(54) Title: USE OF AVOCADO-SOYBEAN UNSAPONIFIABLE PREPARATIONS

(57) Abstract: The present invention provides a preparation, preferably comprising an avocado unsaponifiable, a soybean unsaponifiable or a mixture thereof, for inhibition of apoptosis. In addition the present invention provides a preparation for protection of the skin. In particular it concerns a preparation comprising avocado and soybean unsaponifiables for protection of the skin against negative environmental factors and pollutants. Moreover, the present invention provides a preparation comprising ASU which protects the skin from negative environmental factors and pollutants by stimulating ATP synthesis and preventing the breakdown of the mitochondrial membrane potential in the skin. Furthermore, the present invention provides a preparation, preferably a preparation comprising an ASU complex for prevention of cellular aging.
USE OF AVOCADO-SOYBEAN UNSPAONIFIABLE PREPARATIONS

FIELD AND BACKGROUND OF THE INVENTION

The present invention provides a preparation comprising an avocado and soybean unsaponifiable complex (ASU) for prevention of apoptosis, inhibition of cellular aging and for protection of the skin against negative environmental factors and pollutants.

Apoptosis, or programmed cell death is an evolutionary conserved mechanism essential for the development and maintenance of tissue homeostasis in multicellular organisms. Apoptosis results from the action of a genetically encoded suicide program that leads to a series of characteristic morphological and biochemical changes. These changes include activation of caspases, mitochondrial depolarization, cell volume loss, chromatin condensation and nucleosomal DNA defragmentation. It appears that the downstream biochemical events in apoptosis are the same regardless of the initial death stimulus [1].

A variety of key events in apoptosis focus on mitochondria, including the release of caspase activators (such as cytochrome c), changes in electron transport, loss of mitochondrial transmembrane potential, decrease of intracellular ATP concentration, altered cellular oxidation-reduction and participation of pro- and anti-apoptotic Bcl-2 family proteins [2].

Two major features of human aging clearly have been linked to alterations in the incidence of cell death, immunoseniscence and neurodegeneration. In each of these case apoptosis plays a role in generation of these disorders. There is increasing evidence of the potential role of apoptosis in skin aging [3].

Apoptosis may be induced by various pathological conditions and a variety of agents, such as deprivation of tropic factors, heat shock and various cytotoxic substances [4] [5].

Skin is subjected daily to numerous negative environmental factors and pollutants. These pollutants include, but are not limited to atmospheric factors, chemical pollutants and biological pollutants. Examples of atmospheric factors that effect the skin include but are not limited to radiation such as Ultra-violet (UV)
radiation from the sun, ozone, acid rain and extreme temperatures. Chemical and biological pollutants include pollutants from cars, industry, free radicals, cleaning materials, cosmetics, drugs and toxins. The background art discloses complex synthetic sun block preparations for protection against UV radiation. However, there are no effective preparations available for general protection of the skin against damage by environmental factors. In addition many of the sunblocks available have deleterious effects on the skin.

The vegetable oil unsaponifiables, such as avocado and soybean unsaponifiables are comprised of biologically active compounds, such as sterols, tocopherols, squalene and lipidic furans. These chemical groups exhibit a range of biological effects. Advantageous biological properties have been reported for avocado and soybean unsaponifiables. It was shown that a mixture of avocado and soybean unsaponifiables is beneficial in treatment of various disorders of connective tissue, such as wound healing and scleroderma. The background art contains several references of different cosmetic uses for ASU preparations.

U.S. Patent No. 5,928,659 discloses a cosmetic composition comprising unsaponifiable lipids extracted from avocado for amelioration of skin keratoses that can be caused by aging of the skin and other skin lesions.

U.S. Patent No. 5,468,490 discloses as supporting art that avocado preparations such as non-saponifiable fractions of avocado oil have useful dermatological properties for improving the appearance of aging, dry or wrinkled skin and for moisturizing and refreshing skin. It is further disclosed that lipid furans derived from avocado enhance the metabolism of skin, thereby improving its appearance. The enhanced metabolism results in increased elasticity and thickness of the skin and increased fibroblast population. These compounds are useful in combating dermal atrophy by slowing and even reversing thinning skin, wrinkles and other skin conditions, caused by chronological and photo aging of the skin.


BE Patent No. 886707 discloses compositions containing avocado and soya unsaponifiables for use as cosmetics including skin creams.
FR Patent No. 4174 discloses compositions containing avocado and soya unsaponifiables for use in the treatment of conditions such as eczema. The background art refers to several cosmetic uses of ASU. However, the background art does not teach or suggest uses of ASU preparations for protection against negative environmental factors, for inhibition of cellular aging, or prevention of apoptosis.

There is thus a widely recognized need for a natural derived preparation that can be used for general protection of the skin against negative environmental factors and pollutants, for inhibition of cellular aging and for prevention of apoptosis.

SUMMARY OF THE INVENTION

The present invention provides a preparation, preferably comprising an avocado and soybean unsaponifiable complex (ASU), for prevention of apoptosis. In addition the present invention provides a preparation for protection of the skin. In particular it concerns a preparation comprising avocado and soybean unsaponifiables for protection of the skin against negative environmental factors and pollutants. Moreover, the present invention provides a preparation comprising ASU which protects the skin from negative environmental factors and pollutants by stimulating ATP synthesis and preventing the breakdown of the mitochondrial membrane potential in the skin. Furthermore, the present invention provides a preparation, comprising an ASU complex for inhibition of cellular aging.

According to the teachings of the present invention there is provided in a first embodiment a composition for preventing cell apoptosis, comprising an effective amount of an avocado and soybean unsaponifiable component.

In a preferred embodiment the effective amount of an avocado and soybean unsaponifiable component is in a range of from about 1μg/ml to about 10μg/ml.

