(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 April 2014 (17.04.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/057493 A1

(51) International Patent Classification:
A61K 31/122 (2006.01) *A61K 36/05* (2006.01)

(21) International Application Number:
PCT/IL2013/050822

(22) International Filing Date:
13 October 2013 (13.10.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/713,529 14 October 2012 (14.10.2012) US

(71) Applicant: **ALGATECHNOLOGIES LTD.** [IL/IL]; Kibbutz Ketura, 88840 MP Eilot Corridor (IL).

(72) Inventor: **AYALON, Oran**; 30a Hamagen Street, 76804 Mazkeret Batya (IL).

(74) Agents: **WEBB & CO.** et al.; P.O. Box 2189, 76121 Rehovot (IL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2014/057493 A1

(54) Title: ASTAXANTHIN DERIVATIVES FOR HEAT STRESS PREVENTION AND TREATMENT

(57) Abstract: The present invention relates to the use of natural astaxanthin for enhancing the tolerance to heat stress of a subject suspected to be exposed to heat stress or of a subject already affected by heat stress. Enhancing the tolerance of the subject to heat stress results in preventing or reducing the symptoms and/or conditions associated with heat stress.

ASTAXANTHIN DERIVATIVES FOR HEAT STRESS PREVENTION AND TREATMENT

FILED OF THE INVENTION

The present invention relates to the use of natural astaxanthin and derivatives thereof for enhancing the tolerance of a subject to heat stress thus preventing, treating or reversing heat stress associated conditions.

BACKGROUND OF THE INVENTION

Heat stress is a dangerous condition that can detrimentally affect humans and animals. High air temperatures, high fevers, radiant heat sources, high humidity, direct physical contact with hot objects, or strenuous physical activities are examples of factors that have a high potential for inducing heat stress.

Heat stress is a general description for several conditions including heat stroke, heat exhaustion and more. Heat stroke is an acute condition of hyperthermia, a body temperature of greater than 40.6°C (105.1 °F), due to environmental heat exposure with lack of thermoregulation. Heat stroke is caused by prolonged exposure to excessive heat or to a combination of heat and other factors leading to elevation of the body temperature to the hazardous range. The heat-regulating mechanisms of the body eventually become overwhelmed and unable to effectively deal with the heat, causing the body temperature to climb uncontrollably. Symptoms include hot, dry skin; rapid, strong pulse and dizziness. The skin may become red (heat edema) and hot as blood vessels dilate in an attempt to increase heat dissipation, sometimes leading to swollen lips. An inability to cool the body through perspiration causes the skin to feel dry. In the case of severe heat stroke, the person may become confused or hostile, and may seem intoxicated. Heart rate and respiration rate will increase (tachycardia and tachypnea) as blood pressure drops and the heart attempts to supply enough oxygen to the body. The decrease in blood pressure can then cause blood vessels to contract, resulting in a pale or bluish skin color in advanced cases of heat stroke. Other signs and symptoms vary depending on the cause. The dehydration associated with heat stroke can produce nausea, vomiting, headaches, and low blood pressure. This can lead to fainting (heat syncope) or dizziness.

Heat exhaustion, which can be a precursor of heat stroke, is typically shown by heavy sweating, rapid breathing and a fast, weak pulse. Other conditions associated with heat stress are heat cramps - muscle pains or that happen during heavy exercise in hot weather; heat rash - skin irritation from excessive sweating; and heat tetany – typically resulting from short periods of stress in intense heat. Symptoms may include hyperventilation, respiratory problems, numbness or tingling, or muscle spasms.

Several types of human populations are at high risk to be exposed to environmental conditions that may result in heat stress, including, for example, those who work outside during high temperature, athletes, soldiers and vacationers. Livestock, other farm animals and pets may also be exposed to environmental high temperatures and/or humidity.

Astaxanthin (3,3'-Dihydroxy-β-β-carotene-4,4'dione) is a carotenoid known as powerful antioxidant. Astaxanthin is a strong inhibitor of lipid peroxidation and has been shown to play an active role in the protection of biological membranes from oxidative injury. It has been shown that carotenoids in general and astaxanthin in particular protect the skin from the damaging effects of ultraviolet radiation and ameliorate age-related macular degeneration. In addition, astaxanthin increases high density lipoproteins and protects against cardiovascular diseases. Astaxanthin was also suggested to play a role as hormone precursor, in reproduction, in growth and in maturation.

Astaxanthin is utilized mainly as nutritional supplement, which provides pigmentation in a wide variety of aquatic animals. In Far East it is used also for feeding poultry to yield a typical pigmentation of chicken. It is also a desirable and effective non-toxic coloring for the food industry and is valuable for cosmetics.

It has also been shown that astaxanthin is a potent antioxidant, mainly used as a food additive. It has been reported that astaxanthin is over 500 times more powerful than Vitamin E and 10 times stronger than other carotenoids such as zeaxanthin, lutein, canthaxanthin and beta-carotene. It has also been shown to enhance and modulate the immune system. Astaxanthin current use is mainly as a food additive.

U.S. Patent No. 6,433,025 discloses the use of the antioxidant and immune modulation properties of astaxanthin to prevent and retard sunburn or resulting skin cancers.

Namekawa T. et al. (2010. Reprod Domest Anim. 45(6):e387-91) examined the effect of astaxanthin-containing oil on development and stress-related gene expression of bovine

embryos exposed to heat stress. Early bovine embryos are vulnerable to heat stress during the first few days after fertilization. The inhibitory effect of heat stress on embryonic development is known to be associated with oxidative stress, which can be attenuated by antioxidants. The mRNA expression level of Src homology 2 domain-containing transforming protein C1 (SHC1), an oxidative stress adaptor protein, and of superoxide dismutase 2 (SOD2), a mitochondrial reactive oxygen species (ROS) scavenger, were lower in 5-8 cells embryos that were exposed to heat stress and treated with astaxanthin. These results suggest that astaxanthin added to the culture medium ameliorated the heat stress impaired embryonic development by its altering effects on the expression of stress-related genes.

U.S. Patent Application No. 2001/0217389 discloses methods of treating, reducing, and/or preventing heat stress comprising administering an effective amount of one or more of astaxanthin and milk casein hydrolysate, or a derivative thereof to a subject in need of heat stress treatment or prevention. Also disclosed are methods of reducing the effects of a fever in a subject comprising administering an effective amount of one or more of astaxanthin, chromium and milk casein hydrolysate, or a derivative thereof, to the subject. Pharmaceutical compositions, comprising astaxanthin and one or more of chromium or milk casein hydrolysate are also provided.

The result of heat stress may be acute illness or even death, hence there is a recognized need for efficient means and methods for increasing the tolerance of a subject to heat stress, such that the injuries associated with heat stress are prevented. This need is particularly recognized by worker, soldier and athlete populations frequently exposed to high environmental temperature at the time of physical effort.

SUMMARY OF THE INVENTION

The present invention provides methods of treating, reducing and preventing heat stress and injuries/conditions resulting therefrom. The methods of the present invention are based in part on the unexpected finding that oral administration of astaxanthin-rich oleoresin to test animals before exposure to heat stress increased significantly the animal tolerance to heat stress. The present invention discloses for the first time that pre-conditioning of an animal body by oral administration of natural astaxanthin results in delayed and/or reduced symptoms of heat stress.

According to one aspect, the present invention provides a method of enhancing the tolerance of a subject to heat stress comprising administering to a subject in need thereof natural astaxanthin or a derivative thereof.

According to certain embodiments, the method comprises a period of preconditioning of the subject to heat stress via administration of astaxanthin.

According to certain embodiments, the subject is expected to be affected by heat stress. According to other embodiments, the subject is exposed to heat stress. According to yet additional embodiments, the subject is already suffering from heat stress.

According to certain embodiments, the natural astaxanthin or derivative thereof is administered orally.

