Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2012; 4(2): 17-25

ISSN: 0975-5160

Review Article

Shilajit: A Humic Matter Panacea for Cancer

[1]Kishor Pant, Bimala Singh, Nagendra Thakur

Department of Microbiology, School of Life Sciences, Sikkim University 6th Mile Samdur, Tadong, Gangtok, Sikkim, India

ABSTRACT

Cancer is the leading cause of death after cardiovascular disease. The primary etiologic agents for cancer include mutagens, toxins, free radicals, heavy metals, blood sugar, virus, radiations apart from many other factors including inflammation which can increase the risk of cancer development and progression. Shilajit is a blackish–brown coloured herbomineral medicine, collected from the high altitude mountains of many parts of the world. Shilajit refers to the humic matter that contains 60-80% of fulvic acid (FA) and humic acid (HA). The biological activity of Shilajit is mainly attributed to these humic compounds HA and FA. In this review we have focused on the cancer chemopreventive and therapeutic properties of Shilajit and humic compounds. Shilajit and HA possess anti-inflammatory, antioxidant, antimutagenic, antitoxic, antiviral, heavy metal chelating, antitumor, apoptotic and photo-protective properties. These properties make Shilajit useful agents for cancer therapy and prevention. In addition, Shilajit has no reported side effects and can be administered as a nutritive and rejuvenating tonic and combats age related problems.

INTRODUCTION

Cancer is the second leading cause of death after the cardiovascular disease and is the major public health problem, causing approximately 7 million deaths every year worldwide. The existing treatment approaches and surgical techniques have not been able to cope effectively with this dreaded disease. The conventional therapies like chemotherapy, radiotherapy, immunotherapy and surgical approaches for cancer treatment are not very efficient. Thus, there is an imperative requisite for the development of the mechanism-based approaches for the cancer management and chemoprevention by non-toxic and natural agents. Shilajit is blackish-brown herbal compound and composed mainly of humic acid components namely humic acid and fulvic acid. Humic acid and fulvic acid have been reported to possess cancer preventive properties (23). It has been shown that these compounds can inhibit mutagenesis and have free radicals scavenging, photoprotecting, anti-inflammatory and toxic compound removing properties that can inhibit the cancer development. Shilajit is non-expensive, non-toxic compound which can be taken orally or as a part of the daily diet. Therefore, it is logical that future clinical studies should focus on examining the efficacy of Shilajit and its active constituents in chemoprevention as an alternative to pharmacological agents. In this review, we address the use of Shilajit and its constituents for the prevention and management of cancer.

Chemoprevention is the use of pharmacological or natural agents to check, detain or render the null and void the process of the cancer development (Carcinogenesis) (1). It is accepted that an effective and acceptable chemopreventive agent should have certain properties like: (a), little or no toxic effects in healthy cells; (b), high efficacy against various factors; (c), potential of oral consumption; (d), identified mechanism of action; (e), low cost; and (f), acceptance by humans (1). Shilajit is one such compound, which has been used in Ayurveda for centuries. The humic compounds of Shilajit can be a potential cancer chemopreventive agent. Anti-inflammatory, anti- oxidative, antiviral, anti-mutagenic, immunomodulatory and several other effects of Shilajit have been already reported.

Shilajit: Shilajit is considered as the wonder drug of the ancient Indian medicinal practice (Ayurveda). It is a blackish-brown coloured partly solid matter having sharp odour of the cow's fusty urine and contain the pungent tang, formed by the long-term humification process of many plants (2, 3). Shilajit means 'Shilaras' in Sanskrit (4) and its other meanings are 'conqueror of the rocks' and 'destroyer of the weaknesses'(5). Shilajit is referred to as silajatu in Bengali (4), Uerangyum or Perangyum in Tamil (4, 6), Hajar-ul-musa in Arabic (4), Mummio or Mumie in Russian (7), Asphalt, Mineral Pitch and Jew's pitch in English (4, 6), Asphaltum in Latin (8), and in Botanical terms it called as Bitumen mineral (9). Shilajit is considered as a vital prescription in the ancient Hindu material remedies and presently also extensively used by the Hindu physicians for a variety of diseases. Prehistoric ayurvedic writings 'the Charaka Samhita' and 'Susruta Samhita' explain the use of Shilajit as a treatment for all ailment of body as well as a rasayana (rejuvenative) to increase the longevity (10). Shialjit is usually isolated from the high altitude rocks of the Himalayan regions mainly from Kashmir, Arunachal Pradesh, Uttrakhand, Himachal Pradesh, Australia, Afghanistan, Russia, Nepal, Bhutan and Norway (4, 11). There are several varieties of Shilajit described by the Charaka Samhita namely rajat

Author for correspondence: Email: pant.kishor1@gmail.com

(silver Shilajit), tamra (copper Shilajit), lauha (iron Shilajit) and sarvana (gold Shilajit) (4, 12). Shilajit contains a humic substance fulvic acid (FA) and humic acid (HA) (60-80%), minerals (20-40%) and up to 5% of trace elements (Fe, Ca, Cu, Zn, Mg, Mn, Mo, P) (13, 14). The primary and key active components responsible for the Shilajit activities is the fulvic acid (FA) and humic acid. By the virtue of the FA Shilajit has many biological functions and uses (15, 16), which acts as carrier molecule. The low molecular weight bioactive organic compound such as oxygenated dibenzo-α-pyrones is also present in Shilajit. Shilajit has been used for the treatment of hypersensitivity, diabetes, digestive disorder, nervous ailment, tuberculosis, chronic bronchitis, asthma, anemia, eczema, bone fractures, genitourinary ailment and many other diseases (17, 18). Fulvic acid is the main constituent of Shilajit and along with FA and HA it also contains minerals in ionic form. These minerals are transported to cells and tissues by fulvic acid, which maintains the electric potential of the cells of the body and thus possibly prevents its death, provides the longevity and might act as a rejuvenator. It helps in metabolism and energy production in the body. It can also act as detoxifying agent in the body and can function as immunomodulator and helps in haematopoiesis.