In a preferred embodiment the avocado and soybean unsaponifiable component is in a range of from about 1μg/ml to about 5μg/ml.

In a preferred embodiment the composition comprises from about 0.01% to about 0.15% of the avocado and soybean unsaponifiable component.
In a preferred embodiment the composition comprises from about 0.01% to about 0.05% of the avocado and soybean unsaponifiable component.

In a preferred embodiment the avocado and soybean unsaponifiable component is an avocado unsaponifiable.

In a preferred embodiment the avocado and soybean unsaponifiable component is a soybean unsaponifiable.

In a preferred embodiment the avocado and soybean unsaponifiable component is a lipidic furan derived from ASU.

In a preferred embodiment the avocado and soybean unsaponifiable component is a mixture of avocado unsaponifiables and soybean unsaponifiables.

In a preferred embodiment the avocado and soybean unsaponifiable component is a lipid furan.

In a preferred embodiment the composition further comprises one or a mixture of Ascorbyl Palmitate, fragrance, thickeners, herbal extracts, vitamins, dispersing agents, emulsifiers, binders, colorants, coenzyme-Q, anti-oxidants, natural vegetable oils and polyunsaturated fatty acids.

In a preferred embodiment the composition is administered orally.

In a preferred embodiment the composition is selected from the group consisting of powders or granules, suspensions, emulsions or solutions in water or non-aqueous media, oil or fat, sachets, capsules, tablets, gelcaps, food additives and sustained release formulations

In a preferred embodiment the composition further comprises vitamins, anti-oxidants and a diluent.

In a preferred embodiment the composition further comprises vitamins, anti-oxidants and at least one oil.

In a preferred embodiment the composition is administered topically.

In a preferred embodiment the composition is in the form of a cream.

In a preferred embodiment the cream is a non-ionic oil-in water emulsion.

In a preferred embodiment the composition further comprises an oil phase and a water phase.
In a preferred embodiment the oil phase is selected from the group consisting of one of or a mixture of at least one oil, poly-unsaturated fatty acids, vitamins A, E and F, ascorbyl palmitate and anti-oxidants.

In a preferred embodiment the water phase is selected from the group consisting of water, plant extracts, humectants and non-ionic emulsifiers.

In a preferred embodiment the composition is in the form of lipophilic natural oil.

In a preferred embodiment the composition further comprises vegetable oils, polyunsaturated fatty acids, vitamins A, E and F, anti-oxidants and penetration enhancers.

In a preferred embodiment the penetration enhancers include propylene glycol di-pelargonate and ethoxydiacyglycol.

In a preferred embodiment the vegetable oils include wheat germ oil, sesame oil, soybean oil, avocado oil, olive oil and rice oil.

In a preferred embodiment the polyunsaturated fatty acids include linoleic, linolenic acids-omega 3, gamma linolenic and arachidonic acid-omega 6.

In a second embodiment the present invention provides a composition for protection of the skin against pollutants, comprising an effective amount of an avocado and soybean unsaponifiable component.

In a preferred embodiment of the composition for protection of the skin against pollutants, the pollutant is selected from the group consisting of radiation, sun radiation, ozone, acid rain, extreme temperatures, transport pollutants, industry pollutants, cleaning materials, cosmetics, drugs, toxins and combinations thereof.

In a preferred embodiment of the composition for protection of the skin against pollutants, the avocado and soybean unsaponifiable component is in a range of from about 1µg/ml to about 5µg/ml.

In a preferred embodiment of the composition for protection of the skin against pollutants, the composition comprises from about 0.01% to about 0.05% of the avocado and soybean unsaponifiable component.

In a preferred embodiment of the composition for protection of the skin against pollutants, the avocado and soybean unsaponifiable component is an avocado unsaponifiable.
In a preferred embodiment of the composition for protection of the skin against pollutants, the avocado and soybean unsaponifiable component is a soybean unsaponifiable.

In a preferred embodiment of the composition for protection of the skin against pollutants, the avocado and soybean unsaponifiable component is a lipidic furan derived from ASU.

In a preferred embodiment of the composition for protection of the skin against pollutants, the avocado and soybean unsaponifiable component is a lipidic furan.

In a preferred embodiment of the composition for protection of the skin against pollutants, the avocado and soybean unsaponifiable component is a mixture of avocado unsaponifilables and soybean unsaponifiables.

In a third embodiment the present invention provides a composition for preventing cell anti-aging, comprising an effective amount of avocado and soybean unsaponifiables.

In a preferred embodiment of the composition for preventing cell anti-aging the avocado and soybean unsaponifiable component is in a range of from about 1µg/ml to about 5µg/ml.

In a preferred embodiment of the composition for preventing cell anti-aging the composition comprises from about 0.01% to about 0.05% of the avocado and soybean unsaponifiable component.

In a preferred embodiment of the composition for preventing cell anti-aging the avocado and soybean unsaponifiable component is an avocado unsaponifiable.

In a preferred embodiment of the composition for preventing cell anti-aging the avocado and soybean unsaponifiable component is a soybean unsaponifiable.

In a preferred embodiment of the composition for preventing cell anti-aging the avocado and soybean unsaponifiable component is a lipidic furan derived from ASU.

In a preferred embodiment of the composition for preventing cell anti-aging the avocado and soybean unsaponifiable component is a mixture of avocado unsaponifiables and soybean unsaponifiables.

In a preferred embodiment of the composition for preventing cell anti-aging the composition is administered orally.
In a preferred embodiment of the composition for preventing cell anti-aging the composition is administered topically.

In a fourth embodiment the present invention provides a method of protecting the skin from damage, comprising the step of administering a composition of an avocado and soybean unsaponifiable component on skin cells.

