A method of using Purified Shilajit to promote steroidogenic activity in a mammal is provided.
REGULATION OF STEROIDOGENIC ACTIVITY BY USING PURIFIED SHILAJIT

TECHNICAL FIELD

[0001] The present invention relates to promoting steroidalogenic activity in the body of a mammal, including human, through the use of Shilajit.

[0002] BACKGROUND

[0003] Shilajit is composed of rock humus, rock minerals and organic substances that have been compressed by layers of rock mixed with marine organisms and microbial metabolites. It oozes out of the rocks in the Himalayas at higher altitudes ranging from 1,000–5,000 meters as black mass and is regarded as a mushroom (super-vitalizer) in Ayurveda, the traditional Indian system of medicine (Hinglaja, 1987; Chhabria et al., 1997). Shilajit contains fulvic acids as the main components along with dibenzo-alpha-pyrones ("DBPs") and dibenzo-alpha-pyrene chromoproteins.

[0004] Fulvic acid complex, derived from shilajit, is an assembly of naturally occurring low and medium molecular weight compounds comprising oxygenated dibenzo-alpha-pyrones (DBPs), both in reduced as well as in oxidized form, as the core nucleus, and acylated DBPs and lipids as partial structural units, along with fulvic acids ("FAs"). Fulvic acid complex material derived from alluvial sources lack DBPs; instead, the core nucleus of alluvial fulvic acid is comprised of benzoic acid.


[0007] As discussed, shilajit has been used to treat various ailments. It is also recommended as a performance enhancer. Fulvic acids (FAs) are reported to elicit many important roles in biological systems of plants, in animals as well as humans, including: (a) improvement of bioavailability of minerals and nutrients, (b) serve as electrolytes, (c) detoxification of toxic substances including heavy metals, (d) perform as antioxidants, and (e) improvement of immune function.

[0008] Furthermore, dibenzo-alpha-pyrones have been hypothesized to participate in the electron transport inside the mitochondria, thus facilitating production of more ATP, leading to increased energy. Thus, shilajit is found to increase energy, among other beneficial qualities.

[0009] In view of the above, it would be desirable to provide a method of using shilajit for improvement of mitochondrial function thus increasing energy in a human or animal. If a way could be found to stimulate steroidalogenic gene expression related to skeletal muscle activity to provide increased energy using Shilajit, this would provide a valuable contribution to the medical and nutritional arts.

SUMMARY

[0010] An objective of the present invention is to develop a method of using Shilajit for promoting steroidalogenic activity in the body of a mammal, for example, a human.

[0011] A method for promoting steroidalogenic activity in a mammal is provided, comprising administering to the mammal in need of such treatment an effective amount of a purified Shilajit, wherein energy levels in the mammal are increased.

DETAILED DESCRIPTION

[0012] In one embodiment a gene expression study was conducted on the skeletal muscle of mice with Shilajit, 3,8-dihydroxy-dibenzo-alpha-pyrones (3,8-OH2-DBP), and placebo to determine the effect of these compounds on expression of genes related to skeletal muscle activity.

[0013] In another embodiment, a human clinical study was conducted with supplementation of Purified Shilajit for 8 weeks and skeletal muscle tissue was analyzed for gene expression.

[0014] It is contemplated that the compositions used herein may be administered advantageously in a mammal for inducing or promoting steroidalogenic activity. As used herein, a mammal may include, but is not limited to, a human, a dog, a horse, or a cat.

[0015] Materials: Purified Shilajit (PrimaVie®, Natreon, Inc., New Brunswick, N.J.) is a standardized dietary supplement ingredient extracted and processed from Shilajit bearing rocks, containing not less than about 50% by weight fulvic acids (FAs), at least about 10% by weight dibenzo-alpha-pyrene chromoproteins, and at least 0.3%, or more, by weight dibenzo-alpha-pyrones (DBPs).