According to certain embodiments, the natural astaxanthin is from an algal source. According to certain exemplary embodiments, the alga is *Haematococcus pluvialis.*

According to some embodiments, at least 75% (w/w) of the total amount of astaxanthin is in the form of a fatty acid monoester. According to certain exemplary embodiments, about 80% to about 90% of the total amount of astaxanthin is in the form of a fatty acid monoester. Without wishing to be bound by any specific theory or mechanism of action, the fatty-acid ester form provide for enhanced bioavailability of the astaxanthin. According to other embodiments, the astaxanthin comprises at least 90%, or at least 95% or more of the 3S,3'S astaxanthin stereoisomer. According to certain exemplary embodiments, at least 99% of the astaxanthin is in the form of the 3S,3'S stereoisomer.

According to additional embodiments, the method comprises administering to the subject *Haematococcus pluvialis* oleoresin comprising at least 10% of astaxanthin or a

derivative thereof. According to these embodiments, the oleoresin is essentially free of additional carotenoids. According to exemplary embodiments, the oleoresin comprises at least 98% astaxanthin out of the total carotenoid content. According to further exemplary embodiments, the oleoresin comprises from about 20% to about 40% monounsaturated fatty acids.

According to some embodiments, the astaxanthin is the principal active agent. According to some embodiments, the astaxanthin is the major antioxidant. According to alternative embodiments, the astaxanthin is used in conjunction with other antioxidants.

According to certain embodiments, the heat stress is a result of exposure to high environmental temperatures and/or of physical exertion. It is to be explicitly understood that the heat stress may be the result of high environmental temperature with no exposure to UV or sun light. Accordingly, retarding and preventing sunburns are explicitly excluded from the scope of the present invention.

According to certain exemplary embodiments, the subject is expected to be exposed or is exposed to the heat stress at the time of physical activity, including intense physical activity.

According to certain embodiments, the astaxanthin or derivative thereof is administered in an amount effective in enhancing the tolerance of the subject to heat stress, thereby preventing or reducing a symptom or a condition associated with heat stress. According to certain embodiments, the symptom or a condition associated with heat stress is selected from the group consisting of hyperthermia, headache, dizziness, lightheadedness, fainting, weakness, exhaustion, fever, moist skin, irritability, confusion, dry skin, hot skin, no sweating, loss of consciousness, seizures, convulsions, heat stroke, vomiting and upset stomach. Each possibility represents a separate embodiment of the present invention. According to certain embodiments the astaxanthin or derivative thereof is administered in an amount effective in preventing death from heat stress.

According to some embodiments, the astaxanthin or derivative thereof is administered in an amount of from about 0.02mg/Kg body weight to about 200mg/Kg body weight.

According to certain embodiments, the subject is a mammal. According to certain typical embodiments, the mammal subject is human. The method of the present invention is

particularly applicable to enhance the tolerance of soldiers and athletes expected to be exposed to heat stress during physical exertion.

According to some embodiments, the method of the present invention comprises administering the natural astaxanthin or derivative thereof to a subject expected to be exposed to heat stress. According to certain exemplary embodiments, astaxanthin administration starts at least 10 days before the expected exposure to heat stress. According to specific embodiments, administration starts around at least 10 days prior to exposure and continues up to and including exposure to heat stress. According to other embodiments, the astaxanthin is administered during exposure to heat stress. According to yet additional embodiments, the astaxanthin is administered after exposure to heat stress. According to these embodiments, administration starts at a time point selected from right after the exposure to about 10 days after the exposure. Each possibility represents a separate embodiment of the present invention. According to certain exemplary embodiments, the astaxanthin is administered continuously at least once daily before, during or after exposure to heat stress. According to certain embodiments, the duration of astaxanthin administration is from about 1 day to about 10 days.

According to certain embodiments, the natural astaxanthin is administered at least once daily. According to some embodiments, the subject is a human and the astaxanthin is administered in an amount of from 2mg/day to 2g/day. According to other embodiments, the astaxanthin is administered in an amount of from 12mg/day to 1g/day. According to further embodiments, the astaxanthin is administered in an amount of from 12mg/day to 100mg/day. According to certain exemplary embodiments, the astaxanthin is administered in an amount of over 25mg/day.

According to certain exemplary embodiments, the method of the present invention consists of administering to the subject natural astaxanthin, a derivative thereof or oleoresin comprising same in an amount effective in enhancing the tolerance of said subject to heat stress.

According to another aspect, the present invention provides a method of reducing the effects of a fever in a subject comprising administering to the subject natural astaxanthin or a derivative thereof.

According to certain embodiments, the natural astaxanthin or derivative thereof is administered orally.

According to certain embodiments, the natural astaxanthin is from an algal source. According to certain exemplary embodiments, the alga is *Haematococcus pluvialis.*

According to some embodiments, at least 75% (w/w) of the astaxanthin total amount is in the form of a fatty acid monoester. According to certain exemplary embodiments, about 80% to about 90% of the total amount of astaxanthin is in the form of a fatty acid monoester. According to other embodiments, the astaxanthin comprises at least 90%, at least 95% or more of the 3S,3'S stereoisomer. According to certain exemplary embodiments, at least 99% of the astaxanthin is in the form of the 3S,3'S stereoisomer.

According to additional embodiments, the method comprises administering to the subject *Haematococcus pluvialis* oleoresin comprising at least 10% of astaxanthin or a derivative thereof. According to these embodiments, the oleoresin is essentially free of additional carotenoids. According to exemplary embodiments, the oleoresin comprises at least 98% astaxanthin out of the total carotenoid content. According to further exemplary embodiments, the oleoresin comprises from about 20% to about 40% monounsaturated fatty acids.

According to certain embodiments, the astaxanthin is administered in an amount effective in reducing the effects of the fever. According to some embodiments, the astaxanthin is administered in an amount of from about 0.02mg/Kg body weight to about 200mg/Kg body weight. According to certain exemplary embodiments, the astaxanthin is administered in an amount of from 12mg/day to 1g/day. Astaxanthin may be administered as long as the subject body temperature is above normal.

According to yet additional aspect, the present invention provides a pharmaceutical composition comprising algal oleoresin comprising at least 10% astaxanthin, the oleoresin being purified to a sufficient degree of purity to be suitable for use as a pharmaceutical grade material. According to a further aspect, the present invention provides a dietary supplement composition comprising algal oleoresin comprising at least 10% astaxanthin, the oleoresin being purified to a sufficient degree of purity to be suitable for use as a dietary supplement.

According to certain embodiments, the oleoresin is essentially free of carotenoids, such that astaxanthin is the principal active carotenoid in the pharmaceutical or dietary

supplement composition. According to yet additional embodiments, the pharmaceutical or dietary composition is in a formulation form suitable to enhance the astaxanthin bioavailability. According to certain exemplary embodiments, the astaxanthin containing oleoresin is isolated from the alga *Haematococcus pluvialis*.

According to yet additional aspect, the present invention provides a medicament or a dietary supplement comprising natural astaxanthin, a derivative thereof or oleoresin comprising same for enhancing the tolerance to heat stress, wherein the medicament or dietary supplement is to be administered to a subject for a period of preconditioning of the subject to heat stress.

Other objects, features and advantages of the present invention will become clear from the following description and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the temperature online records (measured via iButtons) for animals pre-treated with astaxanthin vs. vehicle (olive oil) treated controls. Arrows indicate the thermal injury threshold. Horizontal bar shows the period of the hyperthermic plateau.

FIG. 2 demonstrates the effect of pretreatment with astaxanthin on the expression of stress-related markers. Fig. 2A shows the expression of *hsp70* RNA (presented as a ratio of *hsp70* RNA to β actin RNA) over the course of time after exposure to heat shock. FIG. 2B shows the expression of Hsp72 protein (presented as a ratio of Hsp72 protein to β actin protein) over the course of time after exposure to heat shock.

2-W ANOVA test yields highly significant ($P<0.001$) difference between rat fed with oil compared to rats fed with astaxanthin in the expression of both RNA and protein. RNA and protein levels were measured in samples obtained at the indicated time after transforming the rat to normal temperature conditions. Control refers to samples obtained from rats before exposure to heat stress (basal level).

FIG. 3 is a graph showing the expression of heat shock factor 1 (HSF1) before (basal) and at certain time points after exposure to heat shock. Left: HSF1. Right-Phosphorylated HSF1. The difference bewteen the control group and the group of astaxanthin consuming rats was found to be sugnificant (levels 2W ANOVA $p<0.05$).