In a preferred embodiment of the method of protecting the skin from damage the damage is damage from aging.

In a preferred embodiment of the method of protecting the skin from damage the damage is damage from pollutants.

In a preferred embodiment of the method of protecting the skin from damage the damage is damage from apoptosis.

The term ‘apoptosis’ as used herein refers to the process of cell death which results from an orderly pattern of morphological and biochemical changes.

The term ‘negative environmental factors’ as used herein refers to any substance in the water, atmosphere and environment that has deleterious effects on cells, especially skin cells, such as, but not limited to radiation such as UV radiation from the sun, ozone, acid rain and extreme temperatures, pollutants from cars, industry, free radicals, cleaning materials, cosmetics, drugs and toxins.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the protective effect of ASU preparation on human skin fibroblast against PMA, which induces apoptosis;

FIG. 2 shows the protective effect of ASU pretreatment on PMA-induced decrease of SYTO 16 fluorescence in cells;

FIG. 3 shows the protective effect of ASU preparation on human skin fibroblasts against the apoptosis induced stimuli Ca-ionophore A 23187;

FIG. 4 shows the protective effect of ASU preparation on hamster fibroblasts against Ca-ionophore A 23187 and PMA induced decrease of intracellular ATP concentration; and

FIG. 5 shows the protective effect of ASU preparation on hamster fibroblasts against Calcium-ionophore A 23187 and PMA induced decrease in DNA synthesis.
DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides a preparation, preferably comprising an avocado and soybean unsaponifiable complex (ASU), for prevention of apoptosis. In addition the present invention provides a preparation for protection of the skin. In particular it concerns a preparation comprising avocado and soybean unsaponifiables for protection of the skin against negative environmental factors and pollutants. Moreover, the present invention provides a preparation comprising ASU which protects the skin from negative environmental factors and pollutants by stimulating ATP synthesis and preventing the breakdown of the mitochondrial membrane potential in the skin. Furthermore, the present invention provides a preparation, preferably a preparation comprising an ASU complex for inhibition of cellular aging.

Apoptosis may be induced by numerous pathological and physiological factors, such as cell aging and pollutants.

Skin is subjected daily to numerous negative environmental factors and pollutants. These pollutants include, but are not limited to atmospheric factors, chemical pollutants and biological pollutants. Examples of atmospheric factors that affect the skin include but are not limited to radiation such as UV radiation from the sun, ozone, acid rain and extreme temperatures. Chemical and biological pollutants include pollutants from cars, industry, free radicals, cleaning materials, cosmetics, drugs and toxins.

Pollutants cause the development of abnormal skin conditions, such as skin aging, skin irritation, dermatitis and low skin humidity. The general negative effect of such pollutants is changing the regulation of epidermal and dermal homeostasis resulting in cellular stress and the development of cellular apoptosis.

Different environmental pollutants or pathophysiological conditions interfere with the normal function of the mitochondria causing a perturbation of the transmembrane potential and generating superoxides by the uncoupled respiratory chain. Mitochondria are the primary system for ATP generation and therefore, toxic agents which interfere with their normal function would also affect the energy balance of the cells. The alterations of mitochondrial function is important in the cells apoptotic process and even in cell death. Without wishing to be limited by a single hypothesis the ASU complex inhibits apoptosis and thereby protects the skin by
stimulating the ATP synthesis in the cells which stimulates energetic metabolism and ATP dependent reactions in disordered skin, preventing the breakdown of mitochondrial membrane potential. In this way the ASU complex protects cells, such as skin cells from apoptosis, cell aging and the negative effects of pollutants.

The composition of the present invention comprises an avocado unsaponifiable, a soybean unsaponifiable, a lipidic furan derived from ASU or a mixture thereof. Preferably, the composition of the present invention is a mixture of an avocado soybean unsaponifiable (ASU). The ASU composition is effective in an amount of from about 1μg/ml to about 10μg/ml and preferably from about 1μg/ml to about 5μg/ml. A formulation or composition containing the ASU composition contains from about 0.01% to about 0.15% (w/w) ASU and preferably from about 0.01% to about 0.05% (w/w) ASU. It has been shown that the ASU complex at these very low concentrations is very active. Surprisingly, at higher concentrations the ASU complex was found to cause cell damage and even cell death.

The composition of the present invention can optionally contain additional additives, such as Ascorbyl Palmitate, fragrance, thickeners, herbal extracts, vitamins, dispersing agents, emulsifiers, binders, colorants, coenzyme-Q, anti-oxidants, natural vegetable oils, polyunsaturated fatty acids or other suitable components. The polyunsaturated fatty acids are preferably, but not limited to linoleic, linolenic and arachidonic acid-omega 6. The composition of the present invention more preferably comprises the ASU complex, an essential fatty acid and an anti-oxidant.

The composition of the present invention can optionally be in various formulations depending on the route of administration. Optional routes of administration are preferably, but not limited to topical and oral.

Compositions for oral administration, can be in a form which includes powders or granules, suspensions, emulsions or solutions in water or non-aqueous media, oil or fat, sachets, capsules, tablets, gelcaps, food additives and sustained release formulations. A food additive containing the ASU complex can optionally be used as an additive to edible oils in foods, such as but not limited to bread, baking products, biscuits, crackers, dairy products, cakes, chocolate and food fats. Thickeners, diluents, flavorings, vitamins dispersing aids, emulsifiers or binders may be desirable. Preferred formulations for oral administration include gelatin capsules,
preferably comprising ASU complex, vitamins, anti-oxidants and diluents (Example 9). An additional preferred formulation for oral administration comprises ASU complex, vitamins, anti-oxidants and edible oil (Example 10).

