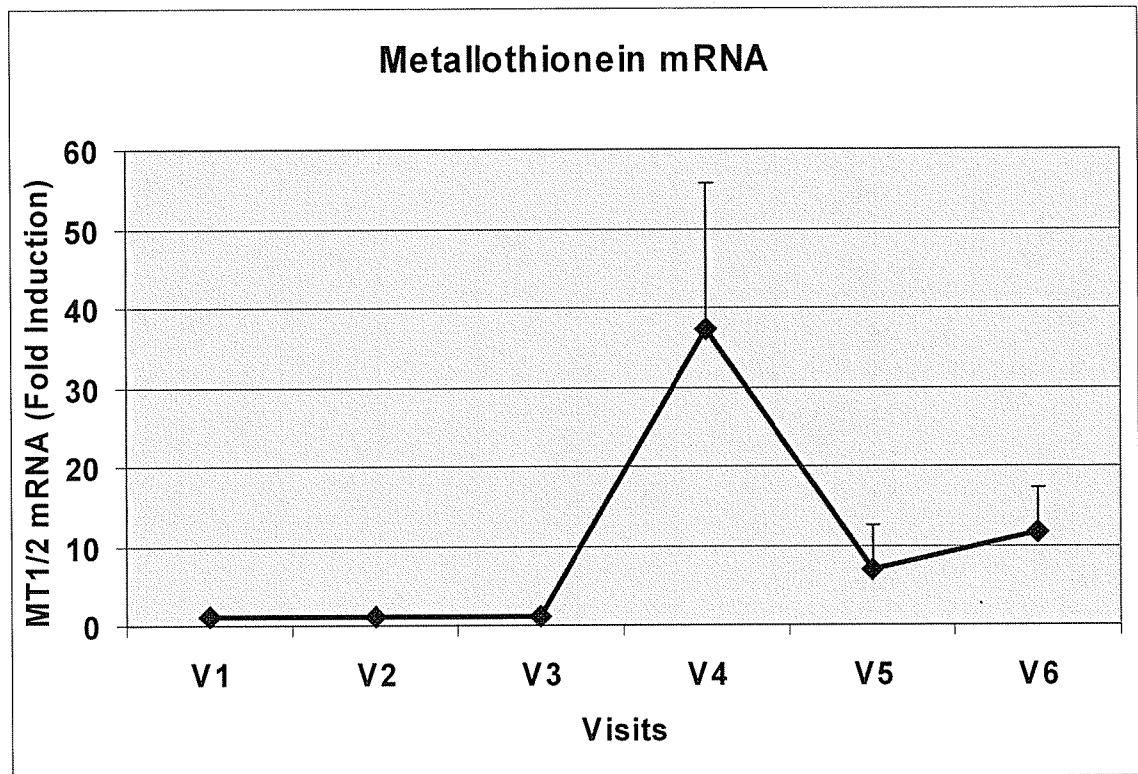


Figure 5

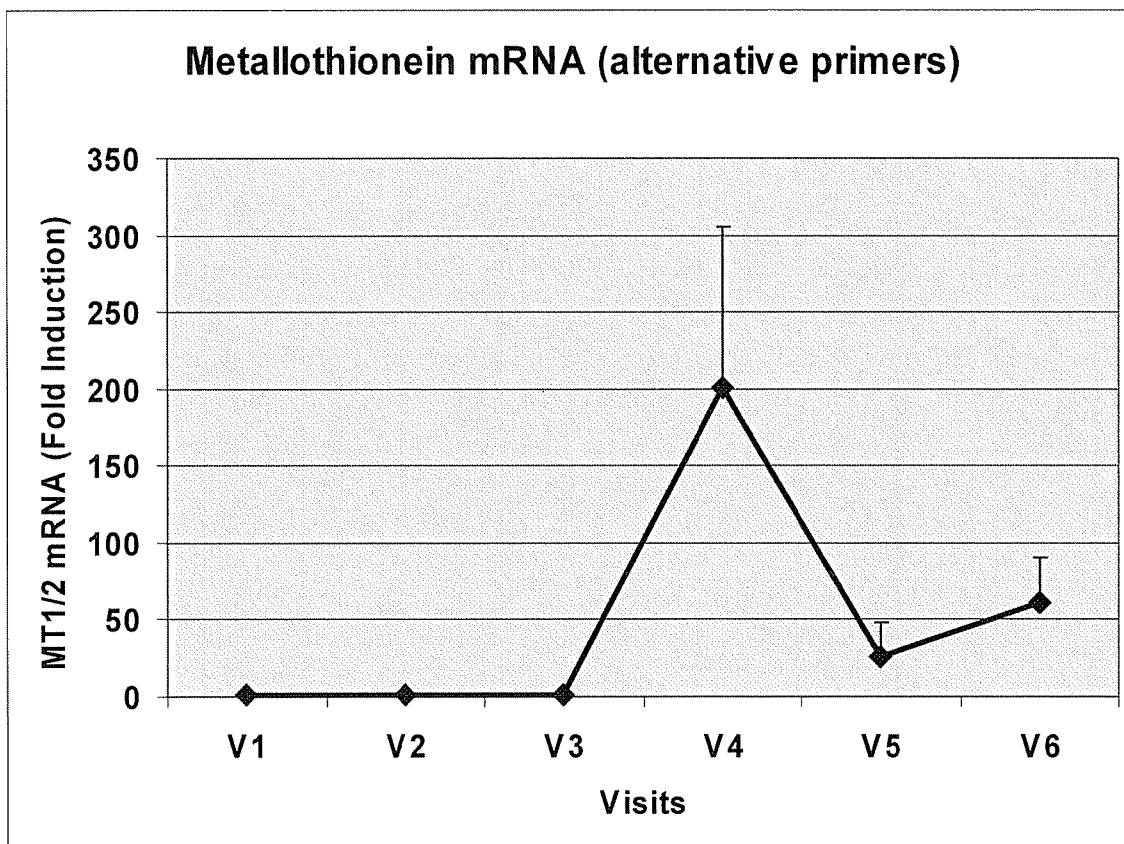


Figure 6

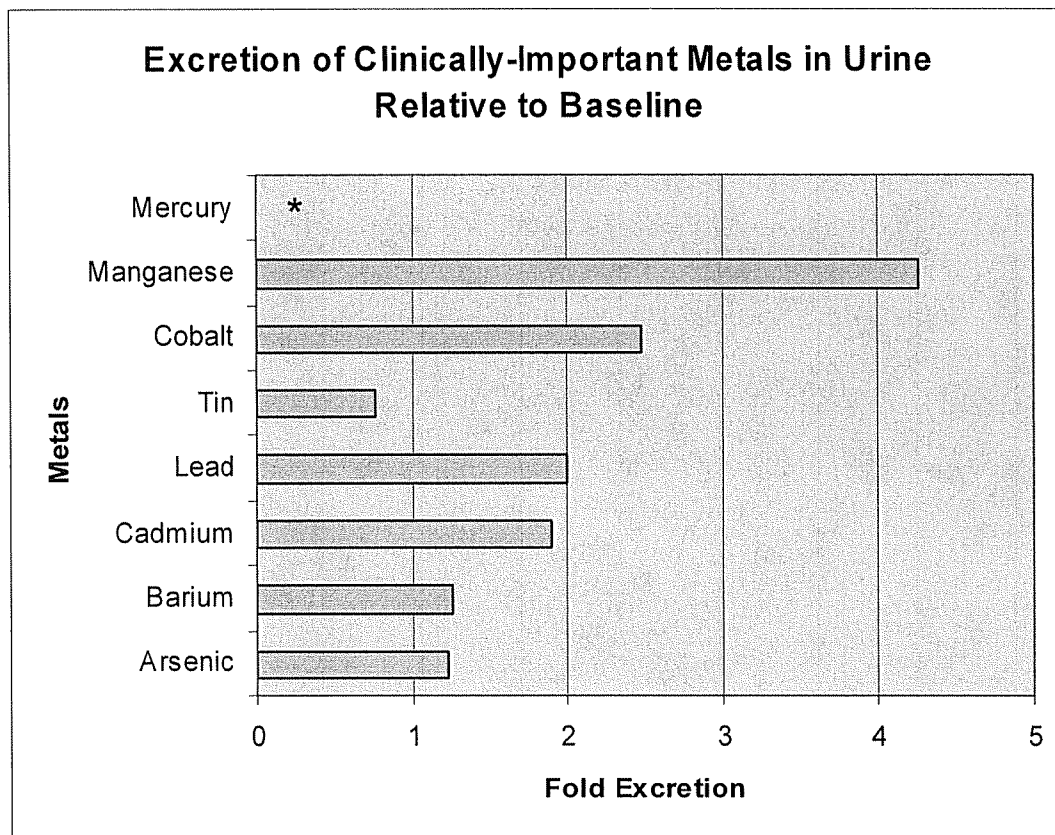


Figure 7

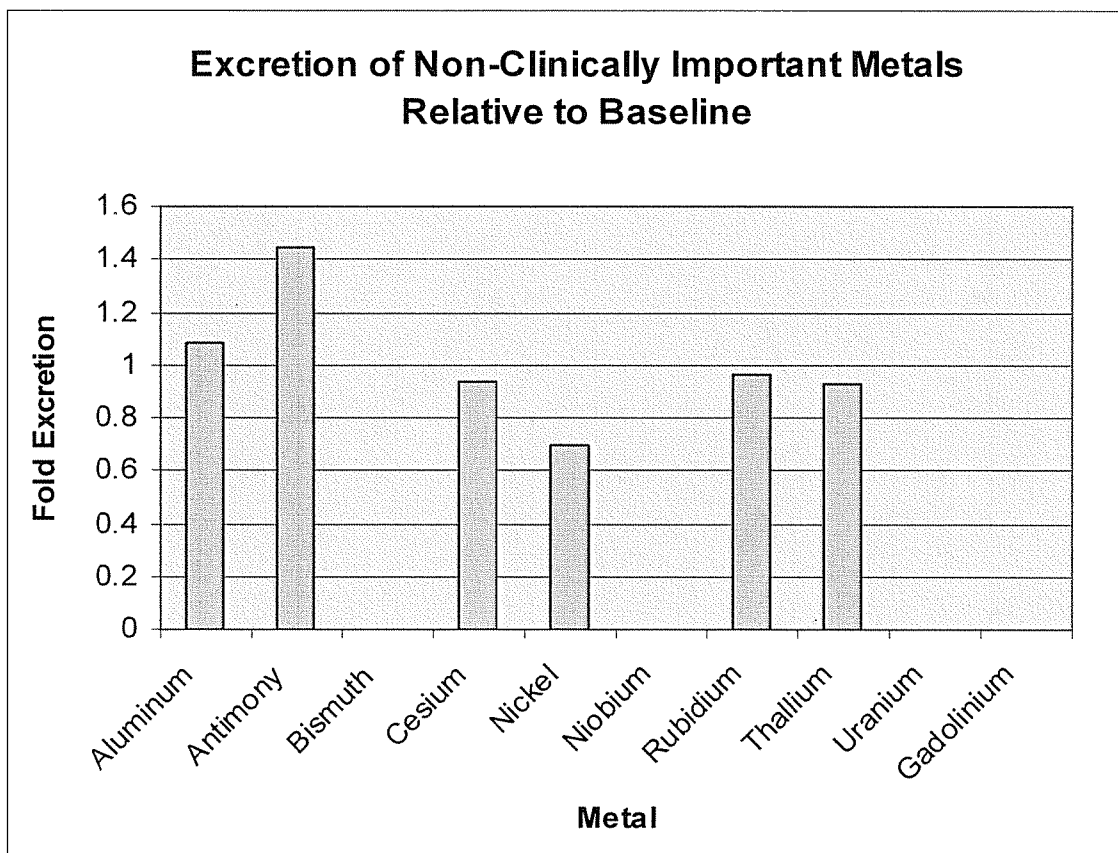


Figure 8

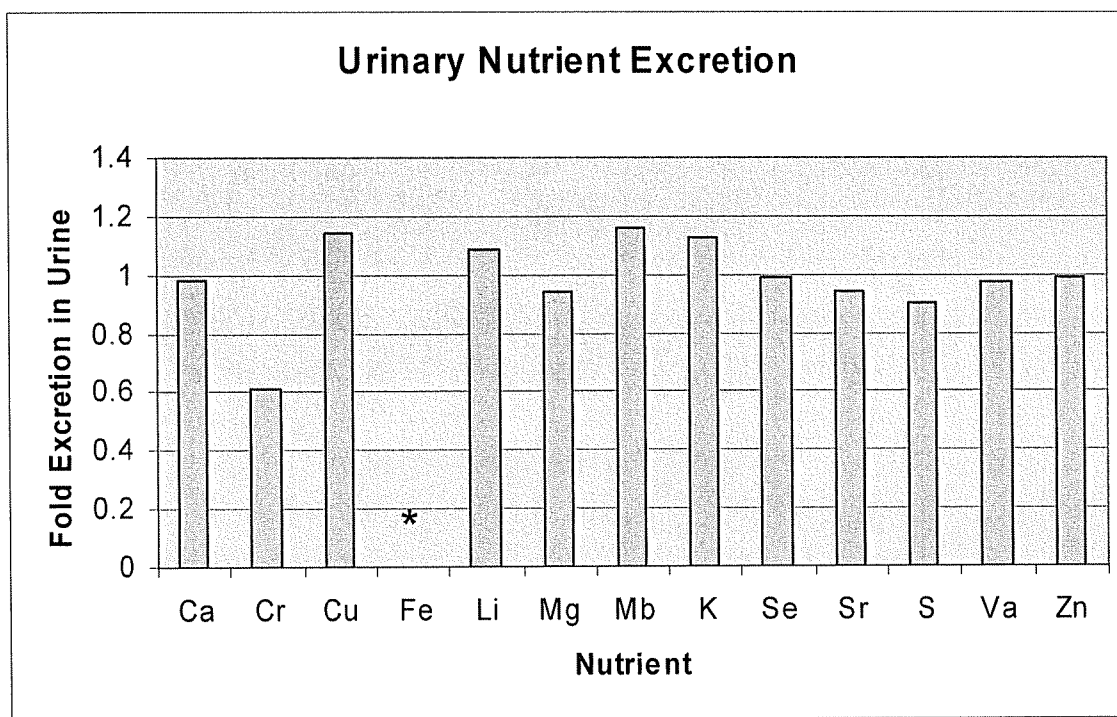


Figure 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/53803

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07D 309/06 (2008.04)

USPC - 568/379

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC-568/379