Pharmacological Actions Of Shilajit And Ha In Cancer Prevention: It is assumed that Shilajit can play an important role in cancer chemoprevention and possibly in its treatment. The general pharmacological actions of Shilajit in cancer therapy are summarised below:

Anti-Oxidant Properties: Free radicals are molecules with incomplete electron shells, which make them more chemically reactive than those with complete electron shells. Exposure to various environmental factors, including tobacco smoke and radiation, can also lead to free radical development. In humans, the most widespread form of free radicals is oxygen. When an oxygen molecule (O_2) becomes electrically charged, it tries to take electrons from other molecules, and thus can cause damage to DNA (20), proteins and cell membrane. Eventually, such damage specially damage in DNA could become irreversible and might lead to ailment including cancer (21). Antioxidants are substances that may

protect cells from the damage caused by unstable molecules (free radicals) by neutralizing their electrical charge and thus can avert the free radical damage in cells (21). Free radicals which are generated during cancer chemotherapy and radiation therapy, can damage the normal cell around the vicinity of the tumor cell, which makes the cancer treatment rather painful. The anti cancer drugs damages the cellular DNA by producing the reactive oxygen species (ROS) hence the antioxidants can be used with the cancer therapy to reduce the pain and the severity of the side effects (21). HA compounds are excellent antioxidants (22, 23) along with anti-lipid peroxidative activity(23). Shilajit has free radical scavenging or antioxidant properties against the NO & OH (24) and this antioxidant activity depends on the increasing concentration of the humic compounds present in Shilajit (22). It has been found that the use of antioxidants are effective in Grave's disease (25).

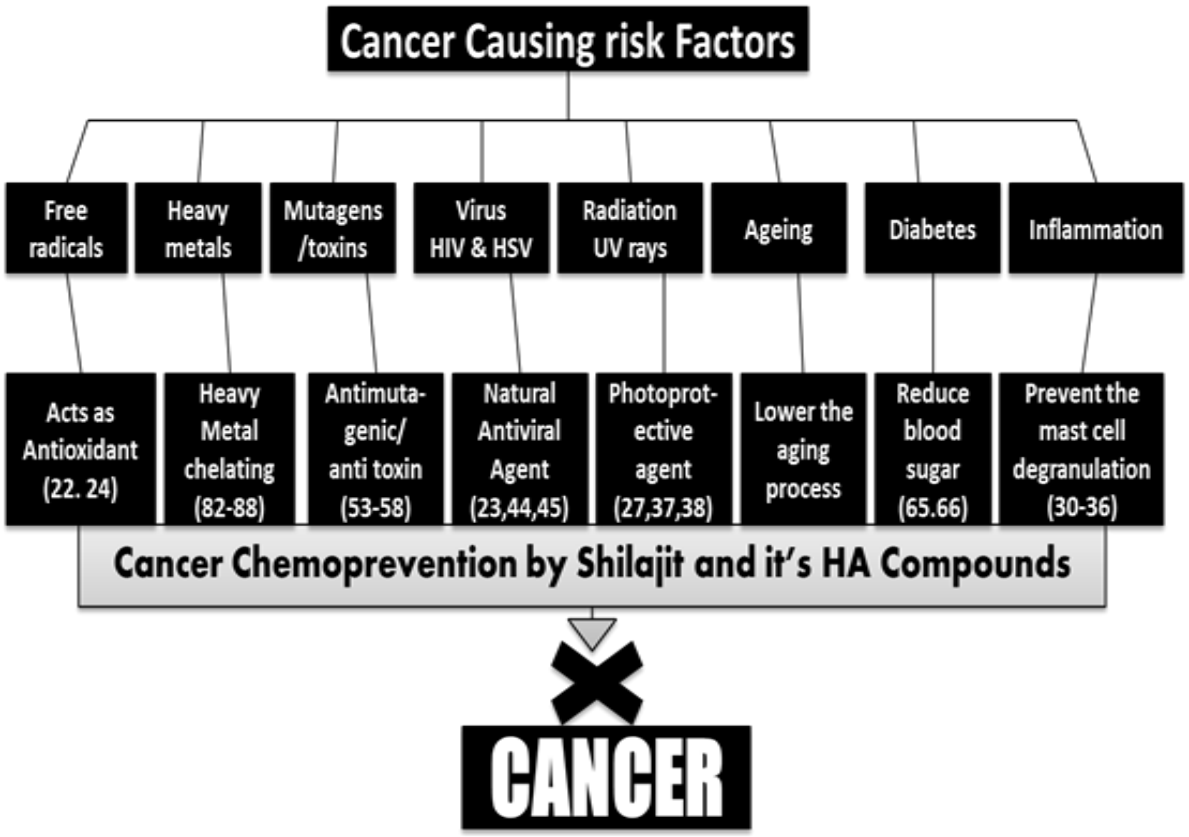
Anti-Aging Properties: The elderly patients (above 65 years old) are more at risk for development of cancer, 12–36 times higher than the 25–44 year old patients and 2–3 times higher than the individuals aged between 45–64 years. Moreover, 70% of total deaths among the people above 65 years of age occur due to cancer (26). DNA repair capacity decreases with increase in age mainly due to decrease in endogenous antioxidants in the body (21) which might lead to the development of cancer. Therefore, intake of antioxidant with increasing age is suggested to arrest the oxidative damage (21). It has been shown that Shilajit can delay the aging process and possibly lower the risk of cell impairment and damage (27, 28). Moreover, Charaka Samhita describes Shilajit as a natural rejuvenator (28) which restore vitality and youthfulness of the body.