FIG. 4 shows the ratio of phosphorylated HSF1 to non-phosphorylated HSF1. No significant difference was found in the ratio in samples obtained from rats receiving astaxanthin compared to control rats (reciving olive oil).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides means and methods for enhancing the tolerance of a subject to heat stress by administering to the subject an effective amount of natural astaxanthin. The methods of the present invention employ all-natural astaxanthin, which is highly effective, non-toxic, and consists essentially of the pharmacokinetic suitable 3S,3S' stereoisomer. The predominant form of the natural astaxanthin is in the fatty-acid monoester.

The method of the invention is of particular importance in enhancing the tolerance of subjects expected to be exposed to heat stress, when the astaxanthin is administered before exposure to heat stress, but is as effective in treating subjects already exposed to heat stress when the astaxanthin is administered during or after exposure. Furthermore, the method of the present invention is highly effective in enhancing the tolerance to heat stress when exposure to heat stress is concomitant with physical activity, including intense physical activity.

The term "subject" as used herein refers to an individual human or non-human animal. The subject may be a vertebrate, more specifically a mammal (e.g., a human, horse, pig, rabbit, dog, sheep, goat, non-human primate, cow, cat, guinea pig or rodent), a fish or a bird. The term does not denote a particular age or sex. Thus, adult and newborn subjects whether male or female, are intended to be included. The astaxanthin administered according to the teachings of the invention significantly enhances the tolerance of the subject to heat stress, and thus can be used to treat, reduce and/or prevent heat stress in human subjects or in animal subjects.

As used herein, the term "astaxanthin" refers to (3,3'-Dihydroxy-β-β-carotene-4,4'dione). Astaxanthin is the principal carotenoid of some fish and most crustaceans (e.g. shrimp, lobster and crab), and is also found in algae, yeast and plants. It belongs to the xanthophyll subfamily of carotenoids, which contain oxygen in addition to the carbon and hydrogen atoms of the carotenes (the carotenes being another subfamily of carotenoids). Astaxanthin can be found in its free form or as a fatty acid mono or di-ester. According to

certain specific embodiments of the present invention, astaxanthin derivative refers to the fatty-acid monoester of astaxanthin. The term astaxanthin derivative further refers to 9z-astaxanthin and 13z-astaxanthin.

Astaxanthin is biosynthesized through the isoprenoid pathway, which initiates at acetyl-Co-A and proceeds through phytoene, lycopene, beta-carotene, and canthaxanthin before the last oxidative steps to astaxanthin. Fatty acids are esterified onto the 3' hydroxyl group(s) of astaxanthin after biosynthesis of the carotenoid, and allow it to have more solubility and stability in the cellular environment. The carotenoid fraction of green vegetative cells consists of mostly lutein (75-80%) and beta-carotene (10-20%), whereas in red cysts, astaxanthin is the predominate carotenoid.

According to one aspect, the present invention provides a method of enhancing the tolerance of a subject to heat stress comprising administering to a subject in need thereof natural astaxanthin or a derivative thereof. According to certain embodiments, the method comprises a period of preconditioning of the subject to heat stress via administration of astaxanthin.

According to alternative aspect, the present invention provides a medicament or dietary supplement comprising natural astaxanthin, derivative thereof or oleoresin comprising same for enhancing the tolerance of a subject to heat stress. According to certain embodiments, the medicament or dietary supplement is to be administered for a period of preconditioning.

According to certain embodiments, the subject is expected to be exposed to heat stress. According to other embodiments, the subject is affected by heat stress. According to yet additional embodiments, the subject was exposed to heat stress.

According to certain embodiments, the natural astaxanthin used according to the teachings of the present invention is isolated from algae. According to certain exemplary embodiments, the alga is *Haematococcus pluvialis.*

Astaxanthin has two chiral centers, at the 3- and 3′-positions. Therefore, there are three stereoisomers; (3R,3′R), (3R,3′S) (meso), and (3S,3′S). Synthetic astaxanthin contains a mixture of the three, in a proportion of approximately 1:2:1. Naturally occurring astaxanthin varies considerably from one organism to another. The configuration of astaxanthin in fish

corresponds to the configuration of the astaxanthin consumed by the fish. The astaxanthin produced by *Haematococcus pluvialis* is in the configuration of the (3S,3'S) stereoisomer.

According to some embodiments, at least 75% (w/w) of the astaxanthin total amount is in the form of a fatty acid monoester. According to other embodiments, the astaxanthin comprises at least 90%, at least 95% or more of the 3S,3'S stereoisomer. According to certain exemplary embodiments, at least 99% of the astaxanthin is in the form of the 3S,3'S stereoisomer.

Astaxanthin is a keto (oxygenated) carotenoid with the molecular formula $C_{40}H_{52}O_4$ and has a molecular weight of 596.86. Astaxanthin exists in several stereochemical forms, including 3S, 3'S; 3R, 3'R; 3S, 3'R and 3R, 3'S depending on the source (Schiedt K et al., Helv Chim Acta (1981) 64:449-457). The natural sources of astaxanthin include the algae *Haematococcus*, the yeast *Phaffia*, Krill, salmon and trout. In *Haematococcus* algae, particularly in *H. pluvialis,* astaxanthin exists as 3S, 3'S isomer, whereas synthetic astaxanthin contains a mixture of stereoisomers. The synthetic mixture contains the 3R, 3'S configuration, which does not appear in astaxanthin isolated from a natural source.

Although natural sources of astaxanthin are numerous, nearly all are found in very low concentrations. Astaxanthin is quite common in nature, especially in the marine environment and is probably best known for providing the pinkish-red hue to the flesh of salmon and trout, as well as to shrimp, lobsters and crayfish. These animals obtain astaxanthin in their diet from zooplankton, insects or crustaceans that have accumulated astaxanthin from phytoplankton.

The green algae *Haematococcus pluvialis* provides the most concentrated natural source of astaxanthin known, (10,000 ppm astaxanthin and more) in addition to other important carotenoids such as beta-carotene, lutein and canthaxanthin. As a comparison, the flesh of wild Atlantic salmon contains only about 5 ppm of astaxanthin, Coho salmon about 14 ppm astaxanthin and sockeye salmon contains an average amount of about 40 ppm. Other sources of astaxanthin include crustacean (krill, shrimp, crab and crawfish), the fermentative yeast *Phaffia rhodozyma* and chemically synthesized astaxanthin. *Haematococcus pluvialis*, also referred to as *Haematococcus lacustris* or *Sphaerella lacustris*, is a ubiquitous green alga of the order Volvocales, family Haematococcaceae. It is now known that the alga occurs in

nature worldwide, where environmental conditions for its growth are favorable. It is most often found in cooler pools of fresh water.

Under nutrient-rich conditions, *Haematococcus* is motile and utilizes the available nitrate, phosphate, and other nutrients to grow and reproduce. However, when nutrients become limiting or the pool begins to dry the alga form a protective cell wall and encyst. High concentrations of astaxanthin are produced, and the cells undergo a dormant stage until the next influx of water and nutrients. Cells can remain viable in this encysted stage with its protective astaxanthin for many years. Red cysts are significantly more resistant to strong light and oxygen radicals than green cells, suggesting significant protective roles for astaxanthin.

Advanced technology has been developed to grow *Haematococcus* and harnesses the unique properties of the algae to produce very high concentrations of natural astaxanthin. The astaxanthin is predominately in the esterified form, which provides the highest stability and bioavailability. Astaxanthin is insoluble in water, and thus can be extracted in organic solvents or supercritical fluids (SCF). Supercritical Fluid Extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids. SFE can be used to either strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils). Carbon dioxide (CO_2) is the most used supercritical fluid. Extraction conditions for supercritical CO_2 are above the critical temperature of 31°C and critical pressure of 74 bars.