Suitable compositions for topical administration include, but are not limited to a gel, cream, mousse, lotion, oil, hydrophyllic oil or other suitable formulation. Preferred compositions for topical administration include a cream or lipophilic natural oil. Preferably the cream composition is a non-ionic oil-in-water emulsion. A preferred cream composition for topical administration is a non-ionic oil-in-water emulsion. The non-ionic oil-in-water emulsion composition preferably comprises from about 30% to about 80% (w/w) oil phase, most preferably from about 35% to about 40% (w/w). The water phase is preferably in a range of from about 19% to about 70% (w/w) and most preferably from about 59% to about 65%. The concentration of the ASU complex is preferably from about 0.01% to about 0.15% (w/w) and most preferably from about 0.01% to about 0.05%. The oil phase is preferably comprised of at least one or a mixture of natural vegetable oils, polyunsaturated fatty acids, vitamins A, E and F, Ascorbyl Palmitate, anti-oxidants and other suitable components. The mixture of natural vegetable oils are preferably, but not limited to jojoba oil, avocado oil, soybean oil, sesame oil, rice oil, wheat germ oil, olive oil, sunflower oil, nut oil, cottonseed oil, coconut oil and corn oil or any other suitable natural vegetable oil.

The water phase of the cream composition is preferably comprised of a mixture of water, natural plant extracts, humectants, non-ionic emulsifiers and other suitable components. The cream can optionally be prepared by an inverse emulsion process by addition of a water phase to an oil phase.

The cream formulation can be optionally administered by topically applying onto the facial skin, neck, scalp, around the eyes and ton the body skin, preferably but not limited to twice daily. Application is especially preferable before exposure of the face and body skin to a hostile environment of toxins, pathogens, UV radiation, negative environmental factors and pollutants.

An additional preferred composition for topical administration is a lipophilic natural oil composition. The lipophilic natural oil composition preferably comprises a mixture of ASU complex, natural vegetable oils, polyunsaturated fatty acids, vitamins
A, E and F, anti-oxidants, penetration enhancers and other suitable components. The
ASU complex is preferably present in a concentration in the range of from about
0.01% to about 0.15% (w/w) and more preferably from about 0.01% to about 0.05%
(w/w). The concentration of natural vegetable oils is preferably from about 20% to
about 80% (w/w) and most preferably from about 65% to about 75% (w/w). The
natural vegetable oils are preferably comprised of one or more of wheat germ oil,
sesame oil, soybean oil, avocado oil, olive oil and rice oil. The fatty acids are
preferably one or more of linoleic acid, linolenic acid, omega 3, gamma linolenic and
arachidonic acid-omega 6 or mixtures thereof. The penetration enhancers are
preferably present in a concentration of from about 0.5% to about 2.0% (w/w) and
most preferably in a concentration of 2.0% (w/w). Preferred penetration enhancers
include, but are not limited to propylene glycol dipelargonate and ethoxydiaglycol.
The propylene glycol dipelargonate is preferably present in a concentration of about
1.60% (w/w) and ethoxydiaglycol is preferably present in a concentration of about
0.4% (w/w). Additional suitable components are optionally present in a range
preferably from about 19% to about 80% (w/w).

The lipophilic natural oil composition can optionally be prepared by premixing
the ASU complex in a part of the natural oils and addition of the premix to the
remaining components of the preparation.

The lipophilic natural oil formulation can be optionally administered by
topically applying onto the facial skin, neck, lips, scalp, around the eyes and on the
body skin, preferably but not limited to twice daily. Application is especially
preferable before exposure of the face and body skin to a hostile environment of
toxins, pathogens, UV radiation, negative environmental factors and pollutants.

The ASU composition of the present invention has been shown to be effective
in hamster and human cells. The ASU composition of the present invention
minimized apoptosis resulting from exposure to toxins or calcium ionophores in
human skin cells. Furthermore, ASU inhibited and protected fibroblast cells in
hamsters from apoptosis.

It is to be understood that the invention is not limited in its application to the
details of construction and the arrangement of the components set forth in the
following description. The invention includes other embodiments and can be practiced or implemented in various ways. Also it is to be understood that the phraseology and terminology employed herein is for the purpose of description only and should not be regarded as limiting.

The present invention may be better understood with reference to the examples and the accompanying description.

Example 1: The protective effect of ASU preparation on human skin against PMA induced apoptosis

Human cultured foreskin fibroblasts were incubated with apoptosis induced stimuli, PMA (100nM) for 15 min after cell pretreatment with ASU in a concentration range of 2-10μg/ml for 1h (treatment group), or without cell pretreatment (negative control group). The mitochondria activity was measured by fluorescent Mito TrackerCMXRos incorporation into the mitochondria. CMXRos fluorescence is proportional to potential across the mitochondria membrane (transmembrane potential) and to absolute amount of Mito Tracker in mitochondria. The results are expressed in Figure 1 as a percentage of the transmembrane potential of the positive control (non-treated) cells.

From Figure 1 it can be seen the CMXRos fluorescence was higher in the group pretreated with ASU, compared to the negative control group only incubated with PMA. There was thus a higher transmembrane potential in ASU pretreated cells. Pretreatment with the ASU complex minimized the negative effect of PMA.

Example 2: A further experiment of the protective effect of ASU preparation on human skin against the apoptosis induced stimuli PMA

Human cultured foreskin fibroblasts were incubated with apoptosis induced stimuli, PMA (100nM) for 15 min after cell pretreatment with ASU in a concentration range of 2-10μg/ml for 1h (treatment group), or without cell pretreatment (negative control group). The apoptosis was detected by cell staining with fluorescent dye SYTO16. The apoptotic cells exhibit reduced fluorescence with SYTO16 [6]. The results are expressed in Figure 2 as a percentage of the fluorescence of positive control (non-treated) cells.
From Figure 2 it can be seen that pretreatment with the ASU complex, resulted in an increase in fluorescence compared to untreated cells incubated with PMA. There was thus less apoptosis in ASU pretreated cells. The ASU complex therefore minimized the negative effect of PMA.