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Google, SciFinder - see keywords below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Google, WEST:terms-GST and curcumin; GST and andrographolide; GST and metal detox; heavy metal; detoxification; spent hops; curcumin; andrographolide; alpha acid; zinc; GST; NQO1
SciFinder: (structure search)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DINKOVA-KOSTOVA et al. Potency of Michael reaction acceptors as inducers of enzymes that protect against carcinogenesis depends on their reactivity with sulfhydryl groups, 2001, Proc. Natl. Acad. Sci., USA, vol 98, pp 3404-3409, Abstract; Table 1, compound 21	1-15
Y	BALLATORI et al. Biliary Secretion of Glutathione and of Glutathione-Metal Complexes, 1985, Fundamental and Applied Toxicology, vol 5, pp 816-831, Abstract	1-15
Y	SINGH et al. Modulatory Influence of Andrographis paniculata on Mouse Hepatic and Extrahepatic Carcinogen Metabolizing Enzymes and Antioxidant Status, 2001, Phytother. Res., vol 15, pp 382-390, Abstract	1-14
Y	WO 2004/037180 A2 (TRIPP et al.) 06 May 2004 (06.05.2004), Claim 171; p 16, ln 16; p 34, ln 4-5; p 48, ln 20-26; p 49, ln 5-11	2-5, 8-11, 15
Y	CHUNG et al. Cytotoxicity of Nitric Oxide Is Alleviated by Zinc-Mediated Expression of Antioxidant Genes, 2006, Exp Biol Med, vol 231, pp 1555-1563, Introduction, para 2	13 and 14

☐ Further documents are listed in the continuation of Box C.



* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 23 May 2008 (23.05.2008)	Date of mailing of the international search report 01 JUL 2008
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774