Current Nutrition & Food Science, 2019, *15*, 104-120

REVIEW ARTICLE

Shilajit (Mumie): Current Status of Biochemical, Therapeutic and Clinical Advances

Tanuja Mishra[a], Harcharan S. Dhaliwal[a], Karan Singh[c] and Nasib Singh[a,b,*]

[a]*Department of Biotechnology, Eternal University, Baru Sahib-173101, Himachal Pradesh, India;* [b]*Microbiology Eternal University, Baru Sahib-173101, Himachal Pradesh, India;* [c]*Chemistry, Eternal University, Baru Sahib-173101, Himachal Pradesh, India*

Abstract: ***Background*:** Shilajit (mumie), a natural multi-component herbomineral ethnomedicinal food, is used as a traditional medicine for enhancing the quality of life and for management of health ailments in many countries of the world. Use of Shilajit as an adaptogen, aphrodisiac, rejuvenator and anti-aging substance is mentioned in many ancient texts. This review aims to provide comprehensive insights into its biochemical aspects, microbial role in biosynthesis, bioactivities and to establish correlation between traditional uses and scientifically validated research findings.

***Methods*:** Scientific literature and ethnopharmacological information were compiled from the published peer-reviewed articles, unpublished materials, thesis, books, patent databases, clinical trial registries and from the websites of research councils of traditional medicine. The scientific databases, thesis repositories and books databases were searched with keywords Shilajit, mumie, mumijo, salajeet, asphaltum, fulvic acid, dibenzo-alpha-pyrones *etc*.

***Results*:** Scientifically validated research and ancient texts suggest multifaceted benefits of Shilajit. It is endowed with anti-stress, memory and energy enhancing, antioxidant, anti-inflammatory, anti-diabetic, spermatogenic, neuroprotective, antiulcer and wound healing activities. These pharmacological effects are mainly attributed to the presence of humic acid, fulvic acid, dibenzo-α-pyrones, dibenzo-α-pyrones chromoproteins and trace elements.

***Conclusion*:** This review summarizes the traditional importance of Shilajit for the treatment and prevention of several acute and chronic diseases and health ailments. Despite numerous health claims, there are still major gaps in our understanding of its mechanism of action, variability in efficacy and toxicity profile. Therefore, a coordinated interdisciplinary approach is needed to establish the underlying mechanisms of action, comprehensive toxicological profile, pharmacokinetics parameters and effects on different organ systems. Regulatory and governmental impetus to basic and clinical research, safety testing and formulations quality control is warranted.

ARTICLE HISTORY

Received: May 19, 2017
Revised: August 01, 2017
Accepted: August 17, 2017

DOI:
10.2174/1573401313666170823160217

Keywords: Dibenzo-α-pyrones, fulvic acid, humic acid, revitalizer, Shilajit, traditional medicine.

1. INTRODUCTION

Traditional Medicine (TM) is an affordable and widely accessible mainstay of basic health needs for millions of people worldwide especially in Southeast Asia, Africa, Europe, Mediterranean region and Latin America [1]. Shilajit is one such natural product whose health applications are mentioned in sacred ancient texts as old as 3000 B.C. of many Asian and Mediterranean countries [2-4]. The word 'Shilajit' is derived from *shila* = rock and *jeet* = win (*Sanskrit*) which means 'Conqueror of mountains and destroyer of weakness' [3]. Shilajit is a pale-brown to blackish-brown, semi-hard and sticky resinous phytocomplex oozed from the layers of rocks in high altitude (1000-5000 m) mountains of Asia, Africa, Australia, Europe, Latin America and Middle-East [3-7]. It is also known as mumie, mumijo, asphaltum punjabinum, mineral pitch, arakul dshibal and Hajar-ul-musa [3, 4, 7]. The name 'mumiyo' finds its origin in Persia and Babylonia and is derived from a French word 'mum' which means wax or a soft balsamic resin [8]. Other names of Shilajit *i.e.* arakul-dzhibol (Arabian), brag-shun (Tibetan) and kao-tui (Myanmar) essentially mean sweat or juice or blood of mountains [4, 9] and its constituents are also found in Indian regional languages. It has many synonyms *i.e.* salajeet, dak-joon, shilajatu, shilaras, shilajita dathusara, shiladhatu, pahar-ki-khoon, pahar-ki-pasina, perangyum, uerangyum, kalmatam *etc*. [3, 4, 10]. Chinese Materia Medica pharmacological compendium dated back to 15th century Ming Dynasty documented the use of 'wujinsan', a fulvic

*Address correspondence to this author at the Department of Microbiology, Eternal University, Baru Sahib, Sirmaur- 173101, Himachal Pradesh, India; Tel: +91-8222836466; Email: kamalgollen@gmail.com

2212-3881/19 $58.00+.00

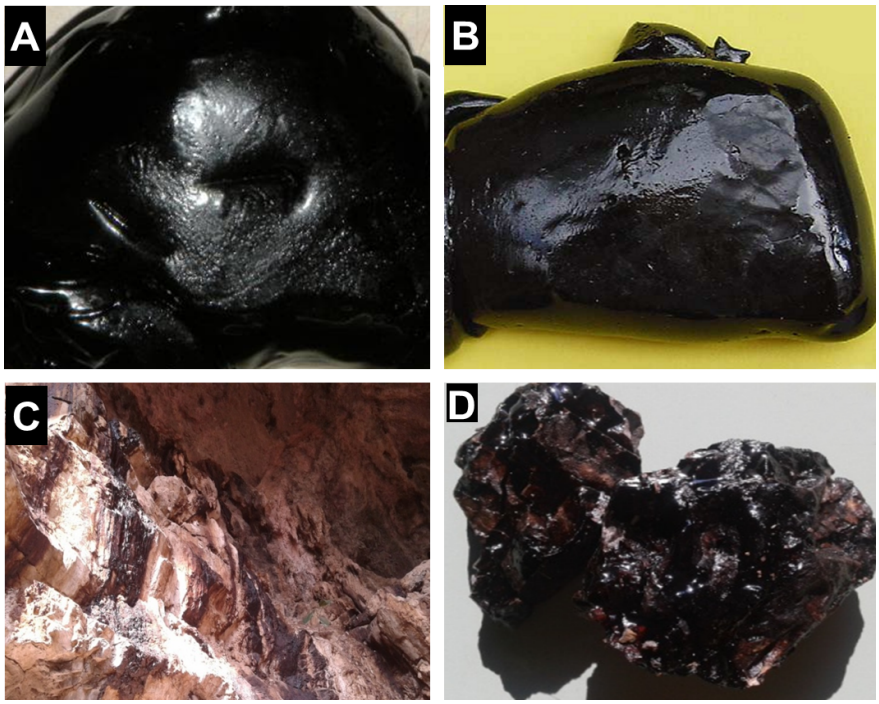

Fig. (1). General characteristics of Shilajit. (**A**) Processed Shilajit available in local market; (**B**) A sample of mumiyo from Kazakhstan (Source: Garedew *et al.* [8]; (**C**) Shilajit excreted from the mountains of Sirmaur, Himachal Pradesh in summer season, and (**D**) A Shilajit sample collected by our research group.

acid containing medicine in the treatment of genito-urinary infections and disorders [2]. Famous Iranian physician Avicenna wrote in Canon Medicinae that mumie possessed the ability to cure pimples and tumors [2]. Mumie was secretly provided to Russian military forces and athletes to enhance their physical performance, accelerate recovery from injuries and to decrease mental stress [7]. In Arabian region, Shilajit was considered a precious aromatic mountain balsam for the treatment of wounds [8]. Historical facts revealed that the knowledge related to Shilajit was kept secret by the families of high ranked physicians or health practitioners and was only verbally handed to blood relations only.

In Indian systems of medicine, Shilajit is considered a therapeutic medicine which has rejuvenating, revitalizing, anti-aging, aphrodisiac and longevity enhancing properties [3, 4, 7, 11]. Indian texts namely *Vedas, Charaka Samhita, Susruta Samhita*, *Rasaratna Samucchya* and *Bhava Prakash* contain description of Shilajit as a *rasayana* or maharasa for health management and enhancing the quality of life [3, 4, 12-15]. In Siddha System of Medicine which is practiced mainly in South India, the use of Shilajit as a natural mineral is described [4, 16, 17]. It is also utilized for preparing many drug formulations in Unani system of medicine [4]. In Himalayan region, Sowa Rigpa or Amchi system of medicine is practiced, in which, Shilajit (local name: Dak-Joon) is considered as an important mineral having anti-allergic, anti-inflammatory, neuroprotective, antidiabetic, aphrodisiac and immunity boosting effects [10, 18]. In India, Ministry of AYUSH (Ayurveda, Yoga, and Naturopathy, Unani, Siddha and Homeopathy) has initiated several programs to promote, strengthen and implement wide scale use of Indian traditional system of medicines for community-health and personal healthcare needs (www.ayush.gov.in).

2. GEOGRAPHICAL DISTRIBUTION

Shilajit is usually excreted and oozed from the rock crevices, caves and clefts on the walls of caves in remote mountain with an altitude of 1000-5000 m under specific environmental conditions *viz.* scorching sunlight, high pressure, presence of vegetation, minerals and extreme temperature in summers and winters [3, 4, 7, 17, 19]. It is an ethnomedicinal food customarily consumed by the native people living in the Himalayan mountain ranges and other parts of the world for the prevention and treatment of chronic diseases and general health maintenance [4, 7, 10, 20]. Shilajit is obtained from India (The Himalayas), Nepal, Bhutan, Chile (Andean mountains), China, Pakistan, Afghanistan, Algeria, Australia, Iran, Russia, Japan, Kazakhstan, Norway, Uzbekistan, Tajikistan, Yemen and some other countries and regions [2-4, 6-8, 21]. In India, it is found in the mountains of several states including Andhra Pradesh, Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, Madhya Pradesh and Uttarakhand [4, 19, 22-24].

3. PHYSICOCHEMICAL PROPERTIES OF SHILAJIT

3.1. Physical Properties

Natural unprocessed Shilajit has variable coloration ranging from pale yellow to black (Fig. **1**). It is highly viscous, semi-hard, resinous and tar-like substance having shiny surface, stale cow urine-like odour and pungent or astringent taste [2-4, 19]. Shilajit samples from different geographical regions sometimes exhibit micro-variations in the pH, coloration, odour and solubility, which might be attributed to deviations in geochemical conditions [2-4, 6, 17, 25]. Its pH varies from 6.2-8.2 and is soluble in water, alcohol and acetone. In Ayurveda, four varieties of Shilajit are described

Table 1. The main organic and inorganic constituents of Shilajit.