An example of astaxanthin that can be used in the compositions and methods disclosed herein is an extract from the microalgae *Haematococcus pluvialis* known by the trade name AstaPure 10% natural astaxanthin oleoresin, purchased from AlgaTechnologies Ltd (Israel). This oleoresin preparation is extracted by carbon dioxide supercritical fluid extraction, and contains significant amount (about 10% w/w) of ultra-pure astaxanthin (at least 98% of astaxanthin out of the total oleoresin content of carotenoids).

Symptoms of heat stress include, but are not limited to, hyperthermia, headache, dizziness, thirst, muscle cramps, tachycardia, oligouria, malaise, hypotension, delirium, renal failure, hyperventilation, pulmonary edema, arrhythmia, shock, lightheadedness, fainting, weakness, exhaustion, fever, moist skin, irritability, confusion, dry skin, hot skin, anhidrosis,

loss of consciousness, seizures, convulsions, heat stroke, vomiting, and upset stomach. Sever heat shock is leading to the subject death.

Besides symptoms associated with a subject experiencing heat stress, heat stress can also cause adverse sequelae. For example, a reduction in egg laying or egg quality can indicate heat stress in a species that lays eggs. Other examples of heat stress sequelae include increased lung disease in humans or cattle, or ulcers in humans. By enhancing the tolerance of a subject to heat stress, the methods of the present invention can beneficially affect the symptoms of heat stress, and can also or alternatively reduce or eliminate any sequelae related to heat stress in a subject. Heat stress also includes heat exhaustion and heat stroke.

Symptoms of heat stress can be detected and can indicate an individual or population of individuals in need of treatment for or prevention of heat stress. Similarly, sequelae can be detected in an individual or population of individuals and can indicate an individual or population of individuals in need of treatment for or prevention of heat stress. Individuals or populations with an increased likelihood of experiencing heat stress can have individual or environmental factors which may predispose an individual or population to heat stress. For example, a subject in need of treatment for or prevention of heat stress can be a subject that is exposed to, or is expected to be exposed to, high environmental temperatures or physical exertion. Other factors, such as, but not limited to, age and heath condition of an individual, can also predispose an individual to heat stress. For example, the elderly, infants, and those with other illnesses may be at higher risk for heat stress. For example, those older than 65 years or younger than one or two years are at a higher risk of heat stress and heat stroke. Moreover, in some geographic regions there is a seasonal increase in risk for heat stress. Such an increase can be due to increased ambient temperatures and/or humidity levels to which subjects are exposed.

Other examples include those who work, live, or are otherwise active outside during high temperature conditions. Athletes and soldiers are non-limiting examples of individuals that are likely to be subjected to high temperatures and physical exertion. Also, many production animals, such as poultry and cattle, can experience high temperature environments that can indicate a need for heat stress treatment or prevention.

Chronic disease, some medications, and poor physical condition can impair a subject's normal mechanisms of dissipating heat, which can result in heat stress or a predisposition to

heat stress. Heat stress can result from a subject's inability or reduced ability to dissipate heat produced by metabolic activity, which often is associated with an increased ambient temperature. Pre-existing conditions that can contribute to heat stress include, but are not limited to alcoholism, anorexia, cardiac disease, cystic fibrosis, dehydration, diabetes insipidus, eating disorders, extremes of age, febrile illness, gastroenteritis, history of heatstroke, hypokalemia, obesity, poor acclimatization, sleep deprivation, sweat gland dysfunction, uncontrolled diabetes, uncontrolled hypertension, thyroid disorder, and upper respiratory tract infection. Medications that can contribute to heat stress include alcohol, alpha adrenergics, anticholinergics, antihistamines, benzodiazepines, beta blockers, calcium channel blockers, neuroleptics, phenothiazine diuretics and tricyclic antidepressants. Subjects having these conditions and/or medication histories can indicate subjects in need of heat stress treatment or prevention.

An example method of determining a subject in need of enhancing the tolerance to heat stress can include determining the heat index of the environment to which a subject has been exposed or will be exposed. Heat index accounts for relative humidity and temperature, with higher values of humidity and temperature equating to a higher heat index. The higher the heat index to which a subject has been or will be exposed indicates a greater likelihood of heat stress. For example, optionally, a subject that has been or will be exposed to a heat index of 30°C or greater indicates a subject in need of heat stress treatment or prevention. Optionally, a subject that has been or will be exposed to a heat index of 30-35 °C, 35-40 °C, 40-45 °C, or 50°C or higher, indicates a subject in need of enhanced tolerance to heat stress or heat stress treatment. In a subject with a predisposition to heat stress, lower heat index levels could be relied upon to initiate therapy.

The present invention now shows that oral administration of natural astaxanthin enhanced the tolerance of test rats exposed to environmental temperature of 41°C for 2 hours. Taking the small body size of the test rats, these conditions are considered as extreme heat stress conditions.

After a subject is identified that is in need of heat stress treatment or prevention, an effective amount of natural astaxanthin, or a derivative thereof can be administered to the subject.

An effective amount of natural astaxanthin is a nontoxic but sufficient amount to provide the desired result. The dosages or amounts of the natural astaxanthin or compositions comprising same described herein are large enough to produce the desired effect. The effective amount can vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the heat stress or fever to which the subject is exposed or expected to be exposed, the particular composition comprising the natural astaxanthin that is used, its mode of administration, and the like. Thus, it is not possible to specify an exact effective amount. However, an appropriate effective amount can be determined by one of ordinary skill in the art, for example, by using the assessment techniques indicated above. The dosage can be adjusted by an individual physician, veterinarian, or other medical or animal professional based on the clinical condition of the subject involved. The dose, schedule of doses, and route of administration can be varied accordingly. According to certain exemplary embodiments of the present invention, the effective amount of the natural astaxanthin or a derivative thereof can be administered to the subject prior to the onset of heat stress symptoms, or prior to the conditions that increase the likelihood that the subject will experience heat stress. Astaxanthin is an orange pigment that provides color to many living organisms, salmon, lobsters, and shrimp their reddish color upon cooking. It exhibits strong free radical scavenging activity by protecting against lipid peroxidation and oxidative damage of LDL-cholesterol, cell membranes, cells, and tissues.

Astaxanthin is typically administered orally to the subject or formulated to be administered orally. The recommended daily intake of natural astaxanthin according to the US-FDA is 12 mg/day; 4 mg/day according to the European FSA and 6 mg/day according to the Japanese regulatory authorities. However, there is no known deleterious effect of astaxanthin consumption at daily amounts far above the recommended daily use. An oral LD 50 of 600 mg/kg body weight was reported in rats; no negative effect in healthy human adults was reported after eight weeks of supplementation at 6 mg per day.

It has been previously disclosed that astaxanthin has a beneficial effect on model rats for obesity and hypertension (Zucker Fatty Rats) exposed to heat stress (U.S. Application Publication No. 2011/0217389). The model rats were, however, exposed to moderate heat (up to about 32°C), and received astaxanthin at 25mg/Kg body weight.

The present invention now shows that feeding normal model rats with higher dose of astaxanthin (100 mg/Kg body weight) results in pre-conditioning of the rats to the heat stress.

A significant elevation was observed in the expression of the heat stress related transcription factor Heat Shock factor-1 (HSF1) and further in the expression of heat shock related RNA and protein (*Hsp/70* and HSP/72, respectively) in samples taken from rats consuming the astaxanthin. The higher expression was measured before heat stress was applied (Figures 2 and 3, control and basal time points, respectively) and maintained throughout the recovery time after exposure to heat stress. Without wishing to be bound by any specific theory or mechanism of action, these results indicate that oral administration of astaxanthin provides for pre-conditioning of the body to heat shock, without the need for massive de-novo synthesis of heat shock associated proteins once the animal is exposed to heat shock. The pre-conditioning leads to a significant increase in the rat tolerance to severe heat stress (41°C), shown by the lower body temperature compared to rats not receiving the natural astaxanthin (Figure 1). These results also indicate that astaxanthin administration can provide long term protection to heat stress after astaxanthin is no longer consumed.

