Regulation of steroidogenic activity by using purified shilajit

Abstract

A method of using Purified Shilajit to promote steroidogenic activity in a mammal provided.

Classifications

A61K35/04 Tars; Bitumens; Mineral oils; Ammonium bituminosulfonate

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WO2015035358A1

WIPO (PCT)

Other languages: French

Inventor: Chandan K. Sen

Worldwide applications

2014 EP AU WO US JP WO ~~US~~ 2016 *HK*

Application PCT/US2014/054705 events

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Info: Patent citations (6), Non-patent citations (3), Cited by (2), Legal events, Similar documents, Priority and Related Applications

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Claims

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CLAIMS:

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, hsdl7b6, srd5al, hsdl7b3, hsdl7bl, hsdl7b8, hsdl2, hsdl7bl0, srd5a2, hsd3b2, hsdl lb2, sral, and hsdll .

Description

REGULATION OF STEROIDOGENIC ACTIVITY BY USING PURIFIED SHILAJIT

TECHNICAL FIELD

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

BACKGROUND

[0002] 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 1000- 5000 meters as black mass and is regarded as a maharasa (super- vitalizer) in Ayurveda, the traditional Indian system of medicine, dating back to 3500 B.C. Shilajit contains fulvic acids as the main components along with dibenzo-a-pyrones ("DBPs") and dibenzo-a-pyrone chromoproteins.

[0003] 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.

[0004] Thus, the active constituents of shilajit contain dibenzo-alpha-pyrones and related metabolites, small peptides (constituting non-protein amino acids), some lipids, and carrier molecules (fulvic acids). See, Ghosal, S., et al, "Shilajit Part 1 - Chemical constituents," J. Pharm. Sci. (1976) 65:772-3; Ghosal, S., et al, "Shilajit Part 7 - Chemistry of Shilajit, an immunomodulatory ayurvedic rasayana," Pure Appl. Chem. (IUPAC) (1990) 62: 1285-8; Ghosal, S., et al, "The core structure of Shilajit humus," Soil Biol. Biochem. (1992) 23:673- 80; and U.S. Patent Nos. 6,440,436 and 6,869,612 (and references cited therein); all hereby incorporated by reference herein.

[0005] Shilajit finds extensive use in Ayurveda, for diverse clinical conditions. For centuries people living in the isolated villages in Himalaya and adjoining regions have used Shilajit alone, or in combination with, other plant remedies to prevent and combat problems with diabetes (Tiwari, V.P., et al., "An interpretation of Ayurvedica findings on Shilajit," J. Res. Indigenous Med. (1973) 8:57). Moreover being an antioxidant it will prevent damage to the pancreatic islet cell induced by the cytotoxic oxygen radicals (Bhattacharya S.K., "Shilajit attenuates streptozotocin induced diabetes mellitus and decrease in pancreatic islet superoxide dismutase activity in rats," Phytother. Res. (1995) 9:41-4; Bhattacharya S.K., "Effects of Shilajit on biogenic free radicals," Phytother. Res. (1995) 9:56-9; and Ghosal, S., et al., "Interaction of Shilajit with biogenic free radicals," Indian J. Chem. (1995) 34B:596- 602). It has been proposed that the derangement of glucose, fat and protein metabolism during diabetes, results into the development of hyperlipidemia. In one study, Shilajit produced significant beneficial effects in lipid profile in rats (Trivedi N.A., et al., "Effect of Shilajit on blood glucose and lipid profile in alloxan-induced diabetic rats," Indian J. Pharmacol. (2004) 36(6):373-376).

[0006] 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.

[0007] Furthermore, dibenzo-a-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.

[0008] 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 steroidogenic 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

[0009] An objective of the present invention is to develop a method of using Shilajit for promoting steroidogenic activity in the body of a mammal, for example, a human.

[0010] A method for promoting steroidogenic 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

[0011] In one embodiment a gene expression study was conducted on the skeletal muscle of mice with Shilajit, 3,8-dihydroxy-dibenzo-a-pyrone (3,8-$(OH)_2$-DBP), and placebo to determine the effect of these compounds on expression of genes related to skeletal muscle activity. [0012] 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.