Major Category	Sub-Category		Constituents
Organic matter (60-80%)	Humic substances		Humic acid, Fulvic acid, Humin
	Non-humic substances	Coumarin derivatives	Dibenzo-α-pyrones, Dibenzo-α-pyrones chromoproteins
		Organic acids	Adipic acid, Benzoic acid, Ellagic acid, Hippuric acid, Succinic acid, Citric acid, Oxalic acid, Tartaric acid, Quinic acid, Mumic acids *etc*.
		Carbohydrates	Polysaccharides, lignins
		Proteins	Amino acids, peptides
		Lipids/steroids	Phenolic lipids, fatty acids, sterols
		Miscellaneous	Alkaloids, diterpenoids, triterpenes, tannoids, polymeric quinones, polyphenols, resins, waxes, carotenoids, indigoids, pyrocatechol, shilajitol, naphsilajitone, shilajityl acetate, shilanthranil, shilacatechol, vitamins, shilaxanthone,
Inorganic matter (20-40%)	Minerals		Si, Cu, Zn, Li, Al, Cr, Pb, Ag, Co, Hg, P, Cd, Br, Fe, Ca, Mg, K, As, Na, Cl, I, Mn, Mo and S.

namely lauha Shilajit (iron, blackish brown), savrana Shilajit (gold, red), rajat Shilajit (silver, white) and tamra Shilajit (copper, blue) [3, 6]. Among these, lauha Shilajit is most commonly available, whereas tamra and savrana Shilajit are rare [3, 19, 26, 27].

3.2. Biochemical Properties

The quest to determine the chemical constituents of Shilajit has remained a matter of extensive research but gained momentum in the 20th century. Shilajit is remarkably famous for its rich chemical composition. Shilajit contains both organic (humic and non-humic substances) and inorganic constituents, as shown in Table **1**. Although its composition varies from region to region, it is generally agreed that Shilajit consists of 60-80% organic matter and 20-40% minerals [4, 7, 9, 17]. Chemical analysis of crude Shilajit revealed the presence of Humic Acid (HA), Fulvic Acid (FA), Dibenzo-α-pyrones (DBPs) and Dibenzo-α-pyrones-chromoproteins (DCPs) as major biochemically relevant constituents [5, 6, 28, 29] (Fig. **2**).

3.2.1. Humic Substances

Humic substances are natural organic matter present in soil, sediments and rivers which are formed by either by secondary synthetic reactions [30, 31] or by progressive decomposition of organic compounds [32]. The humic contents of Shilajit exhibit considerable chemical resemblance with that of soil humus [29]. HA and FA are two major hydroxylated polyphenolic organic compounds present in Shilajit [2, 4, 7, 28]. These are chemically and physically heterogeneous with polyelectrolyte, colloidal and polydispersed characteristics [2, 6, 29].

HA is an amphiphilic macromolecule with higher MW (6500 Da) compared to FA but possessing less oxygen-containing functional groups [30, 33]. It is a complex aggregate with highly variable and undefined chemical composition, dark brown-black colour, insoluble in water at pH<2.0 but shows complete solubility at alkaline pH [25, 30]. These are enriched with functional groups such as carboxylic acids, quinones and phenols which are responsible for their antimicrobial, antioxidant and anti-inflammatory properties [25, 34, 35]. FA is a light yellow, water-soluble and low mol. wt. (700-2500 Da) organic humic substance having weak aliphatic and aromatic organic acids with several hydroxyl and carboxyl groups [6, 30, 36, 37]. It is considered a carrier molecule and powerful natural electrolyte [28, 38]. FA content of Shilajit showed region-specific variability and was measured to be about 21.4%, 19% and 15.5% from India, Russia and Pakistan, respectively [37]. Ghosal *et al.*, [29] reported 21.4% FA and 19.8% HA content in crude Shilajit collected from Kumaon Hills, India. Processed Shilajit, on the other hand, has 50-60% FA content [7, 39]. The presence of hydrophobic core, aromatic rings with lesser condensation, conjugated p-electrons and micropores of 200-1000 A˚ size in HA and FA, facilitates complex formation with minerals, ionic exchange and enhanced bioavailability of drugs [2, 40, 41]. FA showed nearly double oxygen content and much higher exchange capacity than HA which greatly enhances the bioavailability of trace minerals to living cells by facilitating cellular penetration [3, 37]. Its strong antioxidant action, along with the attributes of complement activation makes FA the main bioactive constituent of Shilajit [37, 38]. The available literature point the origin of HA and FA towards lignin [42], quinones [43] and reduced sugars-amino acids [33]. Humin is a macro-organic substance with high

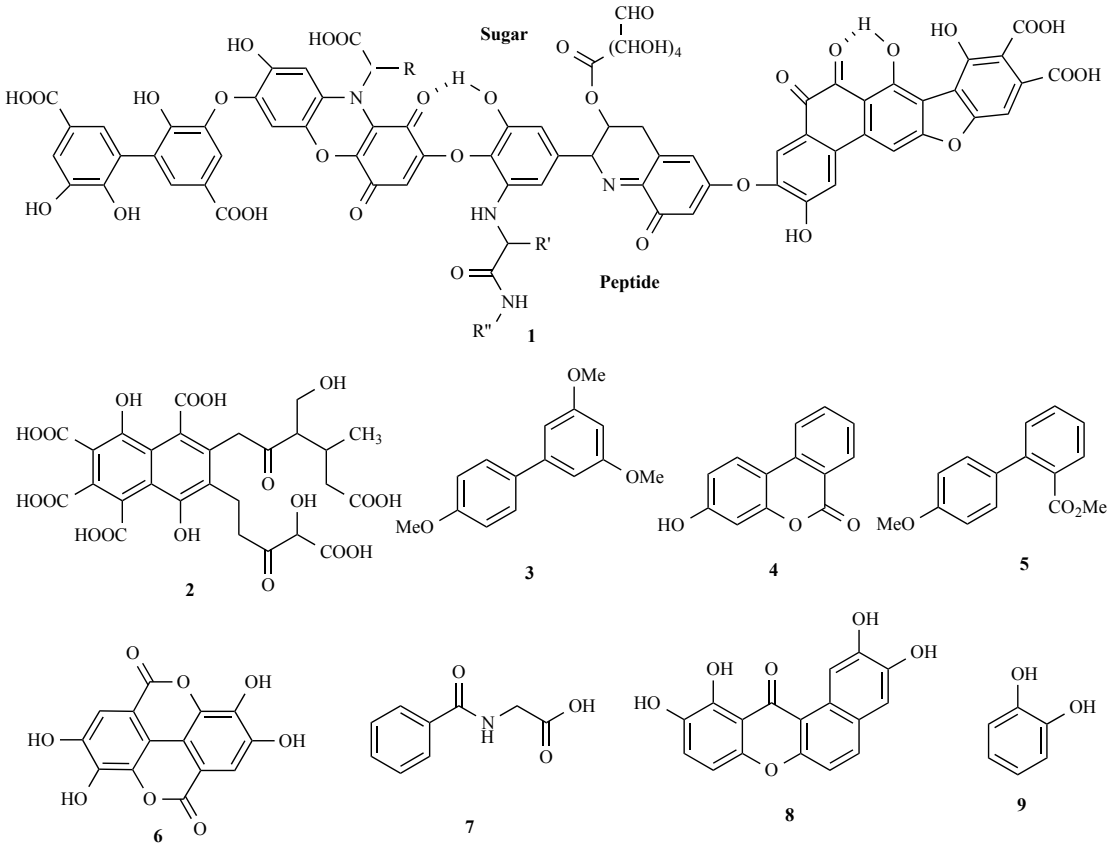

Fig. (2). The major chemical constituents of Shilajit. (**1**) Humic acid representative structure adapted from Stevenson [33]; (**2**) fulvic acid model structure proposed by Buffe; (**3-5**) dibenzo-α-pyrones structures adapted from Schepetkin *et al*. [2]; (**6**) ellagic acid; (**7**) hippuric acid; (**8**) shilaxanthone, and (**9**) pyrocatechol (adapted from Ali *et al*. [52]).

mol. wt. which represents water insoluble fraction of natural crude Shilajit. Ghosal *et al.* [29] reported about 41% humin content in crude Shilajit collected from India.

3.2.2. Non-humic Organic Constituents

DBPs (chemically 6H-dibenzo[b,d]pyran-6-ones) are a group of heptaketide coumarin derivatives that have a fused tricyclic nucleus. These secondary metabolites are produced by plants and microorganisms with more than 53 natural DBPs being reported [44]. A typical processed Shilajit product contains 0.3-0.4% DBPs [7, 39]. DCPs are proteinaceous derivatives of DBPs which also play a role in biochemical activity of Shilajit [45, 46]. Their amount varies from 10-30% in a processed Shilajit sample [7, 39]. The other non-humic organic compounds reported from Shilajit include benzoic acid, uronic acid, ellagic acid, eldagic acid, hippuric acid, triterpenes, 3,4-benzocoumarins, albuminoids, α -glyceryl ethers, phenolic lipids, fatty acids, amino acids, vitamins, resins, gums, ichthyol, albumins and sterols [2, 3, 5, 6, 17, 37, 47-50]. Phaechamud *et al.* [51] found higher level of the total phenolic compounds in Shilajit compared to coal tar. One report indicated the presence of pyrocatechol, shilajitol, naphsilajitone, shilajityl acetate, shilanthranil, shilacatechol and shilaxanthone [52] (Fig. **2**). Similarly, Kiren *et al.* [53] identified five mumic acids A, B, C, D and E (diterpenoids). Further, presence of lignins, polymeric quinones, polysaccharides, mycotoxins, toxic metals and reactive free radicals is also reported from Shilajit [3, 6, 29, 36].

3.2.3. Mineral Matter

Microelemental analysis revealed 20-40% mineral content in crude Shilajit [9, 48] (Table **1**), whereas in a typical processed Shilajit, this value is about 10-15% [2, 5, 7, 54] suggesting about 2-3 mg minerals per 200 mg of crude Shilajit. Chemical studies have indicated the presence of 40-85 different trace elements, although in trace amounts only [9, 55, 56]. The presence of Cu, P, Mg, Se, Zn, Li, Al, Cr, Pb, Ag, Co, Hg, Cd, Br, Fe, Ca, K, As, Na, Cl, I, Mn, Mo, S and Si is reported in Shilajit samples [2, 7, 8, 57]. Over 90% of this mineral content is contributed by Ca, P and Mg [54]. Some authors are of the view that selenium (Se) is responsible for anti-aging activities [27, 37]. Elemental analysis studies by Saper *et al.* [58] and Koch *et al.* [59] indicated the presence of arsenic and lead in some commercial Shilajit products. It is generally accepted that differences in the amounts of Fe, Cu and Ag account for variations in Shilajit's colour and probably in biological activities as well [3, 19].

4. ORIGIN AND BIOSYNTHESIS OF SHILAJIT

The mystery of origin and synthesis of Shilajit in nature have attracted the attention of several traditional medicine practitioners and researchers. Ancient Indian texts *Charaka Samhita*, *Susruta Samhita*, *Rasarangini* and *Dwarishtarang* claim that Shilajit is an exudate of latex gum-resin of plants which ooze from rock layers of mountains under high pressure and scorching heat [4, 5, 47, 60]. Various pioneers in

the field of Shilajit research described it as a substance derived from the centuries-long metamorphosis of fresh and fossilized remnants of vegetation humus admixed with minerals and organic substances in steep rocks mediated by frequent and extreme climatic variations like freezing, thawing, wetting and drying [2-4, 7, 11, 19]. Although concrete scientific evidences are lacking, but the most generally accepted views suggest the origin of Shilajit from long-term decomposition and humification processes of vegetation and rock materials [2, 5, 26, 28, 61] in which unique microbial flora and their metabolites play crucial roles [3, 6, 62]. Further, several reports indicate that it is derived from combination of vegetation, rock humus, marine organisms, rock minerals, organic substances and microbial metabolites which got compressed beneath the intense pressure of rock layers [3, 4, 7, 37, 63]. As the plant degradation and humification processes are believed to occur over centuries, Shilajit is essentially considered a millenary natural product similar to other natural humification products like peat, coal tar, sapropel *etc*. [2, 37, 48]. Some of its samples are considered to be about 3000 years old [61].