According to certain embodiments, the administered effective amount of natural astaxanthin or a derivative thereof is from about 0.02 mg/Kg body weight to about 200mg/Kg body weight. According to some embodiments, the administered effective amount of natural astaxanthin or a derivative thereof can be about 0.05mg/Kg body weight, 0.10 mg/Kg, 0.50 mg/Kg, 1.0 mg/Kg 5.0 mg/Kg body weight or greater. Thus, the administered effective amount of astaxanthin or a derivative thereof can optionally be between about 0.02 mg/Kg and about 200 mg/Kg including any value in between. By way of example, the astaxanthin or a derivative thereof is administered in a daily dosage of or greater than 25 mg/day.

Conversions of dosages for one species to another are known to the person skilled in the art. For example, the doses of astaxanthin fed to a rodent can be converted to human doses based on a ratio of body weights: comparing a 500 g rat to a 70 kg human (delta=140). Surface area rather than body weight can also be used in calculating dosing. The conversion factor for surface area for a rat compared to a human is seven, which makes the delta 20 instead of 140. As exemplified hereinbelow, administering 100 mg of natural astaxanthin per kilogram of rat body weight per day resulted in significant enhancement in the tolerance of the treated rats to heat stress. Taking an average rat body weight of 0.3 kg, the rats eat about 30mg/day. This would approximate a human does of 600 mg natural astaxanthin/day. As described herein above, this amount is highly tolerated by rats as well as by humans.

However, it should be taken in account that the bioavailability of astaxanthin to rodent is significantly lower compared to its bioavailability to human.

For example, an effective amount of natural astaxanthin or a derivative thereof can be administered to the subject once daily. An effective amount of astaxanthin or a derivative thereof can be administered to the subject more frequently than once daily. For example, one or more of an effective amount of astaxanthin or a derivative thereof can be administered twice, three times or more frequently each day. An appropriate dosing regimen can be determined by one of ordinary skill in the art, for example, by using the assessment techniques indicated above.

Optionally, the administration or administrations can be repeated for two or more days. According to certain embodiments, the administration or administrations can be repeated for days, weeks, months or years. Moreover, an administration can be given to the subject prior to, during, or after the subject being exposed to high environmental temperatures, physical exertion, a combination thereof or to any other factor that increases the likelihood of heat stress in that subject. For example, an administration can be repeated once or more daily every day for a week, month or year, or once or more for any subset of a week month or a year prior to, during, or after a heat stress event, or prior to that subject being exposed to conditions that increase the likelihood of a heat stress event. The administration can also be seasonal, wherein it is initiated before high seasonal temperatures and ceased after such seasonal temperatures become more moderate. Thus, an effective dosage of astaxanthin, or a derivative thereof can be administered after a heat stress event or during an acute heat stress event.

Optionally, an effective amount of astaxanthin and an effective amount of another agent are administered to the subject. According to exemplary embodiments, the additional agent is selected from the group consisting of other carotenoids, anti-oxidants and agents for enhancing the astaxanthin bioavailability. Each possibility represents a separate embodiment of the present invention. According to these embodiments, the agents administered can be administered concurrently, separately, or in any other temporal combination. For example, a plurality of administered agents can be administered together in a mixture. Optionally, each administered agent can be administered individually on a given day. Moreover, each administered agent can be administered separately such that one agent is given at a particular administration time (e.g., a first day) and another agent is given at another administration

time (e.g., a second day). Such agents can be given at selected times of day or under selected conditions (e.g., with or without food).

Also provided is a method of reducing the effects of a fever in a subject, comprising administering an effective amount of astaxanthin or a derivative thereof to the subject. According to alterative aspect, a medicament or dietary supplement comprising natural astaxanthin, a derivative thereof or oleoresin comprising same for reducing the effects of a fever in a subject is provided. High fevers, for example in children, can cause serious adverse effects such as seizures, which may or may not be associated with heat stress. Thus, one of skill in the art can administer to the subject at risk of such side effects the disclosed agent or agents. The administration can be performed before adverse symptoms arise or after adverse symptoms arise and can be performed if a particular subject is prone to high fevers or is expected to experience a high fever.

The present invention further provides pharmaceutical compositions comprising natural astaxanthin or a derivative thereof of sufficient purity to be suitable for use as a pharmaceutical grade material. Also provided are dietary supplement acceptable compositions and medical food acceptable compositions comprising the natural astaxanthin of the present invention.

The term “pharmaceutical grade” is used herein according to its meaning known in the art, and refers to a chemical grade of sufficient purity to meet or exceed requirements of the United States Pharmacopeia (USP). A pharmaceutical grade material is acceptable for food, drug, or medicinal use.

As used herein, the term “dietary supplement" refers to a product marketed under food law, containing one or more dietary ingredients in a concentrated form, which may also contain other ingredients, presented in a form intended for single or multiple dose administration, including but not limited to tablets, capsules, powders or liquids.

According to certain embodiments, the pharmaceutical, dietary or medical food compositions further comprise suitable diluents, excipients or carrier. According to certain embodiments, the compositions further comprise compound that enhance the bioavailability of astaxanthin to the animal, particularly to mammals. According to some exemplary embodiments, the compositions further comprise a compound selected from the group consisting of olive oil, tocopherol and rosemary oil extract. Each possibility represents a

separate embodiment of the present invention. According to certain exemplary embodiments, the rosemary extract comprise carnosis acid.

According to certain embodiments, the natural astaxanthin, derivatives thereof or oleoresin comprising same is administered orally. Methods for preparing a composition for oral consumption are known in the art, and depend on the subject consuming the composition.

According to certain exemplary embodiments, the natural astaxanthin of the present invention or the oleoresin comprising same is administered as a food additive or food supplement. The natural astaxanthin, derivatives thereof or oleoresin comprising same can be formulated as food supplements, animal feedstuffs, human foodstuffs and pharmaceutical preparations.

Food supplement products and pharmaceutical preparations comprising the natural astaxanthin of the invention include, *inter alia*, uncoated and coated tablets, and hard and soft gelatin capsules. Preferred food supplement products are soft gelatin capsules in which the astaxanthin is present as oil-containing suspension.

The following examples are presented in order to more fully illustrate some embodiments of the invention. They should, in no way be construed, however, as limiting the broad scope of the invention. One skilled in the art can readily devise many variations and modifications of the principles disclosed herein without departing from the scope of the invention.

EXAMPLES

Material and Methods

In all experiments, natural oleoresin containing 10% natural astaxanthin, extracted from the microalga *Haematococcus pluvialis* by super critical fluid CO_2 extraction was used (AstaPure® Algatechnologies Ltd., Kibbutz Ketura Israel). An exemplary composition of AstaPure® is presented in Table 1 hereinbelow.

Experiments were conducted with Sprague Dawley (SD) rats. Rats were given free access to regular diet and the astaxanthin-containing oleoresin described above (astaxanthin

treatment) or olive oil (control groups) was given by guavas (typically at 0.2 ml) for 10 days. Astaxanthin was given at a dosage of 5 mg/100 Kg body weight or 100mg/Kg body weight.

Following this period, rats from each group were assigned to one of two experimental protocols: (1) subjection to heat stress at a room temperature of 41°C to assess thermal tolerance and injury threshold; (2) subjection to 2hrs of heat stress as above followed by 4hr of recovery period at room temp (24°C).

Rats assigned to the first experiment were implanted with temperature probes (iButtons) 3-4 days prior the astaxanthin or oil administration. Body temperature was recorded online during the experiment. Rats were exposed to heat stress (41°C) until initial signs of impairments in temperature balance (about 370 min).

For measurements of protein and RNA level, rats assigned to the second protocol were transferred in groups into several different recovery periods of 20, 40, 60, 120 and 240 min. at a temperature of 24°C. Samples were taken at the end of the indicated recovery time. HSF1 (heat shock factor 1), Hsp72 and *hsp70* mRNA profiles were measured. The level of Hsp70 and HSF1 proteins was measured by Western immunoblot. For mRNA measurements Real-time PCR was used. The Hsp70 stress machinery was analyzed in the cardiac muscle.