Example 3: The protective effect of ASU preparation against the apoptosis induced stimuli Calcium-ionophore A 23187 in human skin fibroblasts

Human cultured foreskin fibroblasts were incubated with apoptosis induced stimuli A 23187 (Ca$^{2+}$-ionophore) (10nM) for 15 min either after cell pretreatment with ASU in a concentration range of 2-10 µg/ml for 1h (treatment group) or without pretreatment (negative control group). The apoptosis was detected by cell staining with fluorescent dye SYTO16. The results are shown in Figure 3 and are expressed as percentage of fluorescence compared to the positive control (non-treated) cells.

From Figure 3 it can be seen there was less fluorescence in the negative control group than in the ASU pretreated group. This indicates that pretreatment with ASU complex minimized the negative effect (apoptosis development) of the Ca-ionophore.

Example 4: The protective effect of ASU preparation against Calcium-ionophore A 23187 and PMA induced decrease of intracellular ATP concentration in hamster fibroblasts.

Hamster BHK line fibroblasts were pretreated with ASU in a concentration range 2-10µg/ml for 1h followed by treatment with Ca$^{2+}$-ionophore (10µM ) or PMA (100 nM) for 1 h or without pretreatment (negative control group). The effect on the intracellular synthesis of ATP was measured by ATP bioluminescence (Figure 4).

From Figure 4 it can be seen that there was augmentation of intracellular ATP concentration in the cells treated with 2µg/ml ASU. In the cells incubated with Ca$^{2+}$-ionophore or PMA, there was an increase in ATP concentration with pretreatment with ASU 2 and 10µg/ml. The effect was more notable when the lower concentration of 2µg/ml ASU was used. It was therefore shown that cell pretreatment with ASU protected the cells against the decrease in ATP concentration caused by the apoptotic stimuli Ca$^{2+}$ ionophore and PMA.
Example 5: The protective effect of ASU preparation against Ca\textsuperscript{2+} ionophore A 23187 and PMA induced decrease in DNA synthesis in hamster fibroblasts

Hamster BHK line fibroblasts were pretreated with ASU in a concentration range 2-10\mu g/ml for 1 h followed by treatment with Ca\textsuperscript{2+} ionophore (10\mu M) (treatment group), or without treatment (negative control group). DNA synthesis was assayed after 4 h incubation of the cells with \[^{3}\text{H}]-\text{thymidine} by monitoring \[^{3}\text{H}]-\text{thymidine} incorporation into the acid insoluble fraction.

The effect of ASU on DNA synthesis in cells treated with ASU and Ca\textsuperscript{2+} ionophore can be seen in Figure 5. It can be seen from Figure 5 that there was higher DNA synthesis in the ASU pretreated fibroblasts than in the negative control. The ASU protected the cells from the Ca\textsuperscript{2+} ionophore A 23187 induced decrease in DNA synthesis.

Example 6: Preparation of a non-ionic oil-in-water emulsion cream composition containing ASU complex for topical administration

The non-ionic oil-in-water emulsion comprised 37% oil phase (w/w of the composition). The oil phase contained, wheat germ oil, polyunsaturated fatty acids, vitamins A, E and F, Ascorbyl Palmitate and anti-oxidants. The water phase was 62.99% (w/w) and contained a mixture of water, natural plant extracts, humectants and non-ionic emulsifiers. The concentration of the ASU complex was 0.01% (w/w). The cream was prepared by an inverse emulsion process by addition of the water phase to the oil phase. The ASU complex was predissolved into the oil phase and the non-ionic emulsifiers were predissolved into the water phase. Both phases were preheated to 75\degree C. The emulsification was produced by a high speed homogenizer for 20 minutes, continued for another 60 minutes by a low-speed planetary mixer.

Example 7: Preparation of a lipophilic natural oil composition containing ASU complex for topical administration

The lipophilic natural oil composition comprised a mixture of ASU complex, jojoba oil, gamma linolenic acids, vitamins A, E and F, anti-oxidants and propylene glycol and dipelargonate ethoxydiaglycol. The ASU complex was present in a
concentration of 0.05% (w/w). The concentration of natural vegetable oils was 70% (w/w). The propylene glycol dipelargonate was present in a concentration of about 1.60% (w/w) and ethoxydiacyglycol was present in a concentration of about 0.4% (w/w). The additional components were present in a concentration of 27.95% (w/w).

The lipophilic natural oil composition was prepared by premixing the ASU complex in a part of the natural oils, followed by addition of the premix to the remaining components of the preparation.

Example 8: General Method of preparation of a gelatin capsule containing ASU complex for oral administration

The gelatin capsule ASU formulation is made by premixing the components, the ASU complex, vitamins, anti-oxidants and diluents at a suitable temperature, depending on the components. Each gelatin capsule contains from about 200mg to about 250mg of this mixture.

Example 9: A gelatin capsule ASU composition for oral administration

The ASU composition was prepared by mixing the ASU complex and antioxidants in the amounts listed below at a temperature of about 40°C. The gelatin capsule contained about 0.250g of this mixture.