It is reported that Shilajit secreting rocks have several bryophytes (mosses, liverworts and hornworts) in their close vicinity. These include *Anthoceros, Asterella, Dumortiera, Fissidens, Marchantia, Pellia, Plagiochasma* and *Thuidium* [3, 4, 64]. The similarities in their metal and mineral composition (Cu, Ag, Zn, Fe, Pb) with that of Shilajit support their potential role in Shilajit formation [4, 65]. Angiosperm plants viz. *Euphorbia royleana Boiss, Trifolium repens, Caryopteris, Styrax officinalis, Ficus* spp. and *Rhus* spp. are also found in the vicinity of Shilajit exuding rocks and may play significant role in its formation [2, 3, 17, 28, 47, 66]. Further, the presence of latex in these plants and resinous nature of Shilajit also support these assumptions [26]. In addition, Hemadri [22] has suggested the possible role of plant species *Boswellia serrata, Commiphora caudate, Sterculia urens* and *Anogeissus latifolia* in the synthesis of gomutra Shilajit. The above mentioned observations show reasonable agreement with the facts mentioned in ancient texts of traditional system of medicines, and ayurvedic literature. However, concrete and repeatable evidences are still awaited to conclusively link a particular plant species with Shilajit formation under natural geological conditions. High potassium content (10.4 g/100 g) in raw mumiyo may be contributed by the nearby plants and their roots as latter contain high potassium levels [8]. UV-Vis spectroscopic analysis found the presence of porphyrin-like pigments and chlorin-rich chemical entities in crude mumie [48]. As chlorins and porphyrins are derived from chlorophyll, this further support the contribution of plant matter in the synthesis of humic components of Shilajit. Another important evidence in favour of plant origin of Shilajit is the presence of dibenzo[b,d]pyran-6-ones in plant family *Asparagaceae, Combretaceae, Cucurbitaceae, Cupressaceae, Euphorbiaceae, Fabaceae, Juglandaceae, Hypericaceae, Lythraceae, Myrtaceae, Onagraceae, Polygonaceae, Rosaceae, Salicaceae, Sapindaceae,* and *Tamaricaceae* [45, 47]. It is highly likely that DBPs and some other constituents of Shilajit are derived from plant matter by long-term biodegradation process [45].

5. MICROBIAL ASSOCIATION WITH SHILAJIT

It remains a matter of debate whether microorganisms are essentially involved in Shilajit synthesis or represent transient flora which accidently get into it just during sample collection or processing. Most of the currently available evidences points towards the most significant role of fungi in Shilajit formation among different microbial groups. These findings are corroborated by their saprophytic nature, versatile enzymatic machinery and better survival under environmental conditions encountered in Shilajit forming ecosystems. The contribution of microorganisms, mostly fungi in the formation of Andean Shilajit from fossilized plant matters was mentioned by Carrasco-Gallardo *et al.* [37] and Guzman-Martinez *et al.* [67]. Shilajit samples from Kumaon hills of Almora were found to harbour 19 species of fungi, majority of which belonged to order Moniliales. The associated mycoflora was dominated by *Aspergillus niger, Cladosporium oxysporum, Trichothecium roseum* and *Ulocladium chartarum* besides 15 other species [68]. In another study, Ghosal [19] reported *A. niger, A. ochraceus, A. sydowii, Fusarium moniliformae* and *Trichotehecium roseum* from rock Shilajit. Islam *et al.* [69] also found the association of *A. niger* with Shilajit. Intrinsic involvement of microbial flora in Shilajit formation has received further support by the fact that some species of *Alternaria, Acremonium, Arthrinium, Botryosphaeria, Cephalosporium, Chaetomium, Colletotrichum, Hyalodendriella, Nigrospora, Penicillium, Phialophora, Phomopsis, Preussia* and *Stagonospora* produce dibenzo[b,d]pyran-6 which may contribute to DBPs formation through biodegradative and secondary synthesis pathways [45]. It is also feasible that only the microbial species bestowed with exceptional adaptive characteristics can flourish under conditions characterized by extreme temperature variations, high pressure, low oxygen, and high metal contents. The role of bacteria towards Shilajit formation is not explicitly demonstrated so far but considering their rapid metabolic rates and diverse enzyme repertoire, these are certain to play an intimate role in Shilajit synthesis *e.g*. in rock mineralization. Moreover, seasonal fluctuations in the bacterial community dominance from thermophilic to psychrophilic and vice-versa cannot be ruled out. Algae may also be associated with Shilajit formation in some aspects. Cyanobacterium *Nostoc* sp. exists in symbiotic association with hornwort *Anthoceros* spp. which in turn present in close vicinity of Shilajit-excreting rocks [70]. There is a need have greater insights into the community structure, population dynamics and nutrient interactions among microbial species associated with Shilajit synthesis. Towards this goal, the advanced omics technologies can prove useful in ascertaining the microbiome of Shilajit.

6. TRADITIONAL IMPORTANCE AND BIOACTIVITIES OF SHILAJIT

6.1. As Traditional Medicine

Historically, it is evident that people living in the Himalayan region use to consume Shilajit, as a custom, either alone or in combination with other herbal preparations to prevent and treat many health conditions [3, 10, 11, 19]. Its therapeutic properties were well known in South-East Asia, Central Asia, Europe and Middle-East countries. It has been

considered a panacea in the four Indian systems of medicine [3, 7, 19]. It was prominently known for its adaptogenic, rejuvenating, aphrodisiac, revitalizing and anti-aging properties [3, 19]. It can enhance stamina, strength and relieves mental and physical stress [3, 12]. In ayurveda, it is recommended for both internal and external uses for weakness, sexual dysfunctions, neurological ailments, wound healing, ulcers, liver diseases, diabetes, anemia, bronchial asthma, gastrointestinal infections [11-13, 19]. Shilajit is also considered as medha rasayan, an enhancer of learning and memory [29, 37]. It was a highly recommended remedy for neurological disorders like amnesia, epilepsy, radiculitis, plexitises, neuralgias and insanity [4, 71]. As reviewed by Schepetkin *et al.* [2], Shilajit was an excellent medicine for pain, migraine, neuralgia, radiculitis, paralysis, vertigo, arthritis, inflammation, wounds, ulcers, hemorrhages, gastrointestinal diseases and genitourinary diseases in other regions of world also. Shilajit was also extensively used for treating bone fractures, osteoporosis, osteochondrosis, paradontosis, rheumatoid arthritis, muscular hypertrophy and ankylosing spondylitis [2, 36, 72-74]. Shilajit has been found to exhibit beneficial effects against gut ailments such as indigestion, vomiting, haemorrhoids, piles and helminth infections [12]. It has been used for treating liver ailments, jaundice and gall stones [3, 48]. Its use as a kidney tonic and diuretic is mentioned in numerous ancient texts. It exhibited beneficial effects against urinary tract infections, glycosuria, dysuria, spermoruia and lithouria [3, 4, 11, 19]. Sherpas, an ethnic group from the Himalayas known for climbing abilities and survival at high altitudes, are claimed to consume Shilajit regularly in their daily diets [37]. In central Asia, it was extensively used for the treatment of pyogenic wounds caused by species of *Streptococcus, Staphylococcus, Enterococcus, Proteus*, and coliforms [75]. Shilajit is also considered useful in the management of high-altitude related problems [76]. In Europe, weightlifters regularly consumed it for energy, strength and muscular hypertrophy [77]. It was used in Russian military for years as stimulator of bone regeneration and enhancer of muscle mass strength [72].

The rejuvenating and anti-aging effects of Shilajit are mainly attributed to FA which owing to its electrolyte nature, act as a carrier molecule for transporting the essential minerals across cell membranes [5]. Reportedly, FA promotes cell division, enzymatic reactions and increases metabolism [2, 3, 7, 94]. In addition, DBPs are also contributed to these biological actions [7, 78]. Shilajit is known to preserve and enhance the functioning of cells and their organelles. Shilajit restores and sustains cellular energy by enhancing the production of ATP [79]. Shilajit and its constituents (mainly FA and DBPs) boost efficiency of Coenzyme Q10 in the mitochondria and enhance energy production in brain and muscle tissues during exercise [78, 80]. CoQ10 is known to protect mitochondria from free radical damages thus enhancing the life and functions of the cells [78, 81].

6.2. *In vitro* Studies

Pharmacological activities of Shilajit have been studied in *in vitro* systems based on primary cells, continuous cell lines and tissues/organs derived from test animals. Antioxidant property of Shilajit is well demonstrated in *in vitro* assays. Its extracts scavenged free radicals [82] and inhibited lipid peroxidation [3, 83, 84]. Shilajit showed inhibition of cumenehydroperoxide and ADP/Fe^{++} complex-induced lipid peroxidation in a dose-dependent manner [83]. FA, HA, DPBs and DCPs are the main free-radical scavenging constituents of Shilajit [2, 85]. DBPs are known to protect the brain and nerve tissue from free-radical damage as they can permeate through the blood-brain barrier [2]. Two DBPs isolated from Shilajit viz. 3-hydroxydibenzo-α-pyrone and 3,8- dihydroxydibenzo-α-pyrone also exhibited potent radical scavenging activity [78]. Aqueous extract of Shilajit enhanced the phagocytic properties and cytokines release from murine peritoneal macrophages [86]. Similarly, FA enhanced the release of reactive oxygen species and nitric oxide in similar macrophages [48]. It also stimulated lymphocyte proliferation and their migration into lymph nodes, spleen and epithelioid granulomas during experimental tuberculosis [87]. However, these effects may not be considered true immunostimulatory unless certain strict criteria are fulfilled as suggested by Gertsch *et al.* [88]. Shilajit has shown glycine- and GABA-mimetic actions on the brainstem substantia gelatinosa neurons of the trigeminal subnucleus caudalis through the activation of the glycine receptor and GABA receptor [55]. It induced concentration-dependent, non-desensitizing lasting inward currents in preoptic hypothalamic neurons in mice brain slices and exhibited glycine mimetic postsynaptic action [56]. Shilajit holds potential for the treatment of Alzheimer's disease. Andean Shilajit and FA strongly interfered with tau aggregation and also enhanced neurites outgrowth in neural cell cultures [37]. In addition, its fraction rich in glycerol ethers/wax esters displayed growth-promoting activity in neuronal PC12 cells [61]. Shilajit is also known for its bone regeneration potential in Ayurveda and Russian systems of medicine. Its osteoblastic differentiation property was studied in human and murine mesenchymal stem cells. An increased expression of osteocalcin, ERK, core binding factor 1 and alkaline phosphatase was observed at 3-5 µg/ml concentrations. It stimulated the osteoblastic differentiation but inhibited the osteoclastogenesis [72]. In another study, Pant *et al.* [20] reported the anticancer effect of Shilajit on hepatic cancer cells (Huh-7 cells) which was mediated through induction of reactive oxygen species, nitric oxide, apoptosis, increased miRNA-22 expression and decreased miRNA-22 expression. FA is implicated in the stimulation of nitric oxide from RAW 264.7 cells through NF-κB activation and in enhanced cell death of Hep3B, LNCaP, and HL60 human cancer cells lines [89]. In the above study, although FA was not derived from Shilajit, it indirectly suggests anti-cancer activity in Shilajit since the latter contains fulvic acid as one of its active constituents. However, these assumptions need to be ascertained by experimental studies. In addition to various physiological activities, Shilajit is also endowed with antibacterial, antifungal and antiviral activities as indicated by *in vitro* assays. It exhibited antimicrobial potential against *Staphylococcus aureus, E. coli, Candida albicans* [51] and *Salmonella* spp. [90]. It also showed antifungal activity [91]. Its antiviral was evident against herpes simplex virus, human respiratory syncytial virus and human cytomegalovirus with EC_{50} values of <35 µg/ml [92]. In a recent study, humic acid from Shilajit showed potent anti-hepatitis B virus activity in HepG2 cells mediated through apoptosis induction and inhibition of autophagosome formation and cell proliferation [93]. The an-

timicrobial activities of Shilajit may be associated with the presence of FA and benzoic acid [2, 7, 94]. Unfortunately, some health claims of Shilajit are far from fully investigated even at *in vitro* experimental stage.