Table 1: Exemplary Composition of AstaPure® 10% natural Astaxanthin Oleoresin

Astaxanthin Complex	%	(RP-HPLC without de-esterification)
Free Astaxanthin	1-3.5	
Mono-ester	85-90	
Di-ester	7-9	
Astaxanthin	**%**	**(RP-HPLC following de-esterification)**
E-astaxanthin	75-80	
9z-astaxanthin	10-16	
13z- astaxanthin	5-10	
Other Carotenoids	**%**	**(of total carotenoids, RP-HPLC)**
Best carotene	0.1 (±0.1)	
Lutein	0.8 (±0.5)	
Zeaxanthin	≤ 0.06	
Violaxanthin	≤ 0.1	

Fat and Nutritional Values	%
Total fat	98
Saturated fat	15
Mono-unsaturated fatty acids	36.5
Poly-unsaturated fatty acids	46.5

Lipid Profile		%
Palmitic	C16:0	9.2
Palmitoleic	C16:1	0.5
Stearic	C18:0	0.6
Oleic acid	C18:1n9	33.0
Vaccenate	C18:1	3.0
Linoleic	C18:2n6	30.0
γLinolenic	C18:3n3	13.5
Arachidic	C20:0	0.2
Arachidonic	C20:4n6	1.0
EPA	C20:5n3	2.0
Docosanoic (Behenic)	C22:0	5.0

Other Components	%
Proteins	0.2
Sugars	0.5
Dietary fibers	0.7
Ash	0.07
Moisture	0.35
Sodium	0.1

Calories/100 g	880

Trace Elements	ppm
Calcium	55
Phosphorous	0.5
Iron	0.6
Magnesium	11

Zinc	0.2
Iodine	< 10

Example 1: Effect of astaxanthin on body temperature of rats exposed to heat stress

Figure 1 shows the average temperature of rats exposed to heat stress conditions (41°C) for 370 min. As is shown in Figure 1, rats (n=5) that received astaxanthin before the exposure to heat developed a lower basal core temperature and belated failure in the regulation of body temperature compared to control rats (n=4). Body temperature of natural astaxanthin treated rats was significantly lower compared to that of untreated rats during the entire period of hypothermic plateau ($p<0.001$). Fluid loss was significantly greater in the control group in comparison with the astaxanthin group (11.03 ±0.27 vs. 10.35±0.018 respectively, % of body weight; $p<0.016$). Importantly, rats that have received natural astaxanthin reached the thermal injury threshold at a later time compared to the control group.

Example 2: Effect of astaxanthin on heat-sock associated markers

The 70 kilodalton heat shock proteins (Hsp70s) are a family of ubiquitously expressed heat shock proteins. Proteins with similar structure exist in virtually all living organisms. The Hsp70s are an important part of the cell's machinery for protein folding, and help to protect cells from stress. It was thus examined whether oral administration of natural astaxanthin affect the expression level of *hsp70* and the encoded heat shock protein Hsp72. Surprisingly, Figure 2 shows that the orally administered astaxanthin significantly affected the basal levels of both *hsp70* RNA and Hsp72 protein. An elevation of about 44% was found in the group that received Astaxanthin compared to the control-oil receiving group (*P=0.001*; "con" in Figure 2). Furthermore, the level of both RNA and protein remained higher throughout the recovery phase in the astaxanthin group. The control group displayed earlier onset of the accelerated phase of Hsp72 production ($p<0.001$). Without wishing to be bound by any theory or mechanism of action, these results indicate that orally taken natural astaxanthin leads to an increase in the HSP72 kDa reserves, which in turn attenuates the need to its elevation during heat stress. It is suggested that these elevated heat shock protein reserves is associated with the delayed thermal injury.

Heat Shock Factor-1 (HSF1) exists as an inactive monomer in a complex with Hsp40/Hsp70 and Hsp90. Upon stress, such as elevated temperature, HSF1 is released from the chaperone complex and trimerizes. HSF1 is then transported into the nucleus where it is hyperphosphorylated and binds to DNA containing heat shock elements (NGAAN). HSF1's target genes include the heat shock proteins Hsp72. The expression of HSF1 was elevated in the astaxanthin group compared to the control group ($p<0.05$) (Figure 3). The expression profile of HSF1 corresponded with that of *Hsp70*. However, no significant difference was found in the ratio phosphorylated HSF1 to non-phosphorylated HSF1 between samples obtained from rats receiving astaxanthin compared to rats reciving olive oil (control). These results indicate that astaxanthin had no negative effect on the transcrption process within the cells.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed functions may take a variety of alternative forms without departing from the invention.

CLAIMS

1. A method of enhancing the tolerance of a subject to heat stress comprising a period of preconditioning via administering to the subject natural astaxanthin or a derivative thereof.

2. The method of claim 1, wherein the subject is expected to be affected by heat stress.

3. The method of claim 1, wherein the subject is exposed to heat stress.

4. The method of claim 1, wherein the subject is already suffering from heat stress.

5. The method of any one of claims 1-4, wherein the heat stress is a result of exposure to high environmental temperatures and/or of physical exertion.

6. The method of claim 1, wherein the natural astaxanthin or derivative thereof is administered orally.

7. The method of claim 1, wherein the natural astaxanthin is from an algal source.

8. The method of claim 7, wherein the alga is *Haematococcus pluvialis*.

9. The method of claim 7, wherein at least 75% (w/w) of the astaxanthin total amount is in the form of a fatty acid monoester.

10. The method of claim 7, wherein at least 90% (w/w) of the astaxanthin total amount is in the 3S,3'S stereoisomer configuration.

11. The method of claim 7, said method comprising administering to the subject *Haematococcus pluvialis* oleoresin comprising at least 10% of astaxanthin or a derivative thereof.

12. The method of claim 11, wherein the oleoresin comprises at least 98% astaxanthin out of the total carotenoid content of said oleoresin.

13. The method of claim 11, wherein the oleoresin comprises from about 20% to about 40% free monounsaturated fatty acids.

14. The method of any one of claims 1-13, said method consists of administrating to the subject natural astaxanthin, a derivative thereof or oleoresin comprising same.

15. The method of claim 1, wherein the astaxanthin or derivative thereof is administered in an amount effective in preventing or reducing a symptom or a condition associated with heat stress.

16. The method of claim 15, wherein the symptom or condition associated with heat stress is selected from the group consisting of hyperthermia, headache, dizziness, lightheadedness, fainting, weakness, exhaustion, fever, moist skin, irritability, confusion, dry

skin, hot skin, no sweating, loss of consciousness, seizures, convulsions, heat stroke, vomiting and upset stomach.

17. The method of claim 1, wherein the astaxanthin or derivative thereof is administered in an amount of from about 0.02mg/Kg body weight to about 200mg/Kg body weight.

18. The method of claim 2, wherein astaxanthin administration starts at least 10 days before the subject is expected to be exposed to heat stress.

19. The method of claim 3, wherein astaxanthin is administered during exposure to heat stress.

20. The method of claim 4, wherein astaxanthin administration starts at a time point selected from right after the subject was affected by heat stress to about 10 days after said subject was affected by heat stress.

21. The method of any one of claims 18-20, wherein astaxanthin is administered continuously at least once daily.

22. The method of claim 1, wherein the astaxanthin is administered at least once daily, in an amount of from 12 mg/day to 1 gr/day.

23. The method of claim 22, wherein the astaxanthin is administered at least once daily, in an amount of over 25 mg/day.

24. The method of any one of claims 17-23, wherein the subject is human.

25. A method of reducing the effects of a fever in a subject comprising administering to the subject natural astaxanthin or a derivative thereof.

26. The method of claim 25, wherein the natural astaxanthin or derivative thereof is administered orally.

27. The method of claim 26, wherein the astaxanthin is administered in an amount of from about 0.02mg/Kg body weight to about 200mg/Kg body weight.

28. A pharmaceutical composition comprising algal oleoresin comprising at least 10% astaxanthin, the oleoresin being purified to a sufficient degree of purity to be suitable for use as a pharmaceutical grade material, the composition further comprises a therapeutically acceptable diluents or carrier.

29. The pharmaceutical composition of claim 28, wherein the astaxanthin containing oleoresin is isolated from the alga *Haematococcus pluvialis*.

30. The pharmaceutical composition of claim 26, wherein astaxanthin is the principal active carotenoid in said pharmaceutical composition.