Anti-Ulcerative Properties: The relationship between peptic ulcer and gastric cancer is an important aspect, mainly after the infection of Helicobacter pylori which plays an important role in the development of duodenal ulcer, gastric ulcer, as well as gastric cancer. earlier history of gastric ulcer or duodenal ulcers in patients increases the risk of development of gastric cancer or duodenal cancers (29). In duodenal ulcers, Shilajit pre-treatment notably reduced the occurrence of ulcers induce by means of cysteamine in rats and by histamine in guinea pigs. Moreover Shilajit pre-administration orally at the dose of 100mg/kg was found to reduced ulcer index in immobilization and aspirin induced gastric ulcers (17).

Anti-Inflammatory Properties: The inflammation is considered to induce the cancer progression, development and the metastasis (20). It has been shown that the HA matters has anti-inflammatory properties (23). Shilajit has anti-inflammatory properties confirmed in the study of the Goel et al (30). They have shown that the Shilajit can reduce the inflammation such as pedal oedoma and granulama pouch in rats induced by the carrageenan. In addition, Shilajit can also reduce the adjuvant induced arthritis in rats. In another study the humic compounds derived from the coal was found to inhibit the inflammation responded ear swelling in the rats (31).

Lowen et al have described that HA alone or HA combined with other anti-inflammatory drugs such as indomethacin were beneficial for the chronic and acute inflammation in the male Sparague Dwley rats(32). Van Rensburg et al have shown that the presence of potassium humate reduces the level of proinflammatory cytokines like TNF-α, IL-1β, IL-6 and IL-10 produced by mononuclear cells and also the compliment activation (33). HA can inhibit the degranulation of the neutrophils during inflammation (34), moreover it is an anti-allergic agent (34, 35). It has been Oxifulvic acid may be beneficial in the clinical treatment of inflammatory skin conditions in humans as FA possesses anti-inflammatory properties (36).

Photo Protective Properties: The exposure to the radiation as the ultraviolet (UV) rays can leads to the long-term DNA damage by forming the thymine dimer in the DNA which can lead to mutations and cancer. Shilajit can



Figure;1- Cancer risk Factors and the Pharmacological actions of Shilajit and HA Compounds in Cancer Chemoprevention

function as photoprotective agent, as reviewed by Meena et al 2010 (27). It has been shown that Shilajit can save the mountaineers from the sunburn, skin and eye problems by high intensity of the UV light at high altitudes. HA can absorb the luminous radiation including UV and provides photoprotective properties to E. Coli in aqueous solution (37). HA has capacity to absorb radiations and can reduce the amount of energy of radiation reaching to the cells.

Akran et al have reported that the harmful effect of UV radiation on E. coli were reduced in water due to absorption of UV light by humic compounds. Increasing concentrations of fulvic acid emerged to improve its effect with elevated doses of UV radiation tested on the bacterial growth (38). The HA is UVB-absorbing chromophore, which reduces the penetration of the high-energy wavelength lights and thus protects the zooplankton population from UV exposure (39).

Anti-Viral Properties: Several studies have shown that many viral infections are associated with the development of cancer. The possibility of Hodgkin lymphoma is directly related to state of the immunosuppression and HIV (40), whereas AIDS is reported to support the development of the Brukitt's lymphoma (41). Human Papilloma virus (HPV) are directly associated with cervical cancer (41) and Kaposi sarcoma herpes virus (KSHV) infections are also linked to lymphomas and sarcomas in HIV patients (42, 43). It has been suggested that HA can prevent some of these cancers and cancer-causing viruses (23). For example, HA can decrease the HIV infection and replication (44, 45) and also inhibits the viral fusion between the T-cell (44). Furthernore, humic acid can activate the T-lymphocytes (44) and also increases the IL-2 production by TH1 cells in HIV patients (45-47).

In another study, Gupta et al have treated HIV patients with antiretroviral therapy along with Shilajit and they found that the patients improved their appetite, nausea, vomiting, depression, diarrhoea, weight loss, fever, anaemia and they have elevated level of the CD4 along with several other advantages. Therefore, Shilajit and similar humic compounds could be the ray of hope for the immunocompromised patients (48). Herpes simplex virus-1 (HSV-1) can cause genital and oral infections and it is linked to oropharyngeal cancer (49). It has been found that HA posses antiviral and cytotoxic activity in the HSV-1 infected cells (50) and can block the HSV replication (51). The antiviral effect of HA depends upon the increased content of acidic functional groups (carboxyl groups) along with the hydroxyl group (51).