6.3. Experimental Animal Studies

The pharmacological activities of Shilajit have been validated in few animal studies as mentioned in Table **2**. Its oral administration at 30 mg/kg for 4 days significantly enhanced the energy levels in muscle, brain and blood of albino mice subjected to forced swimming test [79]. Similarly, DBPs levels increased inside mitochondria when mice were administered with DBP which might improve the functioning of electron transport chain. Similar observations were reported by Surapaneni *et al.* [95] in rat model of chronic fatigue syndrome. The neurological effects of Shilajit were validated in rats [5, 23, 96-98] and mice [91, 98, 99] at acute and subchronic dosages (Table **2**). However, no *in vivo* study has reported the effects of its long-term administration (6-12 months) on neurological parameters yet. Its potential for the treatment of neurodegenerative conditions was evident in a study where protection against apoptosis inducing amyloid peptide β-fragment was conferred [61]. Similarly, Cornejo *et al.* [100] found that FA can inhibit paired helical filaments formation and also caused disassembly of preformed tau fibrils. Antidiabetic potential of Shilajit was evaluated in streptozotocin-induced diabetic rats [24, 101] and alloxan-induced hyperglycemic rats [102] in which improvement in blood glucose levels and lipid profile was observed. Similar findings were reported by Trivedi *et al.* [103]. Alleviation of free radical induced damage to pancreatic islet cells was reported by Bhattacharya [104] and Bhattacharya *et al.* [24]. In the above reports, Shilajit was administered for 1 or 4 weeks only. Animal studies have also indicated the antioxidant, anti-inflammatory, immunomodulatory, spermatogenic, ovogenic, anti-ulcer, wound healing, memory enhancing, antibacterial, cardioprotective, radiation-protective and analgesic potentials of Shilajit and its constituents (Table **2**). In one study, its cardioprotective efficacy was evaluated after 91 days also [105]. In two studies, the influence of Shilajit at gene expression [106], cytokines and T-cells [74] was also reported.

The traditional health benefits of Shilajit as an energy enhancer, memory booster, spermatogenic, immunomodulatory, antioxidant, antidiabetic, neuroprotective and wound healing substance have been ascertained, although partially, in rat and mice models. These animal studies have only investigated the influence of acute and sub-chronic exposure of Shilajit on physiological and cellular parameters. It is worth exploring its effects at long-term dose schedule to derive a better correlation between *in vivo* outcomes and years-long human consumption habits. Some studies are limited by inadequate number of animals in test groups, fewer time points for observations and recording of lesser parameters. Experimental studies on immunostimulatory aspects of Shilajit have drawbacks such as physiological irrelevant doses, small animal group size, lack of negative control and no endotoxin testing [7]. Such evaluation requires scientific validation based on specific *in vivo* assays as suggested by Gertsch *et al.* [88]. Further, a cautious approach is also needed while interpreting these results. In future, molecular studies aimed at transcriptome, proteome, metabolome and microbiome level of test animals may offer better insights into physiological roles of Shilajit.

6.4. Clinical Trials

Despite great importance of Shilajit in traditional systems of medicine, only less than a dozen well-designed clinical studies have been completed or ongoing (Table **3**). Spermatogenic efficacy of processed Shilajit was tested in 28 oligospermia patients at 100 mg, *p.o.* twice daily for 90 days [39]. In a randomized, double-blind, placebo-controlled clinical study with 38 healthy volunteers, 90 day treatment schedule with 250 mg processed Shilajit given twice daily for consecutive 90 days imparted significant increase in total testosterone, free testosterone and dehydroepiandrosterone levels as compared to placebo [107]. Two clinical trials are related to diabetic condition whereas the same number of trials is listed for Alzheimer's disease (Table **3**). Clinical study of Carrasco-Gallardo *et al.* [108] is based on 9 subjects only. Recently, effect of Shilajit (PrimaVie) on muscle transcriptome of 16 adult overweight subjects was studied by Das *et al.* [109]. Transcriptomic parameters reflected upregulation of enascin XB, decorin, myoferlin, collagen, elastin, fibrillin 1, and fibronectin 1 probe sets in muscles after 8 weeks oral supplementation compared with the expression at the baseline visit. Upregulation of these ECM-related genes is implicated in muscle mechano-transduction properties, elasticity, repair, and regeneration. Some clinical studies discussed above were of short duration, involved only small numbers of subjects and not registered in clinical trial registries. Moreover, the number and frequency of clinical studies on Shilajit are still limited and confined to few countries only whereas it is consumed in many more countries. Nonetheless, clinical findings validated its beneficial pharmacological effects as dietary supplement in diabetes, oligospermia, sexual dysfunctions, neurological disorders and obesity. Long-term, randomized, controlled trials with higher number of subjects are suggested for future research.

7. TOXICITY AND HEALTH RISKS OF SHILAJIT

Processed Shilajit is generally considered safe to human and animals for short-term as well as long-term consumption. A number of animal and clinical studies strongly support these observations [7, 19]. Acute and subchronic studies in rats and mice found it generally safe at physiologically relevant dose regimens (Table **2**). In rats, LD_{50} of FA and 4-methoxy-6-carbomethoxybiphenyl by oral route was 1268 mg/kg and 684 mg/kg, respectively [28]. Oral LD_{50} of >2000 mg/kg was reported by Rubab *et al*. [74]. Velmurugan *et al*. [110] found no major adverse effects on rats at a sub-chronic dose as high as 5000 mg/kg given orally for 91 days. In clinical studies, no toxicity was observed at doses ranging from 100 mg to 1000 mg given daily by oral route (Table **3**). However, consumption of crude or inadequately processed Shilajit may manifest toxicities due to the presence of heavy metals ions, free radicals, polymeric quinones, mycotoxins and plant secondary metabolites [3, 19, 111, 112]. Some recent reports indicated the presence of toxic heavy metals such as lead and arsenic in ayurvedic formulations [58, 59, 113]. Improper and inadequate purification, *i.e*. addition of

Table 2. ***In vivo*** **bioactivities of Shilajit and its major constituents in different animal models.**

Biological Activity	Animal Model	Dose Regimen	Group Size	Treatment Duration	Outcomes	References
Energy enhancing activity	Albino mice	30 mg/kg, p.o.	na	4 days	Improved ATP levels in brain and muscles; increase in adenylate energy charge and total adenine nucleotide levels; synergistic effects with coenzyme Q 10	[79]
Anti-fatigue activity	Charles Foster albino rats	25-100 mg/kg, p.o.	6	21 days	Reduction in stress-induced immobility, SOD, nitric oxide levels; increase in plasma corticosterone and mitochondrial membrane potential	[95]
Spermatogenic and ovogenic activity	Sprague-Dawley albino rats	25-100 mg/kg, p.o.	9	42 days	Increase in sperm counts, serum testosterone level and ovulation frequency	[114]
Learning and memory enhancing effects	Wistar albino rats	5-50 mg/kg/day, p.o.	8-12	5 days	Augmentation of learning acquisition and memory retrieval	[29]
	Male Wistar rats	40 mg/kg, i.p.	4	7 days	Decrease in acetylcholinesterase staining in basal forebrain nuclei; effect on cortical and basal forebrain cholinergic signal transduction cascade	[115]
Anti-inflammatory activity	Wistar albino rats	50 mg/kg, i.p.	10	1 day	77% reduction in carrageenan-induced pedal oedema	[116]
	Wistar albino rats	200 mg/kg as Humic acid complex with aspirin (1:2)	4	4 h	67% inhibition of paw edema after 4 h	[41]
	Swiss albino mice	100-400 mg/kg, p.o.	6	10 days	Inhibition of nitric oxide release from peritoneal macrophages	[74]
Anti-allergic activity	Albino rats	10 mg/kg/day, p.o.	10	14 days	Inhibition of degranulation of the sensitized mast cells	[117]
Immunomodulatory activity	Hens	133 mg/kg, p.o.	3	14 days	Increased titers of IgA, IgG and IgY	[118]
	Swiss albino mice	100-400 mg/kg, p.o.	6	10 days	Increases lymphocyte proliferation, macrophage phagocyte response, IFN-γ, $CD4^+/CD3^+$ ratio and delayed-hypersensitivity response	[74]
	Macrobrachium rosenbergii (prawn)	2-6 g/kg b.i.d.; p.o.	25	30 days	Increase in phagocytosis, respiratory burst, glutathione peroxidase, phenoloxidase, superoxide dismutase activity	[84]
Antioxidant activity	Wistar rats	20 and 50 mg/kg, i.p.	8	21 days	Increase in SOD, catalase and glutathione peroxidase activities in frontal cortex and striatum	[24]
	Swiss albino mice	20 mg/kg of 3-OH-DBP and 3,8-(OH)2-DBP, i.p.	4	Acute exposure	Radical scavenging activity, increase in levels of CoQ 10 and 3,8-(OH)2-DBP	[78]
Anti-diabetic activity	Wistar rats	100 mg/kg, p.o.	na	28 days	Attenuated STZ-induced hyperglycaemia, enhanced the SOD activity in the β-islet cells of pancreas	[24]
	Wistar rats	1.0 mg/kg; b.i.d.; i.p.	6-11	10 days	Inhibited STZ-induced diabetes	[101]
	Albino rats	50-200 mg/kg, p.o.	6	28 days	Reduction in blood glucose levels and improvement in lipid profile	[103]
	Wistar albino rats	50-200 mg/kg Chandraprabha Vati (96 g Shilajit)	5	7 days	>50% reduction plasma glucose in alloxan-induced hyperglycemic animals; decrease in cholesterol and triglycerides	[102]

(Table 2) contd….