[0017] Procedure for Studies in Mice Using Shilajit and DBPs:

[0018] Three groups of adult mice (n=8) were intragastrically supplemented with purified Shilajit (PS), 3,8-(OH2)-DBP, or placebo for 12 weeks. At the end of week 12, skeletal muscle tissue was harvested for gene profiling. Some tissue was stored for histology and HPLC analysis.

[0019] The control group of mice received DMSO in corn oil while the PS group received 100 mg of purified Shilajit/kg
body weight of mice, dissolved in water and the DBP group received 10 mg of 3,8-(OH)2-DBP/kg body weight of mice, dissolved in DMSO/corn oil.

[0020] At week 12, the following tissues were collected from mice: heart, lung, liver, brain, muscles, adipose tissue, skeletal muscle (vastus lateralis) and whole blood.

[0021] Procedure for Human Clinical Study:

[0022] 20 healthy volunteers were recruited following proper procedures for clinical studies. The baseline readings were taken and supplementation with Purified Shilajit 250 mg twice/day dosing was done for 8 weeks. Skeletal muscle biopsy was done and the tissue collected was subjected to gene chip analysis as described below.

[0023] Gene Expression Profiling using GeneChip® Assay


[0025] Results:

[0026] The following genes for steroid biosynthesis were up regulated or induced in mice by Shilajit:

[0027] (1) Hsd3b5: hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 5.

[0028] (2) StarD3: START domain containing 3. START domain-containing protein 3; STAR3, a.k.a. metastatic lymph node 64: MLN64. Expression of MLN64 leads to increased pregnenolone secretion and that steroidogenic activity resides in the C terminus of the protein. Pregnenolone, also known as 3α,5β-tetrahydroprogesterone (3α, 5β-THP), is an endogenous steroid hormone involved in the steroidogenesis of progesterone, mineralocorticoids, glucocorticoids, androgens, and estrogens, as well as the neuroactive steroids.

[0029] (3) Star: steroidogenic acute regulatory protein. Studies of Star in MA10 cells in the absence of hormone stimulation was sufficient to induce steroid production. This study concluded that Star is required for hormone-induced steroidogenesis.

[0030] (4) HSD3B1: 3-beta-hydroxysteroid dehydrogenase 1. 3-hydroxy-3-steroid dehydrogenase catalyzes the oxidation and isomerization of delta-5-3-beta-hydroxysteroid precursors into delta-4-ketosteroids, thus leading to the formation of all classes of steroid hormones.

[0031] The steroidogenic genes may be up-regulated by Shilajit in accordance with an embodiment of the present invention. Other steroidogenic genes that may be upregulated include, but are not limited to: androgen binding protein alpha (Abpa), and oxyester binding protein 2 (Osbp2).

[0032] 3,8-(OH)2-DBP did not show significant effect on steroidogenic activity in mice.

[0033] Table 1 shows fold change results for several representative steroidogenic genes, in accordance with a hierarchical gene cluster array showing genes up-regulated in mice treated with Purified Shilajit. In particular, these genes are demonstrating up-regulation or induction in muscle tissue with Purified Shilajit.

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Title</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsd3b5</td>
<td>hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 5</td>
<td>1.10</td>
</tr>
<tr>
<td>StarD3</td>
<td>START domain containing 3</td>
<td>1.22</td>
</tr>
<tr>
<td>Abpa</td>
<td>androgen binding protein alpha</td>
<td>1.15</td>
</tr>
<tr>
<td>Osbp2</td>
<td>oxyester binding protein 2</td>
<td>1.11</td>
</tr>
</tbody>
</table>

[0034] Table 2 shows fold change for several steroidogenic genes in the human clinical study. These results are based on gene chip analysis of skeletal muscle samples from three subjects out of a total of 20 subjects. In particular, these genes are demonstrating up-regulation or induction in muscle tissue with Purified Shilajit. Gene chip analysis of the samples from the remaining subjects is pending and the statistical significance of these results is expected to improve after the results when all 20 subjects are statistically analyzed.