Anti-Mutagenic And Detoxification Properties: In daily routine we are exposed to several harmful chemicals and some of them are potential carcinogens. Exposure to the polycyclic aromatic hydrocarbons (PAH), pesticides, herbicides, toxins and other carcinogenic compound can occur accidently or occupationally (52), which may lead to cancer development. Mutagens such as aflatoxins or mycotoxins, benzo (a) pyrene and pesticides are the most common types of the carcinogenic compounds.

Marova et al have shown that processed HA can inactivate the mutagens which were tested on the yeast (53). They used 4-nitroquinoline-N-oxide (4-NQO), a mutagen on Saccharomyces cerevisiae D7 to check the anti-mutagenic properties of the sodium or potassium humate and found that the sodium humate have significant antimutagenic property against the 4-NQO. Furthermore, Zhang et al checked the toxicity and bioavailability of the ionic liquids (ILs), 1-butyl-3-methylimidazolium chloride and 1-octyl-3-methylimidazolium chloride, in the presence of the HA (54). These results illustrated that the HA reduces the bioavailability and toxicity of these two ILs and also HA increases the viability around 50% of HepG2 cell line against the two ILs. Moreover, the death rate of the model fish Medaka decreased against the lethal dose of two ILs when the HA was present in the water (54).

Potassium humate was observed to detoxify the mutagenic matters, N-methyl-N-nitrosourea and N-nitrosodium-ethylamine in Arabidopsis thaliana (55). HA can inactivate the aflatoxins produced by the fungi. Rensburg et al have shown that the liver damage, stomach and heart enlargement in boiler chicken which occur after intake of aflatoxins were reduced in presence of oxihumate (56). In addition, the serum toxicity caused by the toxin was also found to be reduced significantly ($P < 0.005$). In another study, Santosa et al (57) checked the absorption of the aflatoxins by the humic acid polymer in the digestive system (oral cavity, stomach and intestines) at different pH of monogastric animals. They found that humic acid can absorb polycyclic aromatic hydro-carbons (58, 59). Humic acid can also inactivate 2, 4, 6-Trichlorophenol which can cause lymphomas, leukemia and liver cancer in animals by means of oral contact (60).

The humic compounds have been proved to be excellent agents for reduction of gene mutation caused by pollutants and mutagens. Toxins, pesticides, radioactive metals, petroleum products, polyaromatic hydro-carbons and heavy metals can be inactivated by HA (61, 62). Moreover, it reduces bioavailability of the hazardous matters (62) and averts the formation of the mutagenic or carcinogenic compound and prevent DNA damage in the cell. Anti-Diabetic Property: Several studies have proved that Diabetes is a key factor contributing to the increase in solid organ malignancies or tumor including liver, pancreas, colorectal, breast, endometrial, uterine, and bladder(63, 64). Shilajit can maintain the blood glucose profile and studies on diabetic rats have shown that Shilajit can reduce the blood sugar level and improve the lipid profile by reducing the total cholesterol, triglyceride and high-density lipoproteins in the rats (65). In another study with 32 type II diabetes patients, Upadhyay et al have shown that the uptake of aqueous extract of Ashwagandha (Withania somnifera)-250mg and pure Shilajit extract-250mg for one month led to a decrease in blood sugar level along with low-density lipids, very low-density lipoprotein and fewer ratios of the total cholesterol/ high-density lipoprotein (66). Anti-Tumor Activity; It has been shown that Shilajit and HA are effective anti-tumor and anti-cancer agents (23, 67) which is effective for both malignant and benign tumors (28). HA can increase the production of active oxygen during the wound healing process and in anti-tumor process. Reports by Jurcsik have shown that when HA was incubated with Hep-2 cancer cell line for 24 hours, the proliferation of the HEp-2 cancer cell line was found to be decreased by 65% as compared to the control cell line (68). HA can inhibit the tumor cell multiplication by intercalating with DNA, blocks the DNA opening and destroys the DNA by producing the reactive oxygen derivatives (68). Hiroshi et al in 2007 have shown that 3% of HA extract has antitumor activity on L1210 lymphocytic leukemia cell line with delay of tumor formation and significant reduction in size of tumor. The mice administered with the HA extract may survive for longer time than untreated mice after development of tumor (69). They concluded that antitumor effect was due to direct killing of L1210 and not due to the stimulation of apoptotic cell. This antitumor activity is due to the activation of the innate immune system by humus extracts (69). In another study on mouse breast cell line (Ptas64), the 1:1 ratio of the HA and glucan was found to inhibit the tumor growth up to 92% in the Ptas64 cell line (70).

It has been shown that HA possesses significant cytotoxic activity in the CEM (acute T lymphoblastic leukaemia) cell line (24). HA have been found to exert anti-proliferative action and growth inhibition on HL-60 cells through induction of apoptosis by activating the caspase-3 and mitochondrial cytochrome-c in these cells (67). HA has also been found to induce the apoptosis and inhibit the growth in the human smooth muscle cells. Hseu et al, have reported that HA has been found to inhibit the proliferation of the smooth cells in the G1 phase of the cell cycle and led to the apoptotic cell death of smooth muscles cells (71).