Biological Activity	Animal Model	Dose Regimen	Group Size	Treatment Duration	Outcomes	References
Anti-ulcer and wound healing activity	Albino rats (Fischer strain)	50-100 mg/kg, p.o.	10	6 days	Decrease in gastric and duodenal ulcer and increase in mucosal mucus	[28]
	Wistar albino rats and male guinea pigs	100 mg/kg x twice daily, p.o.	9-11	3 days	Increased in carbohydrate/protein ratio and decrease in gastric and duodenal ulcer index	[116]
	Swiss mice	200-600 µg/ mouse, i.p.	8	1 day	Increase in number of pseudopodial projections on macrophages; increase in spindle-shaped fibroblast-like cells	[86]
	Wistar albino rats	200 mg/kg as Humic acid complex with aspirin (1:2)	5	4 h	Reduction in gastric ulceration	[41]
	Wistar rats	100 mg/kg, p.o.	10	4 days	Protected against acetic acid-induced gastric ulcer by decreasing pepsin and mucosal damages	[21]
Anti-convulsion activity	Swiss albino mice	10 mg/kg as HA-carbamazepine complex (1:2) and FA-carbamazepine complex (1:2)	6	30 min	75% inhibition of electroshock	[30]
Cardiac effects	*Daphnia*	1, 10, 100 and 1000 ppm	20	Acute exposure	Negative chronotropic effect at 1 ppm dose whereas positive chronotropic effect at 1000 ppm	[119]
	Wistar rats	250 and 500 mg/kg	7-9	7 days	Protection against acute myocardial injuries	[120]
	Wistar albino rats	250 and 500 mg/kg, p.o.	6	91 days	Protection against isoproterenol-induced cardiac myocardial infarction	[105]
Neuro-protective and anti-anxiety activity	Male albino Wistar rats and Swiss albino mice	25 and 50 mg/kg, p.o.	6	15 days	Protection against seizures induced by electric shock, isonicotinyl hydrazine and pentylene-tetrazole	[98]
	Male Wistar albino rats	100-800 µg/ml	5	na	Lowering of mean arterial blood pressure, heart rate and relaxation of corpus cavernosum	[96]
	Male N-MARI albino rats	150 and 250 mg/kg, i.p.	7	3 days	Decrease in brain edema, permeability of blood-brain barrier, intracranial pressure and increase in neurologic outcomes	[97]
	Charles Foster albino rats	10-50 mg/kg, p.o.	5-8	5 days	Decreased levels of 5-hydroxytryptamine and 5-hydroxyindole acetic acid and increased levels of dopamine, homovanillic acid and 3.4-dihydroxyphenyl-acetic acid in brain tissues	[23]
	Swiss albino mice	25 mg/kg, p.o.	7	10 days	Reversal of ethanol withdrawal anxiety; increased levels of dopamine and GABA in brain	[99]
Radiation-protective effect	Female Wistar albino rats	100 mg/kg, p.o.	10	14 days	Decrease in radiation-induced ovary damage and reduced expression of p53, Bax and caspase 3	[106]
Anti-bacterial activity	*Macrobrachium rosenbergii* (prawn)	2-6 g/kg b.i.d.; p.o.	25	30 days	Decrease in mortality from Aeromonas hydrophila infection	[84]
Analgesic effect	Swiss mice	0.1 - 1.0 mg/kg, i.p.	6	5 days	Protection against development of tolerance to morphine	[121]

na: not available; HA: humic acid; FA: fulvic acid; 3-OH-DBP: 3-hydroxydibenzo-α-pyrone; [3,8-(OH)2-DBP]: 3,8- dihydroxydibenzo-α-pyrone; SOD: superoxide dismutase; STZ: streptozotocin; p.o.: *per os*; s.c.: subcutaneous; i.p.: intraperitoneal; GABA: gamma-aminobutyric acid.

Table 3. Clinical trials conducted or ongoing on Shilajit, its constituents and various formulations in different parts of the world. Source: WHO-International Clinical Trials Registry Platform, www.ctri.nic.in, www.clinicaltrials.gov.

Regimen	Trial Identifier	Country	Number of Participants	Duration of Study	Study Design	Phase of Trial	Parameters/ Disease Condition	Present Status of Trial	Reference
Processed Shilajit (500 mg BID)	na	India	61	30 days	na	na	Diabetes	Completed	[122]
Processed Shilajit (100 mg BID)	na	India	28	90 days	na	na	Oligospermia	Completed	[39]
Shilajit (500 mg BID)	CTRI/2012/06/002712	India	60	56 days	Randomized, Parallel group, placebo controlled	Phase II	Diabetic polyneuropathy	Completed	http://ctri.nic.in
Processed Shilajit (1000 mg per day)	CTRI/2016/04/006878	India	128	30 days	Randomized, parallel group trial	na	Hypertension	Recruiting	http://ctri.nic.in
Purified Shilajit (PrimaVie) (250 mg BID)	na	India	38	90 days	Randomise, Placebo controlled, double-blind	na	Testosterone levels	Completed	[107]
Andean Shilajit & complex B vitamins (Brain Up10®) (300 mg BID)	na	Chile	50	168 days	na	Phase II	Alzheimer's disease (memory enhancer)	Completed	[108]
Andean Shilajit + B_6, B_9 and B_{12} vitamins (Brain Up10®) (300 mg BID)	na	Chile	9	168 days	na	Phase II	Alzheimer's disease (dementia)	Completed	[108]
Purified Shilajit (PrimaVie) (250 mg BID)	NCT02026414	USA	40	84 days	Intervention model, single group assignment, open label	na	Obesity (Skeletal muscle)	Ongoing	[109]
Purified Shilajit (PrimaVie) (125 mg and 250 mg BID)	NCT02762032	USA	45	98 days	Randomized, single blind, Intervention model: parallel assignment	Phase 1	Skin functions	Ongoing	https://clinicaltrials.gov

na: not available
BID: *bis in die* (twice daily)

herbal components or metals during processing or formulations is the main source of toxic substances in Shilajit. Hence, cautious approach is needed towards long-term consumption when it is procured from unreliable local seller or lesser known commercial brands. Toxicity and safety assessment of raw Shilajit and its formulations with herbal additives remain problematic due to methodological issues. There is an imperative necessity to purify raw Shilajit samples to acceptable health standards prior to human consumption to safeguard from detrimental health effects.

8. PATENTS ON SHILAJIT AND ITS COMMERCIAL PRODUCTS

Exhaustive search within Indian and international patent databases (from 1970-2016) using keywords viz. shilajit, mineral pitch, shilajatu, mumie, mumijo, Andean compound, fulvic acid, complexing agents *etc*. revealed several patents on Shilajit (Table **4**). These have been either granted or filed on Shilajit alone or its combination with different herbs and minerals. PrimaVie, a purified Shilajit product marketed by Natreon Inc., USA is protected by several patents. Some other patents on its purification, complexing properties, antidiabetic, anti-aging and neuroprotective actions have been filed or granted (Table **4**). Commercial Shilajit-based health supplements and medications containing purified Shilajit alone, fulvic acid, or their combination with other herbs are available for consumers in many countries. A detailed list with brand names, formulation types, manufacturers, and therapeutic indications is provided in Table **5**.

Table 4. The national and international patents on Shilajit and/or its formulations. (Source: United States Patent and Trademark Office (USPTO), World Intellectual Property Organization (WIPO), Indian Patents Advanced Search System (InPASS), Espacenet patent search).

Patent Identifier	Title	Applicant's Name	Country/ Region	Date Published or Granted Status
03/DEL/2002 (Pat No. 226231)	A synergistic herbal composition	Dabur Research Foundation, India	India	Jan 02, 2009 Granted
531/DEL/2005 (Pat. No. 239574)	Fulvic acid as a novel complexing agent and a process of extraction thereof	Jamia Hamdard (Hamdard University), India	India	Mar 25, 2010 Granted
532/DEL/2005 (Pat. No. 249172)	Fulvic acids and humic acids as novel complexing agents and a process thereof	Jamia Hamdard (Hamdard University), India	India	Oct 10, 2011 Granted
111/DEL/2008	Isolation, identification and characterization of specific chemical compound marker of 1000.8 kD in shilajit sample responsible for the therapeutic activity of shilajit prescribed in ayurved as panacea	Arvind Kumar Balkrishna Acharya Ji Patanjali Ayurved, India	India	Aug. 20, 2010
346/DEL/2010	Ayurvedic energy drinks composition as delivery system for nutrients and medications with active amount of herbs including shilajeet and cow urine and *Terminalia arjuna* contents	Arvind Kumar and Patanjali Ayurved, India	India	Jul 27, 2012
2979/MUM/2013	An anti-diabetic composition and a process for preparing the same	Burade Kishorkumar Balkrishna, India	India	Oct 30, 2015
3408/DEL/2015	A dietary supplement formulation natural remedy product	Alliaance Biotech, India	India	Feb 19, 2016
2157/CHE/2015	A novel herbal composition for dissolving kidney stones, bladder stones	T. V. S. R. Varma	India	Nov. 4, 2016
CL2776919	Nutraceutical composition that comprises extract of shilajit, folic acid, vitamin b12 and vitamin b6 and the use thereof for preventing and/or treating neurodegenerative diseases and/or the cognitive deterioration associated with cerebral ageing	Centro internacional de biomedicina, Chile	Chile	Apr. 14, 2011
US6440436 US6869612	Process for preparing purified shilajit composition from native shilajit	Natreon Inc., USA; Indian Herbs Res. & Supply Co. Ltd., India	USA	Aug 27, 2002 March 22, 2005
US2013280291	Mineral Pitch Resin manufactured under a safe and low temperature procedure	Nodari Rizun	USA	Oct. 24, 2013
US6558712	Delivery system for pharmaceutical, nutritional and cosmetic ingredients	Natreon Inc., USA; Indian Herbs Res. & Supply Co. Ltd, India	USA	May 6, 2003
US8784804	Nutraceutical composition that comprises extract of andean shilajit, for preventing and/or treating neurodegenerative diseases and/or the cognitive deterioration associated with cerebral aging	Centro Internacional de Biomedicina, Chile	USA	July 22, 2014
US20160220609	Regulation of steroidogenic activity by using purified shilajit	Natreon, Inc., USA	USA	Published Aug. 04, 2016
US5405613	Vitamin/mineral composition	Creative Nutrition Canada Corp, Canada	USA	April 11, 1995
WO2016186695	Synergistic compositions containing chromium with *Phyllanthus emblica* and shilajit for improving endothelial function and cardiovascular health	Natreon, Inc., USA	WIPO	Nov. 24, 2016

(Table 4) contd….

Patent Identifier	Title	Applicant's Name	Country/ Region	Date Published or Granted Status
US20160095881	Promoting muscle building and repair and treating disorders related to collagen and pertinent proteins by using shilajit	Natreon, Inc., USA	USA	Apr. 7, 2016
US20140079729	Method for improving endothelial function and decreasing cardiovascular morbidity using shilajit	Natreon, Inc., USA	USA	Mar. 20, 2014
UA34953 (U)	Mumie-shiladzhit modified biologically active additive	Ecosvit Syntes Ltd Liability Co., Ukraine	Ukraine	Aug. 26, 2008
KR20030068221	Composition containing russian mumie extract for improving bone-growth and treating osteoporosis	Immunomics Co Ltd, Korea	South Korea	Aug. 21, 2003
DE10253129	Wound healing or skin-care agent for treating skin defects or burns contains sea buckthorn oil, St. John's wort oil, propolis and 'Mumie'	Stierle Nicolaus, Germany	Germany	Aug. 02, 2004
KR20050050072	Composition comprising the extract or fraction isolated from russian mumie for activation of immunity	Immunomics Co Ltd.	South Korea	May 27, 2005
WO2005099739	Oxygenated dibenzo-alpha-pyrone chromoproteins	Natreon Inc., USA	WIPO	Oct. 27, 2005
WO2006007310	Compositions of stable bioactive metabolites of docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids	Natreon Inc., USA	WIPO	Jan. 19, 2006

WIPO: World Intellectual Property Organization; EPO: European Patent Office; USPTO: US Patent Office; AU: Australian Patent Office.