[0035] Additional animal and/or human studies are expected to further demonstrate the steroidogenic activity of Shilajit.

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Title</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsd17b6</td>
<td>hydroxy-steroid dehydrogenase 1 (17-beta)</td>
<td>1.0015</td>
</tr>
<tr>
<td>Srd5a1</td>
<td>steroid-5a-reductase, alphaprotease (3-oxo-5alpha-steroid delta4-3-steroid dehydrogenase alpha1)</td>
<td>1.0024</td>
</tr>
<tr>
<td>Hsd17b3</td>
<td>hydroxy-steroid dehydrogenase 3</td>
<td>1.1010</td>
</tr>
<tr>
<td>Hsd17b1</td>
<td>hydroxy-steroid dehydrogenase 1</td>
<td>1.2007</td>
</tr>
<tr>
<td>Hsd17b8</td>
<td>hydroxy-steroid dehydrogenase 8</td>
<td>1.0015</td>
</tr>
<tr>
<td>Hsd17b2</td>
<td>hydroxy-steroid dehydrogenase 2</td>
<td>1.0027</td>
</tr>
<tr>
<td>Hsd17b7</td>
<td>hydroxy-steroid dehydrogenase 7</td>
<td>1.0024</td>
</tr>
<tr>
<td>Star</td>
<td>steroidogenic acute regulatory protein</td>
<td>1.0003</td>
</tr>
<tr>
<td>Hsd17b10</td>
<td>hydroxy-steroid dehydrogenase 10</td>
<td>1.0005</td>
</tr>
<tr>
<td>Srd5a2</td>
<td>steroid-5a-reductase, alphaprotease (3-oxo-5alpha-steroid delta4-3-steroid dehydrogenase alpha2)</td>
<td>1.0006</td>
</tr>
<tr>
<td>Hsd1b2</td>
<td>hydroxy-sterol dehydrogenase, 3beta-androstenedione 3alpha-reductase</td>
<td>1.0008</td>
</tr>
<tr>
<td>Sr1a1</td>
<td>steroid receptor RNA activator 1</td>
<td>1.0006</td>
</tr>
<tr>
<td>Hsd1l1</td>
<td>hydroxy-delta-4-steroid dehydrogenase alpha1</td>
<td>1.0003</td>
</tr>
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</table>

[0036] The product(s) of the present invention may be formulated into nutraceutical or pharmaceutical dosage forms comprising of tablets, capsules, powders, liquids, chews, gummies, transdermals, injectables, etc. using standard excipients and formulation techniques in the industry. The
product of the subject invention may be administered to the mammal orally in solid dosage form or by parenteral or transdermal administration.

[0037] While in the foregoing specification this invention has been described in relation to certain embodiments thereof, and many details have been put forth for the purpose of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein can be varied considerably without departing from the basic principles of the invention.

[0038] All references cited herein are incorporated by reference in their entirety. The present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof and, accordingly, reference should be made to the appended claims, rather than to the foregoing specification, as indicating the scope of the invention.

1. A method for promoting steroidogenic activity in a mammal, comprising administering to the mammal in need of such treatment an effective amount of a purified Shilajit, wherein energy levels in the mammal are increased.

2. The method of claim 1, wherein the compound is administered orally, intramuscularly, parenterally, or transdermally.

3. The method of claim 1, wherein the mammal is a human, a dog, a horse, or a cat.

4. The method of claim 1, wherein the purified Shilajit is present in a daily dosage of from about 1.0 mg/kg body weight of the mammal to about 20 mg/kg body weight of the mammal.

5. The method of claim 1, wherein energy levels are determined by muscular activity.

6. The method of claim 5, wherein the muscular activity is characterized by increased induction of one or more genes selected from the group consisting of: hsd3b5, stard3, star, abpa, osbp2, hsd7b6, srd5a1, hsd7b3, hsd7b1, hsd7b8, hsd12, hsd7b10, srd5a2, hsd3b2, hsd1b2, sra1, and hsd1.

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