Shilajit and HA matters are immunomodulatory agents (22). Shilajit helps the immune system for increased cytokine production by activated immunological cells and maintains the cell integrity (72). It has been shown that Shilajit can activate the murine peritoneal macrophages and activate splenocytes in tumor bearing animals at initial and later stage of the tumor growth (72). In this study glucan and HA works in synergy for the stimulation of the immune reaction. The glucan and HA were reported to stimulate phagocytosis by stimulating the macrophage and neutrophil. Moreover HA increases the secretion of IL-2 by spleen cells (70, 73). HA and FA can also increase the antibody titre in the plasma (73) and it can stimulate the immune response and extend it for longer period of time (73). HA is reported to induce apoptosis in the Human Primary Fibroblasts and furthermore HA arrest cell growth at G1 phase and also provoke the cell death in the Vascular Smooth Muscle Cells (71). The cancer cells lose the property of apoptotic cell death and thus the application of anti-cancer agents like Shilajit and HA can induce apoptosis thereby providing an alternative in the treatment of cancer (74).

Detoxification of The Heavy Metals: Heavy metals enter the environment by several anthropogenic activities and creates adverse effects on human, animal and plants (75). Accumulation of heavy metals in plant tissues can be

Table 1; - List of the heavy metals inactivated by HA compounds and types of cancer associated with the heavy metals

S.N.	Heavy Metals inactivated by HA	References	Cancers associated to metals
1	Copper (Cu)	(61, 81, 83-88)	Oral, head and neck (89), non-Hodgkin's lymphoma.
2	Lead (Pb)	(61, 78, 83, 88, 90)	Kidney (91), Lung (92), Breast (93) and Stomach cancer (92).
3	Cadmium (Cd)	(61, 82, 84, 87)	Breast (94), Lung (91), Kidney (92), and Prostate cancer (92).
4	Nickel (Ni)	(87)	Nasal (91), Lung (91) and Oral cancer (95).
6	Aluminium (Al)	(96)	Lung (97), Bladder (97), Breast (98).
7	Zink (Zn)	(82, 83, 87)	Prostate cancer (99, 100).
8	Arsenic (As)	(61, 101-103)	Lung (91, 92, 104, 105), Skin (104, 105) and Bladder cancer (92, 105, 106).

hazardous if they are consumed by the humans or animals. This gradual saturation of heavy metals in the human body can lead to mutagenic and carcinogenic effects resulting in many diseases (75). It has been shown that the elevated level of the iron, zinc and copper in the serum could lead to the development of cancer (76). Therefore, it is essential to restrict access of heavy metals from the soil into the plants and the human body.

HA can interact with heavy metals in the soil rendering them as poorly soluble compound and thus can avoid the heavy metal entrance into the organisms (75).

Many experiments support the capacity of the HA to detoxify the heavy metals and lessen the bioavailability in plant and animals. Experiments by Tao et al have shown that the availability of copper was reduced for fish uptake via their gills in presence of fulvic acid in water (77). Zralý et al have demonstrated that the feeding of lead along with HA reduces the accumulation of lead in chicken organs such as bone, muscle, liver and kidney, compared to the chicken feed without HA (78). Similarly, in the other study of Herzig et al states that accumulation of cadmium were reduced in the presence of HA by 39.6% in kidney, 34.2% in liver and 80.8% in muscle as compared to the chicken feed with Cd in the absence of HA. Sanmanee et al have shown that FA treatment reduces the toxicity of copper in the mammalian cell, porcine oviductal epithelial cells (80). In plants, humic compounds have been reported to decrease the toxicity of the copper (Cu) and vanadium (V) in Zea mays (81) and the uptake of the Zinc and Cadmium were reduced in Ceratophyllum demersum (82).

Dosage And Administration: According to Ayurveda, Shilajit should be consumed in a very small amount (500 mg/ml) with milk or water twice a day, to ensure the optimal level of the Shilajit in the body. The optimal level of Shilajit in the body is attained after 10-12 hours post consumption and it is not recommended with a heavy diet and in individuals possessing a high uric acid count (107).

Toxicity of Shilajit: Shilajit has no reported side effects and is non toxic. Velmurugan et al checked the safety profile of the Shilajit. They regularly administrated Shilajit for 3 months (91 days) in mice and concluded that Shilajit is completely safe for long-term usage and can function as a vital drug for anaemia or iron deficiency (108).

CONCLUSION

Shilajit is an ancient medicine and wonder drug of the Ayurveda used by the Hindu physicians for the treatment of several body disorders. The humic matter component of Shilajit has numerous beneficial effects on the human body. Charaka Samhita recommends Shilajit as a cure for numerous human ailments and is highly recommended by the Ayurvedic practitioners. Shilajit and its humic components have anti oxidant, antiviral, anti-inflammatory, anti ulcerative, antimutagenic and heavy metal chelating properties. Therefore, Shilajit and humic compounds with these properties would possibly be a useful cancer chemopreventive agent and may be beneficial in cancer therapy. In addition, Shilajit is useful in arthritis, can function as anti fungal agent as well as nutritive tonic that help in the nutrient transfer to the deeper tissues of our body. Shilajit has immense therapeutic properties and certainly can be proved to be a boon for mankind possibly in cancer prevention and therapy.