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>AMOUNT %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASU complex</td>
<td>0.01</td>
</tr>
<tr>
<td>DL-α-Tocopherol</td>
<td>0.02</td>
</tr>
<tr>
<td>G-Palmitoyl-L-Ascorbic Acid</td>
<td>0.01</td>
</tr>
<tr>
<td>Diluent</td>
<td>99.96</td>
</tr>
</tbody>
</table>

Example 10: ASU composition for oral administration

The ASU composition was prepared by premixing the ASU complex, and antioxidants in the amounts listed below in part of the oil at a temperature of about 40°C. This was followed by continued addition of the premix to the remaining oil.
The ASU composition for oral administration was also made replacing the
soya oil with one or more of the following oils: corn oil, sunflower oil, avocado oil,
sesame oil, cottonseed oil, wheat germ oil, peanut oil, palm oil, walnut oil, olive oil,
coconut oil, cocoa butter and animal fat.

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>AMOUNT % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASU complex</td>
<td>0.02</td>
</tr>
<tr>
<td>DL-α-Tocopherol</td>
<td>0.02</td>
</tr>
<tr>
<td>G-Palmitoyl-L-Ascorbic Acid</td>
<td>0.01</td>
</tr>
<tr>
<td>Soya oil</td>
<td>99.05</td>
</tr>
</tbody>
</table>

Example 11: Method of use of the ASU composition of Example 7
The composition of Example 7 can be used without any other active
ingredients. Optionally the ASU composition of Example 7 can be added to edible oils
in different foods, such as but not limited to salads and salad dressings, mayonnaise,
margarines, breads, confectionery, milk, meat, fish, or any vegetable substitute for
animal protein.

Example 12: Method of use of ASU complex for protection of the skin against
pollutants
The ASU complex of the present invention can be used for protection of the
skin against pollutants and for treatment of the skin for symptoms caused by exposure
to pollutants. Examples of pollutants include, but are not limited to atmospheric
factors, chemical pollutants and biological pollutants. Examples of atmospheric
factors that effect the skin include but are not limited to radiation such as UV radiation
from the sun, ozone, acid rain and extreme temperatures. Chemical and biological
pollutants include pollutants from cars, industry, free radicals, cleaning materials,
cosmetics, drugs and toxins. Exposure of a subject to pollutants can result in
development of abnormal skin conditions, such as skin aging, skin irritation,
dermatitis and low skin humidity.
The ASU complex of the present invention can be administered as a preventative measure or used for the therapeutic activity after exposure to any of the aforementioned pollutants.

Dosing is dependent on the responsiveness of the subject to the ASU complex. The ASU composition is effective in an amount of from about 1µg/ml to about 10µg/ml and preferably from about 1µg/ml to about 5µg/ml. A formulation containing the ASU composition preferably contains from about 0.01% to about 0.15% ASU and more preferably from about 0.01% to about 0.05% ASU. However higher or lower doses are possible. The dose and frequency of dosing would be dependent on the responsiveness of the subject. Persons of ordinary skill in the art can easily determine optimum dosages, dosing methodologies and repetition rates.

Example 13: Method of use of ASU complex for general anti-aging
The ASU complex of the present invention can be used for a general cell anti-aging effect.

The ASU complex of the present invention can be administered as a preventative measure in a young subject or used for the therapeutic activity after symptoms of aging are apparent. Preferred routes of administration of the ASU complex are oral and topical.

Dosing is dependent on the responsiveness of the subject to the ASU complex. The ASU composition is effective in an amount of from about 1µg/ml to about 10µg/ml and preferably from about 1µg/ml to about 5µg/ml. A formulation containing the ASU composition preferably contains from about 0.01% to about 0.15% ASU and more preferably from about 0.01% to about 0.05% ASU. However higher or lower doses are possible. The amount received by the subject is controlled. For example, the dose and frequency of dosing would be dependent on the responsiveness of the subject. Persons of ordinary skill in the art can easily determine optimum dosages, dosing methodologies and repetition rates.

Example 14: Method of use of ASU for prevention of apoptosis
Apoptosis may be induced by numerous pathological and physiological factors, such as cell aging and pollutants.
The ASU complex of the present invention can be administered as a preventative measure or used for the therapeutic activity after exposure to any apoptosis inducing factor. For example, a subject exposed to pollution, may administer the ASU composition of the present invention to prevent apoptosis.

Dosing is dependent on the responsiveness of the subject to the ASU complex. The ASU composition is effective in an amount of from about 1μg/ml to about 10μg/ml and preferably from about 1μg/ml to about 5μg/ml. A formulation containing the ASU composition preferably contains from about 0.01% to about 0.15% ASU and more preferably from about 0.01% to about 0.05% ASU. However higher or lower doses are possible. The amount received by the subject is controlled. For example, the dose and frequency of dosing would be dependent on the responsiveness of the subject. Persons of ordinary skill in the art can easily determine optimum dosages, dosing methodologies and repetition rates.

Example 15: Various compositions of ASU

Topical and oral administration are the preferred routes of administration. Suitable compositions for topical administration include, but are not limited to a gel, cream, mousse, lotion, oil, hydrophyllic oil or other suitable formulation. Preferred compositions for topical administration are a cream or lipophilic natural oil. The cream is preferably a non-ionic oil-in water emulsion.

Compositions for oral administration, can be in a form that include powders or granules, suspensions, emulsions or solutions in water or non-aqueous media, oil or fat, sachets, capsules, tablets, gelcaps, food additives and sustained release formulations. A food additive containing the ASU complex can optionally be used as an additive in edible oils in foods such as but not limited to bread, baking products, biscuits, crackers, dairy products, cakes, chocolate and food fats. Thickeners, diluents, flavorings, vitamins dispersing aids, emulsifiers or binders may be desirable.