Table 5. List of some proprietary products and formulations of Shilajit available in global consumer markets.

Brand Name	Dosage Form	Manufacturer	Therapeutic Indications/Health Benefits	Web Address
Dabur Shila-X Oil	Liquid	Dabur India Ltd. (India)	Weakness of local muscular and nervous tissues	http://www.dabur.com
Shilajit Gold	Capsule	Dabur India Ltd. (India)	General weakness, decreased energy and vigour	http://www.dabur.com
Vigorex SF	Capsule	Zandu (Emami Ltd., India)	Mental and physical tiredness, libido, premature ejaculation	http://www.zanduayurveda.com
Addyzoa	Capsule	Charak Pharma (P) Ltd. (India)	Male infertility	http://charak.com
Shilajit and Ashvashila	Capsule	Patanjali Ayurved Ltd. (India)	Loss of energy, vigour, sexual weakness, joint pain, asthma allergy, urinary disorders	http://www.patanjaliayurved.org
Shilajit Power	Capsule	Dindayal Aushadhi (India)	General weakness, decreased energy and vigor	https://www.dindayalaushadhi.com
Shilajit	Capsule	Ayurvedant Pvt. Ltd. (India)	Intelligence, mental vigour and power	http://www.ayurvedant.com
Shilajit	Capsule	Apollo Pharmacy (India)	Stamina, power, vigour and vitality	http://www.apollopharmacy.in
Qurs Salajeet	Tablet	Hamdard Laboratories (India)	General debility, body weakness, Spermatorrhoea, Leucorrhoea	http://www.hamdard.in
PrimaVie (Purified Shilajit)	Powder	Natreon Inc. (USA)	Anti-aging, male sexual health promoter and mitochondrial energy booster	http://natreoninc.com
Brain Up10®	Capsule	Neuroinnovation SPA (Chile)	Prevention of cognitive disorders (Alzheimer's disease and dementia), neuroprotection, antioxidant and anti-aging	http://www.neuroinnovation.cl
High Mountain Shilajit	Capsule	Dragon Herbs (USA)	Enhancing bioavailability, purifying the body and normal health maintenance	http://www.dragonherbs.com

(Table 5) contd….

Brand Name	Dosage Form	Manufacturer	Therapeutic Indications/Health Benefits	Web Address
Purblack Shilajit Live Resin	Resin paste	Adaptive Energy LLC. (USA)	Mental health, stress, anxiety, bone and cellular energy	https://purblack.com
Shilajit	Tablet	Banyan Botanicals (USA)	Promotes rejuvenation and detoxification	http://www.banyanbotanicals.com
Shilajit Fulvic Acid Complex	Capsule	Jarrow Formulas Inc. (USA)	Supports energy production in brain and muscle tissues and enhances mitochondrial functions	http://www.jarrow.com
Raw Shilajit Powder	Powder	Sunfood Superfoods (USA)	Supports bone health, immunity, blood pressure and antioxidant	http://www.sunfood.com
Super Ubiquinol CoQ10	Softgels	Life Extension (USA)	Increase CoQ10 absorption, promote hearth health and restore cellular energy	http://www.lifeextension.com
Shilajit Extract	Capsule	Swanson Superior Herbs (USA)	Adaptogen and systemic rejuvenator	http://www.swansonvitamins.com
ORIVeDA's Shilaji-Mumijo	Purified raw paste	Oriveda bv (The Netherlands)	Anti-aging, improves stamina and strength, supports the immune system, anti-ulcer and aphrodisiac	http://www.oriveda.com/mumijo.php
Golden Mumie-Altai Shilajit	Tablet	Evalar (Russia)	For fractures, contusions, rheumatism, radiculitis, inflammation, stomach ulcer *etc*.	https://www.evalar.ru
Pure Ladakhi Shilajit	Solid pieces	Future Alchemy (USA)	Promote strength, stamina and stress relief	http://futurealchemy.com
Shilajit	Capsule	Morpheme Remedies (India)	Anti-aging, antioxidant and strengthen immune system	https://www.morphemeremedies.com
A-XR PCT	Capsule	VMI Sports Nutrition (USA)	Increases *libido* and re-ignites testosterone production	https://www.vmisports.com
Shilajit Paste Purified	Paste	Nepal Shilajit Pvt. Ltd (Nepal)	Reduces stress, fatigue, oxidative damage, muscle weakness	http://www.nepalshilajit.com
Mumijo active cream	Cream	Apeiron Handels GmbH & Co. KG (Germany)	Damaged skin, neurodermatitis and psoriasis	http://natur-apeiron.de
Mumijo Tablets	Tablet	Apeiron Handels GmbH & Co. KG (Germany)	Health supplement	http://natur-apeiron.de
Pure Mumio	Tablet	Univerzum Ltd. (Czech Republic)	Adaptogen	http://natur-apeiron.de
Shampoo Shilajit Mumijo	Liquid	Herbals.LV (Latvia)	Hair breakage, hair loss, and hair pigmentation	http://www.herbals.lv
Himalaya Shilajit Resin	Paste	Tibet Duoxiongla Crudedrugs Co., Ltd. (USA)	Aging, stamina, immunity, endurance	http://www.duoxiongla.com
StressCare®	Capsule	Himalaya Herbal Healthcare (USA)	For energy, adrenals, stress, fatigue and frustration	http://himalayausa.com

CONCLUSION AND FUTURE DIRECTIONS

Shilajit is one of most important natural mineral compounds used in the traditional systems of medicine in different continents. In this review, we attempt to summarize and analyze the existing traditional and scientific knowledge of its healthcare applications, biochemical constitution, pharmacological activities and safety evaluation. Due to multiple benefits, it is in demand for use in dietary supplements, polyherbal formulations, health tonics, bone/muscle health mixture, drug delivery complexes, cosmetics *etc*. The available biochemical and molecular evidences reliably indicate that its main physiological effects are due to HA, FA, DBPs, DCPs and minerals. Encouragingly, some health claims of Shilajit are being verified by well-designed and scientifically valid *in vitro* and animal studies. These include energy enhancing, antioxidant, antiinflammatory, antidiabetic, neuroprotective, spermatogenic, wound healing, antiulcer, and learning/memory enhancing properties. Further evidences in support of antidiabetic, spermatogenic and testosterone enhancing are provided by recently conducted clinical studies. In spite of its significant traditional health importance in

India, Russia, Nepal and many other countries, the support towards Shilajit biochemical research, clinical studies and research and development is largely been ignored. Its complete therapeutic potential is still largely unexplored by modern research and much claimed bioactivities lack scientific validation. Authenticity and reliability of many research studies remain doubtful and the number of peer-reviewed quality research publications is far less. Caution is required while interpreting the findings of studies which have been published in non-indexed and obscure journals with no or sub-standard peer-review process without the rigor of a scientifically valid experimental procedures and design. In some reports, the source and chemical composition of Shilajit samples are not mentioned. Similarly, a number of clinical studies carried in 1970s were poorly designed, lacked control groups, and many crucial parameters were missing. Another issue is inconsistencies in chemical constituents of Shilajit samples derived from different geographical regions. This variability and highly substituted nature of humic substances pose challenges in standardization and quality control of Shilajit products. Despite the well accepted fact that few plant species are involved in Shilajit formation, no concrete direct evidences are available yet to conclusively establish the contribution of one particular plant species. Current data and scientific assumptions point towards the indispensable role of bacteria (most likely actinobacteria) and fungi in Shilajit synthesis. These microbes may be crucial in rock bioweathering and plant matter biotransformation owing to their unparalleled and highly versatile enzymatic machineries, adaptation to fluctuating environmental conditions and the limiting oxygen levels encountered under the rock layers in high altitude mountains. Further, the possibility of the involvement of heat-tolerant, microaerophilic or facultative anaerobic microbial species cannot be ruled out. The fine-tuned interplay of interactions among various microbial groups is far from understood at present. So far no serious side effects and toxicity of Shilajit have been reported, but further toxicological studies are still needed to ensure its safety for long-term human consumption. Considerable scope exists for elaborated research towards determining the structural variability in Shilajit constituents with regard to geographical regions, comprehensive dose-response-toxicity correlation studies at preclinical stage and multi-centric, double blind, controlled, well-designed clinical trials. *In vitro* and animal experimental studies which fulfill the stringent criteria of assigning bioactivities and pharmacological action should be encouraged. Another interesting approach will be to explore its effect on gene expression and on microbiome of different organs especially gastrointestinal tract. The rich chemodiversity of Shilajit may be utilized to design novel drugs and medicines. Metagenomics and next-generation sequencing technologies offer immense opportunities to enrich our understanding about microbial role in Shilajit formation. It is also worth focusing attention towards *in vitro* synthesis of FA, HA and DBPs from plant by-products using unique microbial strains derived from Shilajit itself as a cost-effective long-term strategy to ensure its ample availability as health supplement and for drug manufacturing. In addition, strict regulation for the implementation of good manufacturing practices, standardization and quality controls on manufacturing process of its commercial products is strongly suggested. In future, a scientific approach to promote natural products including Shilajit as an accessible and affordable medicinal food may receive encouraging support as Ministry of AYUSH, Govt. of India has initiated many initiatives towards harnessing the potential of diversified ethnomedicinal resources of the country for their easy and wide accessibility to wider sections of society for general and specific health needs in decades to come.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

TM is grateful to Chancellor, Eternal University for providing financial assistance during her doctoral research.

REFERENCES

[1] WHO. WHO Traditional Medicine Strategy 2014-2023. World Health Organization, Geneva, Switzerland. 2013.

[2] Schepetkin IA, Khlebnikov AI, Kwon BS. Medical drugs from humus matter: focus on mumie. Drug Develop Res 2002; 57: 140-59.

[3] Agarwal SP, Khanna R, Karmarkar R, Anwer M, Khar RK. Shilajit: A review. Phytother Res 2007; 21(5): 401-5.

[4] Wilson E, Rajamanickam GV, Dubey GP, *et al.* Review on Shilajit used in traditional Indian medicine. J Ethnopharmacol 2011; 136: 1-9.

[5] Ghosal S. Chemistry of Shilajit, an immunomodulatory Ayurvedic Rasayana. Pure Appl Chem 1990; 62: 1285-88.

[6] Khanna R, Witt M, Anwer MK, Agarwal SP, Koch BP. Spectroscopic characterization of fulvic acids extracted from the rock exudate Shilajit. Org Geochem 2008; 39: 1719-24.

[7] Stohs SJ. Safety and efficacy of shilajit (mumie, moomiyo). Phytother Res 2014; 28: 475-9.

[8] Garedew A, Feist M, Schmolz E, Lamprecht I. Thermal analysis of mumiyo, the legendary folk remedy from the Himalaya region. Thermochim Acta 2004; 417: 301-9.

[9] Frolova LN, Kiseleva TL. Chemical composition of mumijo and methods for determining its authenticity and quality (a review). Pharma Chem J 1996; 30: 543-7.

[10] Angmo K, Adhikari BS, Rawat GS. Changing aspects of traditional healthcare system in Western Ladakh, India. J Ethnopharmacol 2012; 143: 621-30.