REFERENCES

1. Mukhtar H, Ahmad, N. Cancer chemoprevention: future holds in multiple agents: contemporary issues in Toxicology. Toxicol Appl Pharmacol 1999;158:207-10.
2. Agarwal SP, Khanna, R., Karmarkar, R., Anwer, M.K., Khar, R.K. Shilajit: a Review. Phytother Res. 2007;21:401-5.
3. Ghosal S, Lal, J., Singh,S.K., Goel, R.K., Jaiswal, A.K., Bhattacharya,S.K. The Need for Formulation of Shilajit by Its Isolated Active Constituents-9. Phytother Res. 1991b;5:211-6.
4. Chopra RN, Chopra, I.C., Handa,K.L., Kapur,L.D. Chopra's Indigenous drugs of india 2nd ed. B Calcutta India; K Dhur of academic Publishers. 1958.
5. Murkherjee B. Traditional Medicine, New delhi. proceedings of an international seminar, Calcutta India Oxford and IBH Publishing. november 1992:308-19.

6. Nadkarni KM. Indian Materia Medica. 3rd edition. Popular Prakashan Private Ltd Bombay, India. 1954;2:23.
7. Bucchi LR. Selected herbals and human exercise performance. American Society for Clinical Nutrition. 2000;72(2):624S-36S.
8. Tirtha SSS. The Ayurvedic Encyclopedia. Bayville, NY: Ayurveda Holistic Center Press; 1998.
9. Puri HS. Rasayana. Taylor & Francis London, England. 2003.
10. Talbert R. Shilajit a materia medica monograph, Degree paper Grass Valley, California: California College of Ayurveda; 2004.
11. Srivastava RS, Kumar Y, Singh SK, Ghosal S. Shilajit, its source and active principles. Proc 16 IUPAC (Chemistry of Natural Products). Kyoto Japan. 1988:524.
12. Sharma PV. In Darvyaguna Vijnan, 4th edn. Chaukkhamba Sanskrit Sansthan Varanasi. 1978:63.
13. Ghosal S, Lal J, Singh SK. The Core Structure of Shilajit Humus. Soil Biol Biochem. 1991a;23:673–80.
14. Frolova LN, Kiseleva TL. Chemical Composition of Mumie and Methods for Determination of Its Authenticity and Quality. ChemPharm J. 1996;8:49–53.
15. Ghosal S, Singh SK, Kumar Y, Srivastava RS, Goel RK Dey, Bhattacharya SK. Anti-Ulcerogenic Activity of Fulvic Acids and 4′-Methoxy-6-Carbomethoxybiphenyl Isolated From Shilajit. Phytother Res. 1988;2:187-91.
16. Schepetkin I, Khlebnikov A, Kwon BS. Medical Drugs From Humus Matter: Focus on Mumie. Drug Dev Res. 2002;57:140-59.
17. Goel RK, Banerjee RS, Acharya SB. Antiulcerogenic and Antiinflammatory Studies With Shilajit. J Ethnopharmacol. 1990;29:95-103.
18. Acharya SB, Frotan MH, Goel RK, Tripathi SK, Das PK. Pharmacological Actions of Shilajit. Indian J Exp Biol. 1988;26:775-7.
19. Islam KMS, Schuhmacher A, Gropp J.M. Humic Acid Substances in Animal Agriculture. Pakistan Journal of Nutrition. 2005;4(3):126-34.
20. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species : role in inflammatory disease and progression to cancer. Biochem J 1996;313:17-29.
21. Borek C. Dietary Antioxidants and Human Cancer. integrative cancer therapies 2004;3(4):333-41.
22. Schepetkin IA, Khlebnikov AI, Ah SY, Woo SB, Jeong CS, Klubachuk ON, Kwon BS. Characterization and Biological Activities of Humic Substances from Mumie. J Agric Food Chem 2003;51:5245-54.
23. Peña-Méndez EM, Havel, J, Patočka J. Humic substances - compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine. Appl Biomed. 2005;3:13-24.
24. Vašková J, Veliká B, Pilátová M, Kron I, Vaško L. Effects of humic acids in vitro. In Vitro CellDevBiol—Animal. 2011;47:376-82.
25. Guerra LN, Moiguer, S, Karner M, de Molina MRC, Sreider CM, Burdman JA. Antioxidants in the Treatment of Graves Disease. IUBMB Life. 2001;51:105-9.
26. Caruso C, Lio D, Cavallone L, Franceschi C. Aging, Longevity, Inflammation, and Cancer. Ann NY Acad Sci. 2004;1028:1-13.
27. Meena H, Pandey HK, Arya MC, Ahmed Z. Shilajit: A panacea for high-altitute problems. International Journal of Ayurveda Research. 2010;1(1):37-40.
28. Murthy KRS. Astanga Hrdayam: Krishnadas Academy, Varanasi, India,; 2001.
29. Molloy RM, Sonnenberg A. Relation between gastric cancer and previous peptic ulcer disease. Gut 1997;40:247-52.
30. Goel RK, Banerjee RS, Achrarya SB. Antiulcerogenic and antiinflammatory studies with Shilajit. J Ethnopharmacol. 1990;29:95-103.
31. Van Rensburg CEJ, Snyman J R, Mokoele T, Cromarty A D. Brown Coal Derived Humate Inhibits Contact Hypersensitivity; An Efficacy, Toxicity and Teratogenicity Study in Rats. Inflammation. 2007;30(5).
32. Lown JF, Gill K, Cutler SJ, Cutler HG, Pollock SH, inventor; Anti-inflammatory humate compositions and metohs of use thereof. Dallas, TX (US)2006.
33. van Rensburg CEJ, and Naude PJ. Potassium Humate Inhibits Complement Activation and the Production of Inflammatory Cytokines In Vitro. Inflammation. 2009;32(4):270-6.
34. Gisela K, Elizabeth C, Rensburg JV. An In Vitro Investigation of the Anti-Inflammatory Properties of Potassium Humate. . inflammation. 2004;28:169-74.
35. Amada PY, Isoda H, Yamaguchi T, Talorete TPN, Abe Y . Inhibitory Effect of Fulvic Acid Extracted from Canadian Sphagnum Peat on Chemical Mediator Release by RBL-2H3 and KU812 Cells. . Biosci Biotechnol Biochem 2007;71:1294-305.
36. Van Rensburg CEJ, Malfeld SCK, Dekker J. Topical Application of Oxifulvic Acid Suppresses the Cutaneous Immune Response in Mice. Drug development research. 2001;53:29-32.
37. Muela A, Garcia-Bringas JM, Barcina AI. Humic Materials Offer Photoprotective Effect to Escherichia coli Exposed to Damaging Luminous Radiation. Microb Ecol. 2000;40:336-44.
38. Alkan U, Teksoy A A, Baskaya HS. Influence of humic substances on the ultraviolet disinfection of surface waters. Water and Environment Journal 2007;21:61-8.
39. Clair TA, Ehrman J, Kaczmarska I, Locke A, Tarasick DW, Day KE, Maillet G. Will reduced summer UV-B levels affect zooplankton populations of temperate humic and Clearwater lakes? Hydrobiologia. 2001;462:75-89.
40. Biggar RJ, Jaffe, E. S., Goedert, J. J., Chaturvedi, A., Pfeiffer, R., Engels, E. A.,. Hodgkin lymphoma and