[0013] It is contemplated that the compositions used herein may be administered advantageously in a mammal for inducing or promoting steroidogenic activity. As used herein, a mammal may include, but is not limited to, a human, a dog, a horse, or a cat.

[0014] Materials: Purified Shilajit (PrimaVie ®, Natreon, Inc., New Brunswick, New Jersey) 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-a-pyrone chromoproteins, and at least 0.3%>, or more, by weight total dibenzo-a-pyrones (DBPs).

[0015] 3,8-$(OH)_2$-DBP (99.0% pure, Natreon, Inc., New Brunswick, NJ).

[0016] Procedure for Studies in Mice Using Shilajit and DBPs:

[0017] Three groups of adult mice (n=8) were intragastrically supplemented with purified Shilajit (PS), 3,8-$(OH)_2$-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.

[0018] 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.

[0019] At week 12, the following tissues were collected from mice: heart, lung, liver, brain, muscles, adipose tissue, skeletal muscle (vastus lateralis) and whole blood.

[0020] Procedure for Human Clinical Study:

[0021] 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.

[0022] Gene Expression Profiling using GeneChip® Assay

[0023] Affymetrix GeneChip® technology (Affymetrix, Santa Clara, California) was used for transcriptome profiling of skeletal muscle tissue. Gene chip assays were performed in accordance with the following references: Roy, S., Biswas, S., Khanna, S., Gordillo, G., Bergdall, V., Green, J., Marsh, C.B., Gould, L.J., Sen, C.K., "Characterization of a preclinical model of chronic ischemic wound," Physiol. Genomics (2009) May 13;37(3):211-24; Roy, S., Khanna, S., Rink, C, Biswas, S., Sen, C.K., "Characterization of the acute temporal changes in excisional murine cutaneous wound inflammation by screening of the wound-edge transcriptome," Physiol. Genomics (2008) Jul 15;34(2): 162-84; and Roy, S., Patel D, Khanna, S., Gordillo, G.M., Biswas, S., Friedman, A., Sen, C.K., "Transcriptome -wide analysis of blood vessels laser captured from human skin and chronic wound-edge tissue," Proc. Natl. Acad. Sci. USA (2007) Sep 4;104(36): 14472-7; herein incorporated by reference.

[0024] Results:

[0025] The following genes for steroid biosynthesis were up regulated or induced in mice by Shilajit:

[0026] (1) Hsd3b5: hydroxy-delta-5 -steroid dehydrogenase, 3 beta- and steroid delta- isomerase 5.

[0027] (2) Stard3: START domain containing 3. Start domain-containing protein 3; STARD3, 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 3 ,5P-tetrahydroprogesterone (3α,5β-THP), is an endogenous steroid hormone involved in the steroidogenesis of progestogens, mineralocorticoids, glucocorticoids, androgens, and estrogens, as well as the neuroactive steroids.

[0028] (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.

[0029] (4) HSD3B1 : 3-beta-hydroxysteroid dehydrogenase 1. 3-Beta-hydroxysteroid 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.

[0030] 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 oxysterol binding protein 2 (Osbp2).

[0031] 3,8-$(OH)_2$-DBP did not show significant effect on steroidogenic activity in mice.

[0032] 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 1 : Steroidogenic Genes Up Regulated in Mouse Skeletal Muscle by Shilajit

Gene Symbol	Gene Title	mean	p value
Hsd3b5	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 5	1.10	0.020
Stard3	START domain containing 3	1.22	0.024
Star	steroidogenic acute regulatory protein	1.22	0.010
Abpa	androgen binding protein alpha	1.15	0.002
Osbp2	oxysterol binding protein 2	1.11	0.026

[0033] 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.

[0034] Additional animal and/or human studies are expected to further demonstrate the steroidogenic activity of Shilajit.