31. The pharmaceutical composition of claim 28, said pharmaceutical composition is formulated to enhance the astaxanthin bioavailability.

32. A dietary supplement composition comprising algal oleoresin comprising at least 10% astaxanthin, the oleoresin being purified to a sufficient degree of purity to be suitable for use as a dietary supplement, the composition further comprises a dietary acceptable diluents or carrier.

33. The dietary supplement composition of claim 32, wherein the astaxanthin containing oleoresin is isolated from the alga *Haematococcus pluvialis*.

34. The dietary supplement composition of claim 32, wherein astaxanthin is the sole active carotenoid in said dietary supplement composition.

35. The dietary supplement of claim 32, said dietary supplement composition is formulated to enhance the astaxanthin bioavailability.

36. A medicament or a dietary supplement comprising natural astaxanthin, a derivative thereof or oleoresin comprising same for enhancing the tolerance to heat stress, wherein the medicament or dietary supplement is to be administered to a subject for a period of preconditioning of the subject to heat stress.

37. The medicament or dietary supplement of claim 36, to be administered to a subject expected to be affected by heat stress.

38. The medicament or dietary supplement of claim 36, to be administered to a subject exposed to heat stress.

39. The medicament or dietary supplement of claim 36, to be administered to a subject already suffering from heat stress.

40. The medicament or dietary supplement of any one of claims 36-39, wherein the heat stress is a result of exposure to high environmental temperatures and/or of physical exertion.

41. The medicament of claim 36, said medicament is formulated for oral administration.

42. The medicament or dietary supplement of claim 36, wherein the natural astaxanthin, derivative thereof or oleoresin comprising same is from an algal source.

43. The medicament or dietary supplement of claim 42, wherein the alga is *Haematococcus pluvialis*.

44. The medicament or dietary supplement of claim 42, wherein at least 75% (w/w) of the astaxanthin total amount is in the form of a fatty acid monoester.

45. The medicament or dietary supplement of claim 42, wherein at least 90% (w/w) of the astaxanthin total amount is in the 3S,3'S stereoisomer configuration.

46. The medicament or dietary supplement of claim 42, wherein the oleoresin comprises at least 98% astaxanthin out of the total carotenoid content of said oleoresin.

47. The medicament or dietary supplement of claim 46, wherein the oleoresin comprises from about 20% to about 40% free monounsaturated fatty acids.

48. The medicament or dietary supplement of claim 36, wherein the astaxanthin, derivative thereof or oleoresin comprising same is in an amount effective in preventing or reducing a symptom or a condition associated with heat stress.

49. The medicament or dietary supplement of claim 48, wherein the symptom or condition associated with heat stress is selected from the group consisting of hyperthermia, headache, dizziness, lightheadedness, fainting, weakness, exhaustion, fever, moist skin, irritability, confusion, dry skin, hot skin, no sweating, loss of consciousness, seizures, convulsions, heat stroke, vomiting and upset stomach.

50. The medicament or dietary supplement of claim 37, to be administered at least 10 days before expected exposure to heat stress.

51. The medicament or dietary supplement of claim 38, to be administered during exposure to heat stress.

52. The medicament or dietary supplement of claim 39, to be administered at a time point from right after the subject was affected by heat stress to about 10 days after said subject was affected by heat stress.

53. The medicament or dietary supplement of any one of claims 50-52, to be administered at least once daily.

54. The medicament or dietary supplement of claim 53, to be administered continuously.

55. The medicament or dietary supplement of claim 53, wherein said medicament or dietary supplant comprises astaxanthin in an amount of from 12 mg to 1 gr.

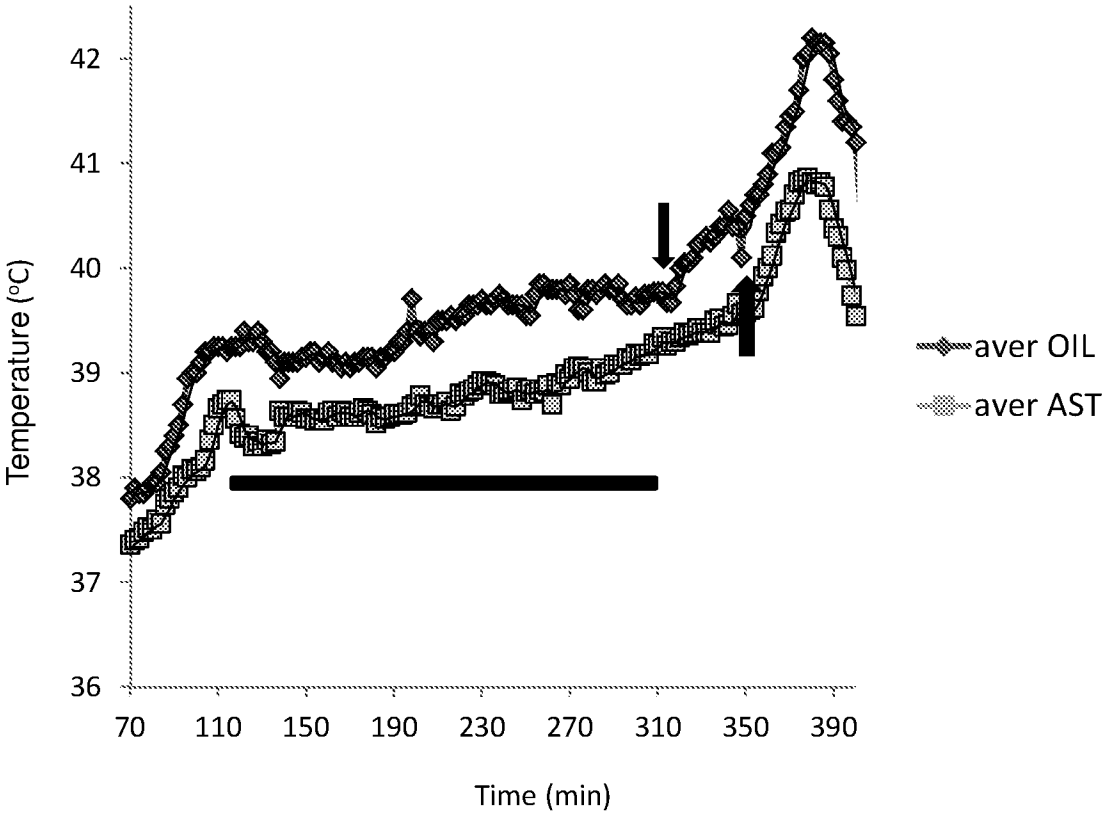
42
41
40
39
38
37
36
Temperature (°C)
70
110
150
190
230
270
310
350
390
Time (min)
aver OIL
aver AST