immunodeficiency in persons with HIV / AIDS,. Blood. 2011;108:3786-91.
41. Harrington WJ, Wood, C. AIDS and Associated Malignancies. DNA Tumor Viruses2009. p. 683-702.
42. Franceschi S, Maso LD, Rickenbach M, Polesel J. Hersal B, Cavassini M, Bordoni A, Elzi L, et al. Kaposi sarcoma incidence in the Swiss HIV Cohort Study before and after highly active antiretroviral therapy. British Journal of Cancer. 2008;99:800-4.
43. Emuss V, Boshoff C. Kaposi ' s sarcoma and the lymphatics.: Springer Science+Business Media B.V; 2009.
44. Mauizio Z, inventor; Treatment of HIV infection with Humic acid. Paris2002.
45. Dekker J, Medlen CE, inventor; Oxihumic acid and its use in the treatment of the various conditions2003.
46. Botes ME, Dekker J, van Rensburg CEJ. Phase I Trial With Oral Oxihumate in HIV-Infected Patients. Drug Development Research 2002;57:34-9.
47. Jooné GK, DekkerJ , van Rensburg CE. Investigation of the immunostimulatory properties of oxihumate. Z Naturforsch C. 2003;58:3-4.
48. Gupta GD, Sujatha, N., Dhanik, A., Rai, N.P. Clinical Evaluation of Shilajatu Rasayana in patients with HIV Infection. AYU. 2010;31(1):28-32.
49. Starr JR, Janet R, Daling E, Fitzgibbons F, Margaret M, Ashley MR, Denise A, Galloway DA, Schwartz SM. Serologic Evidence of Herpes Simplex Virus 1 Infection and Oropharyngeal Cancer Risk. Cancer res. 2001;61:8459-64.
50. Helbig B, Klöcking R, Wutzler P. Anti-herpes simplex virus type 1 activity of humic acid-like polymers and their o-diphenolic starting compounds. Antiviral chemistry & chemotherapy. 1997;8(3):265-73.
51. Klöcking R, Helbig B, Schötz G, Schacke M, Wutzler P. Anti-HSV-1 activity of synthetic humic acid-like polymers derived from p-diphenolic starting compounds. Antiviral Chemistry & Chemotherapy. 2002;13:241-9.
52. Boffetta P, Jourenkova N, Gustavsson P . Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. Cancer Causes and Control. 1997;8:444-72.
53. Marova I, Kucerik J, Duronova K, Mikulcova A, Vlckova Z. Antimutagenic and/or genotoxic effects of processed humic acids as tested upon S. cerevisiae D7. Environ Chem Lett 2011;9:229-33.
54. Zhang Z, Liu J, Cai X, Jiang W, Luo W. Jiang G. Sorption to Dissolved Humic Acid and Its Impacts on the Toxicity of Imidazolium Based Ionic Liquids. Environ Sci Technol. 2011;45:1688-94.
55. Gichner T, Badaev SB, Pospisil F, Velemimnsky J. The Effect of Humic Acids, Fractionated According to Molecular Mass, on the Formation and Mutagenicity of N-MethyI-N-Nitrosourea. Biologia plantarum (Praha). 1989;31(5):392-9.
56. Van Rensburg CJ, Van Rensburg, CEJ, Van Ryssen JBJ, Casey NH, Rottinghaus GE. In Vitro and In Vivo Assessment of Humic Acid as an Aflatoxin Binder in Broiler Chickens. Poultry Science 2006;85:1576-83.
57. Santosa SS, Vermeulena, S., Haritovab, A., Fink-Gremmels, J. Isotherm modeling of organic activated bentonite and humic acid polymer used as mycotoxin adsorbents. Food Additives and Contaminants 2011;28(11):1578-89.
58. Kollist-Siigur K, Nielsen T, Grøn C, Hansen PE, Helweg C, Jonassen KEN, Jørgensen O, Kirso U. Sorption of Polycyclic Aromatic Compounds to Humic and Fulvic Acid HPLC Column Materials. Published in J Environ Qual 2001;30:526-37.
59. Nielsen T, Siigur K, Helweg C, Jørgensen O, Hansen PE, Kirso U. Sorption of Polycyclic Aromatic Compounds to Humic Acid As Studied by High-Performance Liquid Chromatography. Environ Sci Technol 1997;31:1102-8.
60. Wang XP, Shan XQ, Luo L, Zhang SJ, Wen B. Sorption of 2,4,6-Trichlorophenol in Model Humic Acid-Clay Systems. J Agric Food Chem 2005, 53, . 2005;53:3548-55.
61. Yates LM, Wandruszka, R.V. Decontamination of Polluted Water by Treatment with a Crude Humic Acid Blend. Environ Sci Technol. 1999;33:2076-80.
62. Perminova IV, Hatfield, K. . Remediation Chemistry of Humic Substances: Theory and Implications for Technology. In: Perminova IV, editor. Use of Humic Substances to Remediate Polluted Environments: From Theory to Practice. NetherlandS: Springer; 2005. p. 3-36.
63. Huxley R, Moghaddam AA, deGonzalez AB, Barzi F, Woodward M. Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies. British Journal of Cancer. 2005;92:2076-83.
64. Smith U, Gale EAM. Does diabetes therapy influence the risk of cancer? Diabetologia. 2009.
65. Trivedi NA, Mazumdar, B J, Bhatt D, Hemavathi K G. Effect of Shilajit on blood glucose and lipid profile in alloxaninduced diabetic rats. Indian J Pharmacol 2004;36(6):373-6.
66. Upadhyay AK, Kumar, K., Mishra, H. Effects of combination of Shilajit extract and Ashwagandha (Withania somnifera) on fasting blood sugar and lipid profile. Journal of Pharmacy Research 2009;2(5):897-9.
67. Yanga HL, HseuYC, Yi-Ting Hseua, Luc FJ, Linb E, Laid JS. Humic acid induces apoptosis in human premyelocytic leukemia HL-60 cells. fe Sciences. 2004;75(15):1817-31.
68. Jurcsik I. Possibilities of applying humic acid in the medicine (wond healing and cancer theraphy). In: Senesi N, Miano, T.M., editor. Humic substances in the global Environment and implications on human health: Elsevier Science B.V.; 1994.
69. Hiroshi K, Denso. Antitumor Effect of Humus Extract on Murine Transplantable L1210 Leukemia. J Vet Med Sci. 2007;69(10):1069-71.