TABLE 2: Steroidogenic Genes Up Regulated in Human Skeletal Muscle by Shilajit

Gene Symbol	Gene Title	Fold Change
HSD17B6	hydroxysteroid(17-beta)dehydrogenase6homolog (mouse)	1.0015
SRD5A1	steroid-5-alpha-reductase,alphapolypeptide1(3-oxo-5alpha-steroiddelta4-dehydrogenasealpha1)	1.0024
HSD17B3	hydroxysteroid(17-beta)dehydrogenase3	1.0010
HSD17B1	hydroxysteroid(17-beta)dehydrogenase1	1.0009
HSD17B8	hydroxysteroid(17-beta)dehydrogenase8	1.0032
HSD17B8	hydroxysteroid(17-beta)dehydrogenase8	1.0024
HSD17B8	hydroxysteroid(17-beta)dehydrogenase8	1.0022
HSD17B8	hydroxysteroid(17-beta)dehydrogenase8	1.0015
HSD17B8	hydroxysteroid(17-beta)dehydrogenase8	1.0027
HSD17B8	hydroxysteroid(17-beta)dehydrogenase8	1.0024
HSDL2	hydroxysteroiddehydrogenaselike2	1.0062
STAR	steroidogenicacuteregulatoryprotein	1.0003
HSD17B10	hydroxysteroid(17-beta)dehydrogenase10	1.0035
SRD5A2	steroid-5-alpha-reductase,alphapolypeptide2(3-oxo-5alpha-steroiddelta4-dehydrogenasealpha2)	1.0008
HSD3B2	hydroxy-delta-5-steroiddehydrogenase,3beta-andsteroiddelta-isomerase2	1.0006
HSD11B2	hydroxysteroid(11-beta)dehydrogenase2	1.0087
SRA1	steroidreceptorRNAactivator1	1.0006
HSDL1	hydroxysteroiddehydrogenaselike1	1.0093

[0035] 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.

[0036] 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.

[0037] 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.

Patent Citations (6)

Publication number	Priority date	Publication date	Assignee	Title
US5433363A *	1991-02-07	1995-07-18	Simhaee; Ebrahim	Plastic bag dispenser
Family To Family Citations				
US20050282781A1 *	2004-06-18	2005-12-22	Shibnath Ghosal	Compositions of stable bioactive metabolites of docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids
US8894993B2 *	2006-08-04	2014-11-25	Natreon Inc.	Mitochondria-targeted antioxidants
TW200904454A	2007-03-22	2009-02-01	Squibb Bristol Myers Co	Methods for treating obesity employing an SGLT2 inhibitor and compositions thereof
EP3278800B1 *	2010-12-23	2019-04-10	Amazentis SA	Compositions and methods for improving mitochondrial function and treating muscle-

				related pathological conditions
KR102163091B1	2012-06-27	2020-10-08	아마젠티스 에스에이	Enhancing autophagy or increasing longevity by administration of urolithins or precursors thereof

* Cited by examiner, † Cited by third party

Non-Patent Citations (3)

Title
BHATTACHARYYA, S ET AL.: "Beneficial Effect Of Processed Shilajit On Swimming Exercise Induced Impaired Energy Status Of Mice.", PHARMACOLOGYONLINE., vol. 1, 2009, pages 817 - 825 *
BISWAS, T ET AL.: "Clinical Evaluation Of Spermatogenic Activity Of Processed Shilajit In Oligospermia.", ANDROLOGIA., vol. 42, no. 1, February 2010 (2010-02-01), pages 48 - 56 *
YOSHIOKA, M ET AL.: "Effects Of Dihydrotestosterone On Skeletal Muscle Transcriptome In Mice Measured By Serial Analysis Of Gene Expression.", J MOL ENDOCRINOL., vol. 36, no. 2, April 2006 (2006-04-01), pages 247 - 259 *

* Cited by examiner, † Cited by third party

Cited By (2)

Publication number	Priority date	Publication date	Assignee	Title
Family To Family Citations				
JP2017210444A *	2016-05-26	2017-11-30	株式会社ダイセル	Lipase inhibitor containing urolithin
JP7046304B2 *	2017-07-20	2022-04-04	学校法人 中村産業学園	A method for producing a 5α-reductase inhibitor, and a method for producing a composition for preventing and treating alopecia or promoting hair growth.