A preferred composition for topical administration is a non-ionic oil-in-water emulsion. The non-ionic oil-in-water emulsion composition preferably comprises from about 30% to about 80% (w/w) oil phase, most preferably from about 35% to about 40% (w/w). The water phase is preferably in a range of from about 19% to about 70% (w/w) and most preferably from about 59% to about 65% (w/w). The
concentration of the ASU complex is preferably from about 0.01% to about 0.15% (w/w) and most preferably from about 0.01% to about 0.05%. The oil phase is preferably comprised of at least one or a mixture of natural vegetable oils, polyunsaturated fatty acids, vitamins A, E and F, Ascorbyl Palmitate, anti-oxidants and other suitable components. The mixture of natural vegetable oils are preferably, but not limited to jojoba oil, avocado oil, soybean oil, sesame oil, rice oil, wheat germ oil or any other suitable natural vegetable oil. The water phase of the cream composition is preferably comprised of a mixture of water, natural plant extracts, humectants, non-ionic emulsifiers and other suitable components.

The cream formulation can be optionally administered by topically applying onto the facial skin, neck, scalp, around the eyes and ton the body skin, preferably but not limited to twice daily. Application is especially preferable before exposure of the face and body skin to a hostile environment of toxins, pathogens, UV radiation, negative environmental factors and pollutants.

The lipophilic natural oil composition preferably comprises a mixture of ASU complex, natural vegetable oils, polyunsaturated fatty acids, vitamins A, E and F, anti-oxidants, penetration enhancers and other suitable components. The ASU complex is preferably present in a concentration in the range of from about 0.01% to about 0.15% (w/w) and more preferably from about 0.01% to about 0.05% (w/w). The concentration of natural vegetable oils is preferably from about 20% to about 80% (w/w) and most preferably from about 65% to about 75% (w/w). The natural vegetable oils are preferably comprised of one or more of wheat germ oil, sesame oil, soybean oil, avocado oil, olive oil and rice oil. The fatty acids are preferably one or more of linoleic acid, linolenic acid, omega 3, gamma linolenic and arachidonic acid-omega 6 or mixtures thereof. The penetration enhancers are preferably present in a concentration of from about 0.5% to about 2.0% (w/w) and most preferably in a concentration of 2.0% (w/w). Preferred penetration enhancers include, but are not limited to propylene glycol dipelargonate and ethoxydiacyglycol. The propylene glycol dipelargonate is preferably present in a concentration of about 1.60% (w/w) and ethoxydiacyglycol is preferably present in a concentration of about 0.4% (w/w).

Additional suitable components are optionally present in a range preferably from about 19% to about 80% (w/w).
The lipophilic natural oil formulation can be optionally administered by topically applying onto the facial skin, neck, lips, scalp, around the eyes and on the body skin, preferably but not limited to twice daily. Application is especially preferable before exposure of the face and body skin to a hostile environment of toxins, pathogens, UV radiation, negative environmental factors and pollutants.

Preferably, once the goal of treatment is achieved, treatment can be stopped until the signs and symptoms reappear.

It will be appreciated that the above examples and descriptions are intended only to serve as examples, and that many other embodiments are possible within the spirit and the scope of the present invention.
REFERENCES CITED


WHAT IS CLAIMED IS:

1. A composition for reducing cell apoptosis, comprising an amount of an avocado and soybean unsaponifiable component effective in reducing cell apoptosis.

2. The composition of claim 1, wherein said effective amount of an avocado and soybean unsaponifiable component is in a range of from about 1µg/ml to about 10µg/ml.

3. The composition of claim 1, wherein said avocado and soybean unsaponifiable component is in a range of from about 1µg/ml to about 5µg/ml.

4. The composition of claim 1, wherein said composition comprises from about 0.01% to about 0.15% of said avocado and soybean unsaponifiable component.

5. The composition of claim 1, wherein said composition comprises from about 0.01% to about 0.05% of said avocado and soybean unsaponifiable component.

6. The composition of claim 1, wherein said avocado and soybean unsaponifiable component includes at least one item selected from the group consisting of an avocado unsaponifiable and a soybean unsaponifiable.

7. The composition of claim 1, wherein said avocado and soybean unsaponifiable component is a lipidic furan derived from ASU.

8. The composition of claim 1, wherein said avocado and soybean unsaponifiable component is a mixture of avocado unsaponifiables and soybean unsaponifiables.

9. The composition of claim 1, wherein said avocado and soybean unsaponifiable component is a lipid furan.
10. The composition of claim 1, further comprising one or a mixture of Ascorbyl Palmitate, fragrance, thickeners, herbal extracts, vitamins, dispersing agents, emulsifiers, binders, colorants, coenzyme-Q, anti-oxidants, natural vegetable oils and polyunsaturated fatty acids.

11. The composition of claim 1, wherein said composition is administered orally.

12. The composition of claim 11, wherein said composition is in a form selected from the group consisting of a powder, granules, a suspension, an emulsion, a solution in water, a solution in a non-aqueous media, an oil, a fat, a sachet, a capsule, a tablet, a gelcap, a food additive and a sustained release formulation.

13. The composition of claim 11, further comprising vitamins, anti-oxidants and a diluent.

14. The composition of claim 11, further comprising vitamins, anti-oxidants and at least one oil.

15. The composition of claim 1, wherein said composition is administered topically.

16. The composition of claim 15, wherein said composition is in the form of a cream.

17. The composition of claim 16, wherein said cream is a non-ionic oil-in water emulsion.

18. The composition of claim 17, further comprising an oil phase and a water phase.
19. The composition of claim 18, wherein said oil phase is selected from the group consisting of one or a mixture of at least one oil, poly-unsaturated fatty acids, vitamins A, E and F, ascorbyl palmitate and anti-oxidants.

20. The composition of claim 18, wherein said water phase is selected from the group consisting of water, plant extracts, humectants and non-ionic emulsifiers.

21. The composition of claim 15, wherein said composition is in the form of lipophilic natural oil.

22. The composition of claim 21, further comprising at least one item selected from the group consisting of a vegetable oils, a polyunsaturated fatty acid, vitamin A, vitamin E, vitamin F, an anti-oxidant and a penetration enhancer.