FIGURE 1

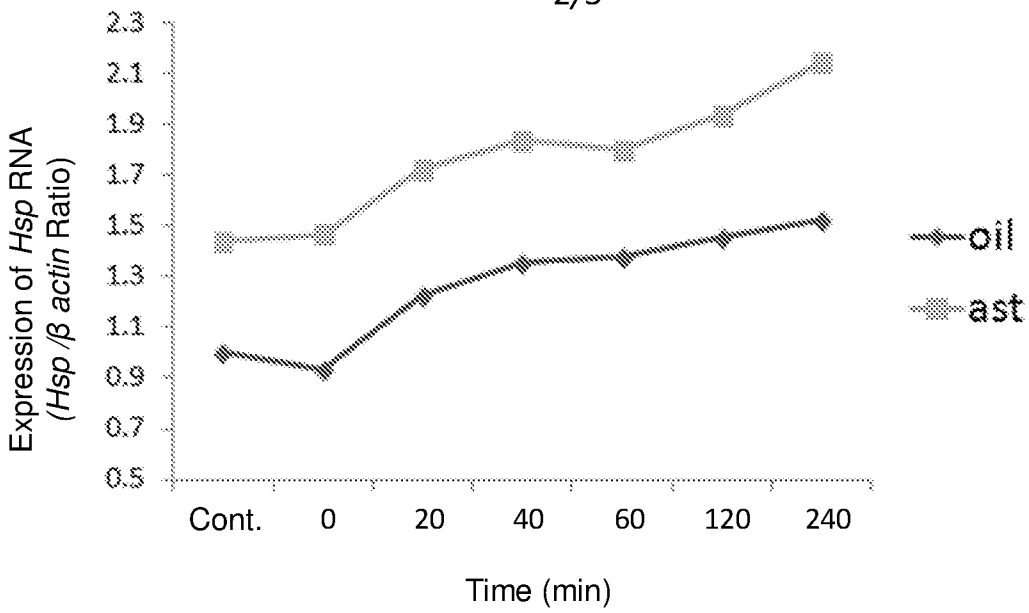

FIGURE 2A

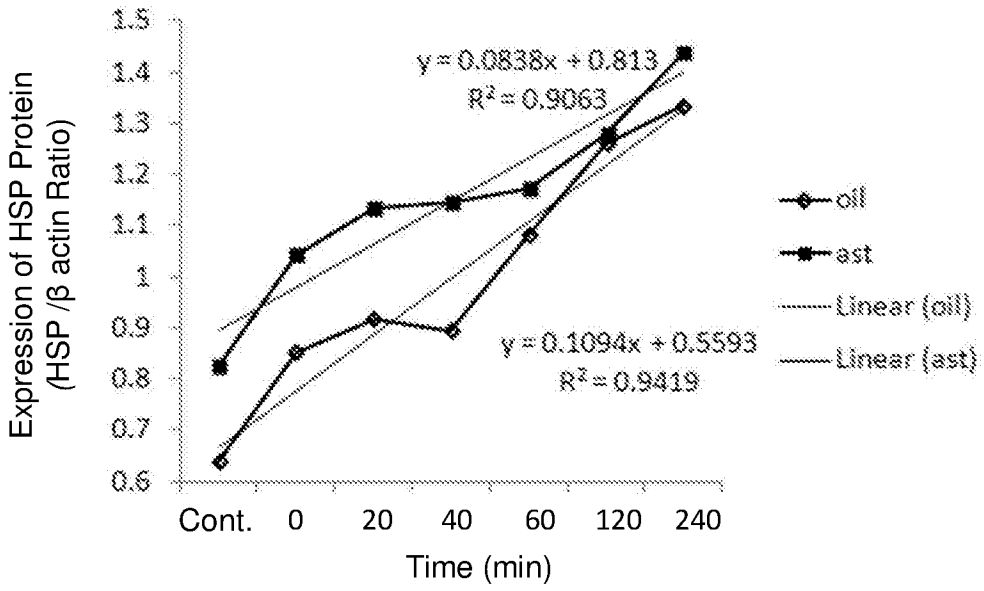

FIGURE 2B

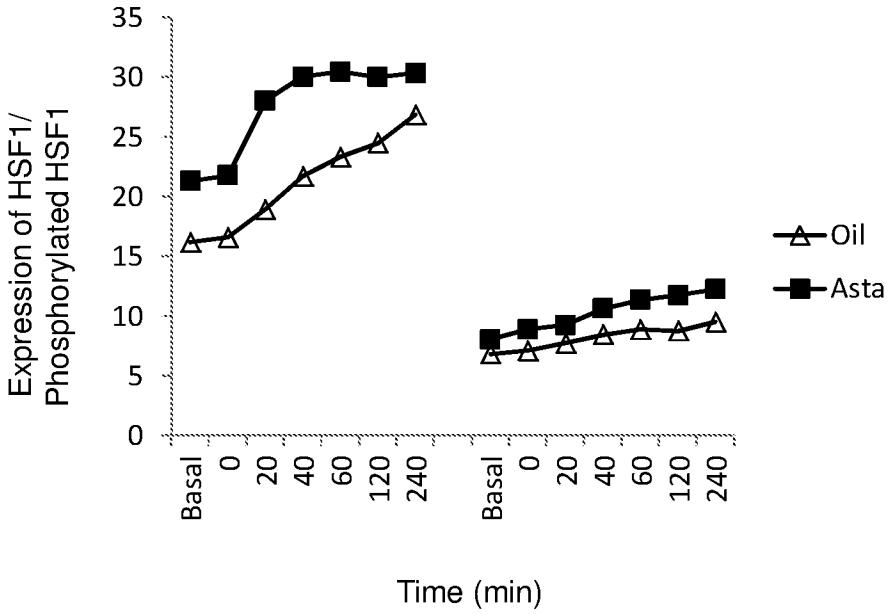

FIGURE 3

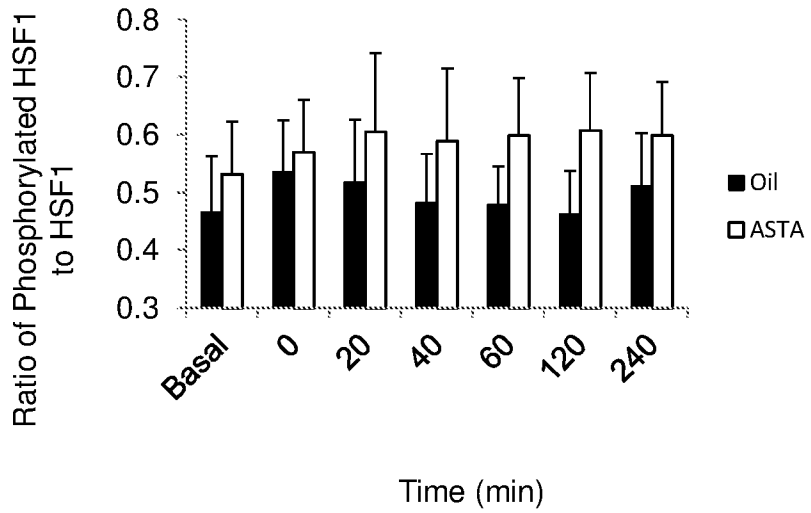

FIGURE 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2013/050822

A. CLASSIFICATION OF SUBJECT MATTER
IPC (2013.01) A61K 31/122, A61K 36/05

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)
IPC (2013.01) A61K 31/122, A61K 36/05

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See extra sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2010039676 A1 UNIV GEORGETOWN [US]; PREUSS HARRY GEORGE [US] 08 Apr 2010 (2010/04/08) whole document	1-27,36-55
Y	"Cosmetic benefits of astaxanthin on humans subjects" Kumi Tominaga et-al., Acta Biochim Pol. 2012;59(1):43-7. Epub 2012 Mar 17 17 Mar 2012 (2012/03/17) abstract	1-27,36-55
Y	"Dietary Supplementation with Astaxanthin-Rich Algal Meal Improves Strength Endurance", Curt L. Malmsten et-al., Carotenoid Science, 2008, Vol.13: 20-22 31 Dec 2008 (2008/12/31) abstract; p.20, right column, 3rd para.	1-27,36-55
Y	"Effects of Astaxanthin Supplementation on Exercise-Induced Fatigue in Mice", Mayumi IKEUCHI et-al., Biol Pharm Bull 29(10): 2106–2110 09 Aug 2006 (2006/08/09) abstract; p. 2106, left column, MATERIALS AND METHODS	1-27,36-55

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"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)
"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
"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
"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
"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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 Jan 2014	20 Jan 2014
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	Authorized officer AMITAY Noam Telephone No. 972-2-5651725

B. FIELDS SEARCHED:

* Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases consulted: SCIRUS, PATENTSCOPE, THOMSON INNOVATION, Esp@cenet, CAPLUS, WPI Data, EPODOC, Google Scholar, Web of Science

Search terms used: astaxanthin, Carotenoid, xanthophyll, Oleoresin, Alga*, Haematococcus, heat stress, heat shock, dry skin, heat stroke, Fever, pyrexia

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2013/050822

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2010043032 A FUJI CHEM IND CO LTD 25 Feb 2010 (2010/02/25) whole document (translation to English)	28,29,32,33
Y		28-35

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL2013/050822

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
WO 2010039676 A1	08 Apr 2010	US 2011217389 A1	08 Sep 2011
		WO 2010039676 A1	08 Apr 2010
JP 2010043032 A	25 Feb 2010	JP 2010043032 A	25 Feb 2010
		JP 5334492 B2	06 Nov 2013