* Cited by examiner, † Cited by third party, ‡ Family to family citation

Similar Documents

Publication	Publication Date	Title
Moreno et al.	2006	Effects of Arachis hypogaea nutshell extract on lipid metabolic enzymes and obesity parameters
Rom et al.	2012	Identification of possible cigarette smoke constituents responsible for muscle catabolism
Smirin et al.	2010	Sarcopoterium spinosum extract as an antidiabetic agent: in vitro and in vivo study
CN1964728A	2007-05-16	Compositions for correcting age related changes of a human endocrine system and methods for producing a pharmaceutical form bases on said compositions
Tinkov et al.	2015	Adipose tissue chromium and vanadium disbalance in high-fat fed Wistar rats
Latham et al.	2021	Vitamin D promotes skeletal muscle regeneration and mitochondrial health
Maghrani et al.	2004	Effects of an aqueous extract of Triticum repens on lipid metabolism in normal and recent-onset diabetic rats
Tan et al.	2014	Ginsenoside Rb1 improves energy metabolism in the skeletal muscle of an animal model of postoperative fatigue syndrome
Sabo et al.	2010	Pharmacodynamic action of a commercial preparation of the mushroom Coprinus comatus in rats
WO2015035358A1	2015-03-12	Regulation of steroidogenic activity by using purified shilajit
Jain et al.	2013	Metabolic effect of short term administration of Hoodia gordonii, an herbal appetite suppressant
Hocking et al.	2018	Administering fixed oral doses of curcumin to rats through voluntary consumption
Park et al.	2020	Dioscorea nipponica extracts enhance recovery from skeletal muscle atrophy by suppressing NF-κB expression
Jiajun et al.	2011	Regulation of organic nucleic acids and serum biochemistry parameters by dietary chromium picolinate supplementation in swine model
Tadjeddine et al.	2013	Antidiabetic effect of Anacyclus valentinus L. aqueous extract in normoglycemic and streptozotocin induced-diabetic rats
Ahn et al.	2007	Antiobesity effects of Isaria sinclairii by repeated oral treatment in obese Zucker rats over a 4-month period
Weli et al.	2021	The Effects of the Aqueous Extract of the Leaves of the Local Plant Eruca sativa on Lipid Profile and Some Minerals in the Blood of Male White Mice
Duangnin et al.	2018	In vitro investigation of Mucuna pruriens seed extracts to treat erectile dysfunction
Chavhan	2018	Study of Anti-psoriatic activity and Evaluation of E2A Gene Expression for Psoriasis Levels by RT-PCR of Thespesia populnea L. Methanolic Leaf Extract
Haider et al.	2021	Parabens as Endocrine Disrupting Chemicals and Their Association with Metabolic Disorders

US11382879B2	2022-07-12	Methods for improving physical performance and capsicum compositions used therein
JP5877731B2	2016-03-08	UCP-3 expression promoter
Ayotte et al.	1999	The detection of new synthetic drugs in athlete's urine samples
Almohanna et al.	2020	Role of Oral Supplements: When and How to Choose
Liu et al.	2014	A study of the pseudo-aldosteronism toxicity oflicorice flavonoid dispersible tablets

Priority And Related Applications

Priority Applications (2)

Application	Priority date	Filing date	Title
US201361875513P	2013-09-09	2013-09-09	*US Provisional Application*
US61/875,513		2013-09-09	

Applications Claiming Priority (1)

Application	Filing date	Title
US14/917,873	2014-09-09	Regulation of steroidogenic activity by using purified shilajit

Legal Events

Date	Code	Title	Description
2015-04-29	121	Ep: the epo has been informed by wipo that ep was designated in this application	**Ref document number**: 14842141 **Country of ref document**: EP **Kind code of ref document**: A1
2016-03-09	NENP	Non-entry into the national phase	**Ref country code**: DE
2016-03-09	WWE	Wipo information: entry into national phase	**Ref document number**: 14917873 **Country of ref document**: US
2016-10-05	122	Ep: pct application non-entry in european phase	**Ref document number**: 14842141 **Country of ref document**: EP **Kind code of ref document**: A1

Concepts

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Name	Image	Sections	Count	Query match
steroidogenetic		title,claims,abstract,description	18	0.000
biological regulation		title,description	2	0.000
Mammalia		claims,abstract,description	15	0.000
effects		claims,description	10	0.000
STARD3		claims,description	7	0.000
increased		claims,description	7	0.000
promoting		claims,description	5	0.000
body weight		claims,description	4	0.000
inductive effect		claims,description	4	0.000
compounds		claims,description	3	0.000
Felis catus		claims,description	2	0.000
muscular		claims	2	0.000
HSDL2		claims	1	0.000

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