23. The composition of claim 22, wherein said penetration enhancers include propylene glycol di-pelargonate and ethoxydiaglycol.

24. The composition of claim 22, wherein said vegetable oil includes at least one item selected from the group consisting of wheat germ oil, sesame oil, soybean oil, avocado oil, olive oil and rice oil.

25. The composition of claim 22, wherein said polyunsaturated fatty acid includes at least one item selected from the group consisting of linoleic acid, linolenic acids-omega 3, gamma linolenic acid and arachidonic acid-omega 6.

26. A composition for protection of the skin against pollutants, comprising an effective amount of an avocado and soybean unsaponifiable component.

27. The composition of claim 26, wherein said pollutant is selected from the group consisting of radiation, sun radiation, ozone, acid rain, extreme
temperatures, transport pollutants, industry pollutants, cleaning materials, cosmetics, drugs, toxins and combinations thereof.

28. The composition of claim 26, wherein said avocado and soybean unsaponifiable component is in a range of from about 1μg/ml to about 5μg/ml.

29. The composition of claim 26, wherein said composition comprises from about 0.01% to about 0.05% of said avocado and soybean unsaponifiable component.

30. The composition of claim 26, wherein said avocado and soybean unsaponifiable component is an avocado unsaponifiable.

31. The composition of claim 26, wherein said avocado and soybean unsaponifiable component is a soybean unsaponifiable.

32. The composition of claim 27, wherein said avocado and soybean unsaponifiable component is a lipidic furan derived from ASU.

33. The composition of claim 27, wherein said avocado and soybean unsaponifiable component is a lipid furan.

34. The composition of claim 27, wherein said avocado and soybean unsaponifiable component is a mixture of avocado unsaponifiables and soybean unsaponifiables.

35. A composition for retarding cellular aging, comprising an amount of avocado and soybean unsaponifiables effective in retarding cellular aging.

36. The composition of claim 35, wherein said avocado and soybean unsaponifiable component is in a range of from about 1μg/ml to about 5μg/ml.
37. The composition of claim 35, wherein said composition comprises from about 0.01% to about 0.05% of said avocado and soybean unsaponifiable component.

38. The composition of claim 35, wherein said avocado and soybean unsaponifiable component is an avocado unsaponifiable.

39. The composition of claim 35, wherein said avocado and soybean unsaponifiable component is a soybean unsaponifiable.

40. The composition of claim 35, wherein said avocado and soybean unsaponifiable component is a lipidic furan derived from ASU.

41. The composition of claim 35, wherein said avocado and soybean unsaponifiable component is a mixture of avocado unsaponifiables and soybean unsaponifiables.

42. The composition of claim 35, wherein said composition is administered orally.

43. The composition of claim 35, wherein said composition is administered topically.

44. A method of protecting the skin from damage, comprising the step of administering a composition of an avocado and soybean unsaponifiable component on skin cells.

45. The method of claim 44, wherein said damage is damage from aging.

46. The method of claim 44, wherein said damage is damage from pollutants.
47. The method of claim 44, wherein said damage is damage from apoptosis.

48. The composition of claim 26, further comprising natural vegetable oils.

49. The composition of claim 35, further comprising natural vegetable oils.

50. The composition of claim 26, wherein said composition is a food additives.

51. The composition of claim 35, wherein said composition is a food additives.

52. The composition of claim 26, wherein said composition is in the form of a non-ionic oil-in water emulsion comprising an oil phase and a water phase and wherein said oil phase is poly-unsaturated fatty acids.

53. The composition of claim 35, wherein said composition is in the form of a non-ionic oil-in water emulsion comprising an oil phase and a water phase and wherein said oil phase is poly-unsaturated fatty acids.

54. The composition of claim 26, wherein said composition is in the form of a non-ionic oil-in water emulsion comprising an oil phase and a water phase and wherein said water phase is plant extracts.

55. The composition of claim 35, wherein said composition is in the form of a non-ionic oil-in water emulsion comprising an oil phase and a water phase and wherein said water phase is plant extracts.
56. The composition of claim 26, wherein said composition is administered topically further comprising vegetable oils.

57. The composition of claim 35, wherein said composition is administered topically further comprising vegetable oils.

58. The composition of claim 56, wherein said vegetable oils include rice oils.

59. The composition of claim 57, wherein said vegetable oils include rice oils.

60. The composition of claim 56, wherein said polyunsaturated fatty acids include linolenic acids-omega 3.

61. The composition of claim 57, wherein said polyunsaturated fatty acids include linolenic acids-omega 3.

62. A method useful in reducing cell apoptosis, the method comprising the step of administering an amount of an avocado and soybean unsaponifiable component which is effective in reducing cell apoptosis.

63. The method of claim 62, wherein said step of administering refers to an administration route selected from the group consisting of topical administration and systemic administration.

64. A method useful in retarding cellular aging, the method comprising the step of administering an amount of avocado and soybean unsaponifiables effective in retarding cellular aging.
65. The method of claim 62, wherein said step of administering refers to an administration route selected from the group consisting of topical administration and systemic administration.
Figure 1. The protective effect of ASU pretreatment on PMA-induced decrease of CMXROS fluorescence in mitochondria.

Figure 2. The protective effect of ASU pretreatment on PMA-induced decrease of SYTO 16 fluorescence in cells.

Figure 3
Figure 5. The effect of ASU on DNA synthesis in cells treated with ASU and Ca²⁺-ionophore
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 35/78
US CL: 424/757

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)

U.S.: 424/757

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)

WEST, STN (CAS)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

Date of the actual completion of the international search
30 MARCH 2001

Date of mailing of the international search report
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