[11] Bhavsar SK, Thaker AM, Malik JK. Shilajit. In: Gupta RC, Ed. Nutraceuticals: Efficacy, Safety and Toxicity. 1st ed. Academic Press: New York 2016; pp. 707-16.

[12] Chopra RN, Chopra IC, Handa KL, Kapur LD. Chopra's Indigenous Drugs of India. 1st ed. Academic Publishers, Calcutta India. 1958.

[13] Tiwari VP, Tiwari KC, Joshi PJ. An interpretation of Ayurvedika findings on Shilajit. J Res Indigenous Med 1973; 8: 57.

[14] Ghosal S. Shilajit: Its origin and vital significance. In: Mukherjee B, Ed. Traditional Medicine. Oxford - IBH: New Delhi 1993; pp. 308-19.

[15] Sharma RK, Dash B. Charaka Samhita. Chowkhamba Sanskrit Series Office: Varanasi, India 2000.

[16] Thiyagarajan R, Sunderrajan A. Gunapadam Thathu Jeeva Vaguppu. 4th ed. Directorate of Indian Medicine and Homeopathy: Chennai, India, 1992.

[17] Ghosal S, Lal J, Singh SK. The core structure of Shilajit humus. Soil Biol Biochem 1991; 23: 673-80.

[18] Gurmet P. Sowa-Rigpa: Himalayan art of healing. Indian J Tradit Knowl 2004; 3: 212-8.

[19] Ghosal S. Shilajit in Perspective, 1st ed. Alpha Science International Limited: Oxford, United Kingdom 2006.

[20] Pant K, Gupta P, Damania P, *et al.* Mineral pitch induces apoptosis and inhibits proliferation *via* modulating reactive oxygen species in hepatic cancer cells. BMC Complement Altern Med 2016; 16: 148.
[21] Shahrokhi N, Keshavarzi Z, Khaksari M. Ulcer healing activity of mumijo aqueous extract against acetic acid induced gastric ulcer in rats. J Pharm Bioall Sci 2015; 7: 56-9.
[22] Hemadri K. Discovery of gomutra silajit from South India. Anc Sci Life 1987; 8: 104.
[23] Jaiswal AK, Bhattacharya SK. Effects of Shilajit on memory, anxiety and brain monoamines in rats. Indian J Pharmacol 1992; 24: 12-7.
[24] Bhattacharya SK, Sen AP, Ghosal S. Effect of Shilajit on biogenic free radicals. Phytother Res 1995; 9: 56-9.
[25] de Melo BA, Motta FL, Santana MH. Humic acids: Structural properties and multiple functionalities for novel technological developments. Mater Sci Eng C Mater Biol Appl 2016; 62: 967-74.
[26] Lal VK, Panday KK, Kapoor ML. Literary support to the vegetable origin of Shilajit. Anc Sci Life 1988; 7: 145-8.
[27] Mittal P, Kaushik D, Gupta V, Bansal P, Khokra S. Therapeutic potentials of Shilajit rasayana- A review. Int J Pharma Clin Res 2009; 1: 47-9.
[28] Ghosal S, Singh SK, Kumar Y, *et al.* Anti-ulcerogenic activity of fulvic acids and 4′-methoxy-6-carbomethoxybiphenyl isolated from shilajit. Phytother Res 1988; 2: 187-91.
[29] Ghosal S. Lal J. Jaiswal AK, Bhattacharya SK. Effects of Shilajit and its active constituents on learning and memory in rats. Phytother Res 1993; 7: 29-34.
[30] Mirza MA, Agarwal SP, Rahman MA, *et al.* Role of humic acid on oral drug delivery of an antiepileptic drug. Drug Dev Ind Pharm 2011; 37: 310-9.
[31] Tang WW, Zeng GM, Gong JL, *et al.* Impact of humic/fulvic acid on the removal of heavy metals from aqueous solutions using nanomaterials: A review. Sci Total Environ 2014; 468: 1014-27.
[32] Lehmann J, Kleber M. The contentious nature of soil organic matter. Nature 2015; 2528: 60-8.
[33] Stevenson FJ. Humus chemistry: Genesis, composition, reactions. John Wiley & Sons; 1994.
[34] Siddiqui Y, Meon S, Ismail R, Rahmani M, Ali A. *In vitro* fungicidal activity of humic acid fraction from oil palm compost. Int J Agric Biol 2009; 11: 448-52.
[35] Khilko SL, Efimova IV, Smirnova OV. Antioxidant properties of humic acids from brown coal. Solid Fuel Chem 2011; 45: 367-71.
[36] Schepetkin IA, Xie G, Jutila MA, Quinn MT. Complement-fixing activity of fulvic acid from Shilajit and other natural sources. Phytother Res 2009; 23: 373-94.
[37] Carrasco-Gallardo C, Guzman L, Maccioni RB. Shilajit: A natural phytocomplex with potential procognitive activity. Int J Alzheimers Dis 2012; 2012: 674142.
[38] Pettit RE. Organic matter, humus, humate, humic acid, fulvic acid and humin: Their importance in soil fertility and plant health. CTI Research 2004.
[39] Biswas TK, Pandit S, Mondal S, *et al.* Clinical evaluation of spermatogenic activity of processed Shilajit in oligospermia. Andrologia 2010; 42: 48-56.
[40] Steinberg CEW, Haitzer M, Bruggemann R, Perminova IV, Yashenko NY, Petrosyan VS. Towards a Quantitative Structure Activity Relationship (QSAR) of dissolved humic substances as detoxifying agents in freshwaters. Int Rev Hydrobiol 2000; 85: 253-66.
[41] Anwer MK, Agarwal SP, Ali A, Sultana Y. Molecular complexes of aspirin with humic acid extracted from Shilajit and their characterization. J Incl Phenom Macrocycl Chem 2010; 67: 209-15.
[42] Waksman SA. Humus: Origin, chemical composition, and importance in nature. Williams and Wilkins Co: Baltimore. 1938.
[43] Kononova MM. Soil organic matter. Pergamon Press, Inc: Oxford 1966.
[44] Mao Z, Sun W, Fu L, Luo H, Lai D, Zhou L. Natural dibenzo-α-pyrones and their bioactivities. Molecules 2014; 19: 5088-108.
[45] Garazd YL, Garazd MM. Natural dibenzo[b,d]Pyran-6-Ones: Structural diversity and biological activity. Chem Nat Comp 2016; 52: 1-18.
[46] Rodriguez FJ, Schlenger P, Garcia-Valverde M. Monitoring changes in the structure and properties of humic substances following ozonation using UV-Vis, FTIR and 1H NMR techniques. Sci Total Environ 2016; 541: 623-37.
[47] Ghosal S, Reddy JP, Lal VK. Shilajit I: Chemical constituents. J Pharm Sci 1976; 65: 772-3.
[48] Schepetkin IA, Khlebnikov AI, Ah SY, *et al.* Characterization and biological activities of humic substances from mumie. J Agric Food Chem 2003; 51: 5245-54.
[49] Agarwal SP, Khanna R, Karmarkar R, *et al.* Physico-chemical, spectral and thermal characterization of Shilajit, a humic substance with medicinal properties. Asian J Chem 2008; 20: 209-17.
[50] Takeuchi K, Amagase K. Evaluation of gastric ulcerogenic and healing impairment effects of bisphosphonates: Adverse gastric reactions of bisphosphonate. Curr Protoc Toxicol 2012; 21: 21-29.
[51] Phaechamud T, Charoenteeraboon J, Wetwitayaklung P, Limmatvapirat C, Srichan T. Some biological activities and safety of mineral pitch. Silpakorn Univ Sci Technol J 2008; 2: 7-17.
[52] Ali M, Sahrawat I, Singh O. Phytochemical investigation of Shilajit. Indian J Chem 2004; 43B: 2217-22.
[53] Kiren Y, Nugroho AE, Hirasawa Y, *et al.* Mumic acids A-E: New diterpenoids from mumiyo. J Nat Med 2014; 68: 199-205.
[54] Raju S. PrimaVie. Technical Data Report. Natreon, Inc: New Brunswick, NJ 08901, 2012.
[55] Yin H, Yang EJ, Park SJ, Han SK. Glycine- and GABA-mimetic actions of Shilajit on the substantia gelatinosa neurons of the trigeminal subnucleus caudalis in mice. Korean J Physiol Pharmacol 2011; 15: 285-9.
[56] Bhattarai JP, Cho DH, Han SK. Activation of strychnine-sensitive glycine receptors by Shilajit on preoptic hypothalamic neurons of juvenile mice. Chinese J Physiol 2016; 59: 39-45.
[57] Peerzada N, Saramani S, Tariq SA. Silajit. Part II. Determination of metals in Silajit and biological significance of some metals. Sci Int 1999; 11: 79-82.
[58] Saper RB, Phillips RS, Sehgal A, *et al.* Lead, mercury, and arsenic in US- and Indian-manufactured ayurvedic medicines sold *via* the internet. JAMA 2008; 300: 915-23.
[59] Koch I, Moriarty M, House K, *et al.* Bioaccessibility of lead and arsenic in traditional Indian medicines. Sci Total Environ 2011; 409: 4545-52.
[60] Ghosal S. Shilalit: Its origin and significance. Indian J Indg Med 1992; 9: 1-4.
[61] Aiello A, Fattorusso E, Menna M, *et al.* Mumijo traditional medicine: fossil deposits from antarctica (chemical composition and beneficial bioactivity). Evid Based Complement Alternat Med 2011; 2011: 738131.
[62] Kelleher BP, Simpson AJ. Humic substances in soils: Are they really chemically distinct? Environ Sci Technol 2006; 40: 4605-11.
[63] Ghosal S. The aroma principles of gomutra and karpuragandha Shilajit. Indian J Indg Med 1994; 11: 11-4.
[64] Joshi GC, Tiwari KC, Pande NK, Pande G. Bryophytes, the source of the origin of Shilajit - a new hypothesis. BMEBR 1994; 15: 106-11.
[65] Saroya AS. Ethnopharmacology of bryophytes. In: Herbalism, Phytochemistry and Ethnopharmacology. Science Publishers and CRC Press: USA 2011.
[66] Saroya AS. Regulatory and pharmacological bases of ayurvedic formulations. CRC Press: Boca Raton 2016.
[67] Guzman-Martinez L, Farias GA, Maccioni RB. Tau oligomers as potential targets for Alzheimer's diagnosis and novel drugs. Front Neurol 2013; 4: 167.
[68] Kumar R. Census of mycoflora associated with the Shilajit rocks. Curr Sci 1993; 65: 984-5.
[69] Islam A, Ghosh R, Banerjee D, Nath P, Mazumder UK, Ghosal S. Biotransformation of 3-hydroxydibenzo-α-pyrone into 3, 8 dihydroxydibenzo-α-pyrone and aminoacyl conjugates by Aspergillus niger isolated from native Shilajit. Elect J Biotechnol 2008; 11: 1-10.
[70] Costa JL, Paulsrud P, Rikkinen J, Lindblad P. Genetic diversity of Nostoc symbionts endophytically associated with two bryophyte species. Appl Environ Microbiol 2001; 67: 4393-6.
[71] Doosti F, Dashti S, Tabatabai SM, Hosseinzadeh H. Traditional Chinese and Indian medicine in the treatment of opioid-dependence: A review. Avicenna J Phytomed 2013; 3: 205-15.
[72] Jung CR, Schepetkin IA, Woo SB, Khlebnikov AI, Kwon BS. Osteoblastic differentiation of mesenchymal stem cells by mumie extract. Drug Develp Res 2002; 57: 122-33.
[73] Labban NY. Shilajit, a novel regulator of bone/cartilage healing. Doctoral dissertation, Indiana University. Shilajit, a novel regulator of bone/cartilage healing. pp. 197, 2013.

[74] Rubab I, Routray I, Mahmood A, *et al.* Mineral pitch stimulates humoral, cellular and innate immune responses in mice. Pharm Biol 2013; 51: 997-1007.
[75] Shakirov DS. Treatment of infected wounds with Mumie asil in experiment. Exp Surg Anesthes 1969; 6: 36-9.
[76] Meena H, Pandey HK, Arya MC, Ahmed Z. Shilajit: A panacea for high-altitude problems. Int J Ayurveda 2010; 1: 37-40.
[77] Bucci LR. Selected herbals and human exercise performance. Am J Clin Nutr 2000; 72(2 suppl): 624S-36S.
[78] Bhattacharyya S, Pal D, Banerjee D, *et al.* Shilajit dibenzo-α-pyrones: Mitochondria targeted antioxidants. Pharmacol Online 2009; 2: 690-8.
[79] Bhattacharyya S, Pal D, Gupta AK, Ganguly P, Majumder UK, Ghosal S. Beneficial effect of processed Shilajit on swimming exercise induced impaired energy status of mice. Pharmacol Online 2009; 1: 817-25.
[80] Duberley KE, Abramov AY, Chalasani A, Heales SJ, Rahman S, Hargreaves IP. Human neuronal coenzyme Q10 deficiency results in global loss of mitochondrial respiratory chain activity, increased mitochondrial oxidative stress and reversal of ATP synthase activity: implications for pathogenesis and treatment. J Inherit Metab Dis 2013; 36: 63-73.
[81] Aboul-Fotouh S. Coenzyme Q10 displays antidepressant-like activity with reduction of hippocampal oxidative/nitrosative DNA damage in chronically stressed rats. Pharmacol Biochem Behav 2013; 104: 105-12.
[82] Ghosal S. Interaction of Shilajit with free radicals. Ind J Chem 1995; 34B: 596-602.
[83] Tripathi YB, Shukla S, Chaurasia S, Chaturvedi S. Antilipid peroxidative property of Shilajit. Phytother Res 1996; 10: 269-73.
[84] Musthafa MS, Jawahar Ali AR, Hyder Ali AR, *et al.* Effect of Shilajit enriched diet on immunity, antioxidants, and disease resistance in *Macrobrachium rosenbergii* (de Man) against *Aeromonas hydrophila*. Fish Shellfish Immunol 2016; 57: 293-300.
[85] Aeschbacher M, Graf C, Scwarzenbach RP, *et al.* Antioxidant properties of humic substances. Environ Sci Technol 2012; 46: 4916-25.
[86] Bhaumik S, Chattopadhyay S, Ghosal S. Effect of Shilajit on mouse peritoneal macrophages. Phytother Res 1993; 7: 425-7.
[87] Agzamov RA, Arifkhanova SI, Vakhidova GA. Mumie as a pathogenetic agent in the treatment of tuberculosis. Probl Tuberk 1988; 7: 49-52.
[88] Gertsch J, Viveros-Paredes JM, Taylor P. Plant immunostimulants-scientific paradigm or myth? J Ethnopharmacol 2011; 136: 385-91.
[89] Jayasooriya RG, Dilshara MG, Kang CH, *et al.* Fulvic acid promotes extracellular anti-cancer mediators from RAW 264.7 cells, causing to cancer cell death *in vitro*. Int Immunopharmacol 2016; 36: 241-8.
[90] Enkh-Oyun T, Narangerel B. Results for antibiotic-likeness activity of the mumio against *Salmonella* spp. Mongolian J Agric Sci 2014; 13: 27-8.
[91] Mirza MA, Ahmad N, Agarwal SP, Mahmood D, Anwer MK, Iqbal Z. Comparative evaluation of humic substances in oral drug delivery. Results Pharma Sci 2011; 1: 16-26.
[92] Cagno V, Donalisio M, Civra A, Cagliero C, Rubiolo P, Lembo D. *In vitro* evaluation of the antiviral properties of Shilajit and investigation of its mechanisms of action. J Ethnopharmacol 2015; 166: 129-34.
[93] Pant K, Yadav AK, Gupta P, Rathore AS, Nayak B, Venugopal SK. Humic acid inhibits HBV-induced autophagosome formation and induces apoptosis in HBV-transfected Hep G2 cells. Sci Rep 2016; 6: 34496.
[94] van Rensburg CEJ, Van Straten A, Dekker J. An *in vitro* investigation of the antimicrobial activity of oxifulvic acid. J Antimicrob Chemother 2000; 46: 853-4.
[95] Surapaneni DK, Adapa SR, Preeti K, Teja GR, Veeraragavan M, Krishnamurthy S. Shilajit attenuates behavioral symptoms of chronic fatigue syndrome by modulating the hypothalamic-pituitary-adrenal axis and mitochondrial bioenergetics in rats. J Ethnopharmacol 2012; 143: 91-9.
[96] Kaur S, Kumar P, Kumar D, Kharya MD, Singh N. Parasympathomimetic effect of Shilajit accounts for relaxation of rat corpus cavernosum. Am J Mens Health 2013; 7: 119-27.
[97] Khaksari M, Mahmmodi R, Shahrokhi N, Shabani M, Joukar S, Aqapour M. The effects of Shilajit on brain edema, intracranial pressure and neurologic outcomes following the traumatic brain injury in rat. Iran J Basic Med Sci 2013; 16: 858-64.
[98] Durg S, Veerapur VP, Thippeswamy BS, Ahamed SM. Antiepileptic and antipsychotic activities of standardized Śilājatu (Shilajit) in experimental animals. Anc Sci Life 2015; 35: 110-7.
[99] Bansal P, Banerjee S. Effect of Withinia somnifera and shilajit on alcohol addiction in mice. Pharmacogn Mag 2016; 12(Suppl 2), S121-8.
[100] Cornejo A, Jimenez JM, Caballero L, Melo F, Maccioni RB. Fulvic acid inhibits aggregation and promotes disassembly of tau fibrils associated with Alzheimer's disease. J Alzheimers Dis 2011; 27: 143-153.
[101] Kanikkannan N, Ramarao P, Ghosal S. Shilajit-induced potentiation of the hypoglycemic action of insulin and inhibition of streptozotocin induced diabetes in rats. Phytother Res 1995; 9: 478-81.
[102] Wanjari MM, Mishra S, Dey YN, Sharma D, Gaidhani SN, Jadhav AD. Antidiabetic activity of Chandraprabha vati- A classical Ayurvedic formulation. J Ayurveda Integr Med 2016; 7: 144-50.
[103] Trivedi NA, Mazumdar B, Bhatt JD, Hemavathi KG. Effect of shilajit on blood glucose and lipid profile in alloxan-induced diabetic rats. Indian J Pharmacol 2004; 36: 373-6.
[104] Bhattacharya SK. Shilajit attenuates streptozotocin induced diabetes mellitus and decrease in pancreatic islet superoxide dismutase activity in rats. Phytother Res 1995; 9: 41-4.
[105] Vivek B, Wilson E, Nithya Devi SV, Velmurugan C, Kannan M. Cardioprotective activity of Shilajit in isoproterenol-induced myocardial infarction in rats: A biochemical and histopathological evaluation. Int J Res Photochem Pharmacol 2011; 1: 28-32.
[106] Kececi M, Akpolat M, Gulle K, Gencer E, Sahbaz A. Evaluation of preventive effect of Shilajit on radiation-induced apoptosis on ovaries. Arch Gynecol Obstet 2016; 293: 1255-62.
[107] Pandit S, Biswas S, Jana U, De RK, Mukhopadhyay SC, Biswas TK. Clinical evaluation of purified Shilajit on testosterone levels in healthy volunteers. Andrologia 2016; 48: 570-5.
[108] Carrasco-Gallardo C, Farias GA, Fuentes P, Crespo F, Maccioni RB. Can nutraceuticals prevent Alzheimer's disease? Potential therapeutic role of a formulation containing Shilajit and complex B vitamins. Arch Med Res 2012; 43: 699-704.
[109] Das A, Datta S, Rhea B, *et al.* The human skeletal muscle transcriptome in response to oral Shilajit supplementation. J Med Food 2016; 19: 701-9.
[110] Velmurugan C, Vivek B, Wilson E, Bharathi T, Sundaram T. Evaluation of safety profile of black Shilajit after 91 days repeated administration in rats. Asian Pac J Trop Biomed 2012; 2: 210-4.
[111] Ghosal S, Lata S, Kumar Y. Free radicals of Shilajit humus. Indian J Chem 1996; 34B: 591-95.
[112] Genuis SJ, Schwalfenberg G, Siy AKJ, Rodushkin I. Toxic element contamination of natural health products and pharmaceutical preparations. Plos One 2012; 7: e49676.
[113] Giacomino A, Abollino O, Malandrino M, Karthik M, Murugesan V. Determination and assessment of the contents of essential and potentially toxic elements in Ayurvedic medicine formulations by inductively coupled plasma-optical emission spectrometry. Microchemical J 2011; 99: 2-6.
[114] Park JS, Kim GY, Han K. The spermatogenic and ovogenic effects of chronically administered Shilajit to rats. J Ethnopharmacol 2006; 107: 349-53.
[115] Schliebs R, Liebmann A, Bhattacharya SK, Kumar A, Ghosal S, Bigl V. Systemic administration of defined extracts from Withania somnifera (Indian Ginseng) and Shilajit differentially affects cholinergic but not glutamatergic and GABAergic markers in rat brain. Neurochem Int 1997; 30: 181-90.
[116] Goel RK, Banerjee RS, Achrarya SB. Antiulcerogenic and anti-inflammatory studies with Shilajit. J Ethnopharmacol 1990; 29: 95-103.
[117] Ghosal S, Lal J, Singh SK, *et al.* Mast cell protecting effects of Shilajit and its constituents. Phytother Res 1989; 3: 249-52.
[118] Bizanov G, Barinova N, Jonauskie I. Adjuvant effect of Shilajit and plant extracts on the immune responses to bovine serum albumin in hens. Cen Eur J Immunol 2012; 37: 91-5.

[119] Gaikwad NS, Panat AV, Deshpande MS, Ramya K, Khalid PU, Augustine P. Effect of Shilajit on the heart of Daphnia: A preliminary study. J Ayurveda Integr Med 2012; 3: 3-5.

[120] Joukar S, Najafipour H, Dabiri S, Sheibani M, Sharokhi N. Cardioprotective effect of mumie (Shilajit) on experimentally induced myocardial injury. Cardiovasc Toxicol 2014; 14: 214-21.

[121] Tiwari P, Ramarao P, Ghosal S. Effects of Shilajit on the development of tolerance to morphine in mice. Phytother Res 2001; 15: 177-9.

[122] Saxena N, Upendra DN, Raj SK, *et al.* Modulation of oxidative and antioxidative status in diabetes by *Asphaltum panjabinum*. Diabetes Care 2003; 26: 2469-70.