70. Vetvicka V, Baigorri, R., Zamarreiio, A.M., Garcia-Mina, J.M., Yvin, J.C. Glucan and Humic Acid: Synergistic Effects on the Immune System. J of Med Food. 2010;13(4):863-9.
71. Hseu YC, Lin E, Chen JY, Liua R, Huang CY, Lu FJ, Liao JW, Chen SC, Yang HL. Humic Acid Induces G1 Phase Arrest and Apoptosis in Cultured Vascular Smooth Muscle Cells. Environmental Toxicology. 2008;24:243-58.
72. Ghosal S. Chemistry of Shilajit, an immunomodulatory Ayurvedic rasayan. Pure & Appl Chern. 1990;62(7):1285-8.
73. Vucskits AV, Hulla, I., Bersenyi, A., Andrasofszky, E, Kulcsar M, Szabo J. Effect of fulvic and humic acids on performance, immune response and thyroid function in rats. Journal of Animal Physiology and Animal Nutrition 2010;94:271-728.
74. Gerl R, Vaux, D.L. Apoptosis in the development and treatment of cancer. Carcinogenesis 2005;26(2):263-70.
75. Gorova A, Skvortsova, T., Klimkina, I., Pavlichenko, A. . Cytogenetic effects of humic substances and their use for remediation of polluted environments. Use of Humic Substances to Remediate Polluted Environments: From Theory to Practice. Netherland: Springer; 2005. p. 311-28.
76. Wu T, Sempos CT, Freudenheim JOL, Muti P, Smit E. Serum Iron , Copper and Zinc Concentrations and Risk of Cancer Mortality in US Adults. Ann Epidemiol 2004;14:195-201.
77. Tao S, Xu S, Cao J, Dawson R. Bioavailability of Apparent Fulvic Acid Complexed Copper to Fish Gills. Bull Environ Contam Toxicol. 2000;64:121-7.
78. Zralý Z, Písaříková B, Trčková M, Navrátilová M. Effect of Humic Acids on Lead Accumulation in Chicken Organs and Muscles. ACTA Vet brno. 2008;77:439-45.
79. Herzig I, Navratilova, M, Suchy P, Vecerek V, Totus J. Model trial investigating retention in selected tissues using broiler chicken fed cadmium and humic acid. Veterinarni Medicina. 2007;52:162-8.
80. Sanmanee N, Areekijseree, M. The Effects of Fulvic Acid on Copper Bioavailability to Porcine Oviductal Epithelial Cells. Biol Trace Elem Res 2010;135:162-73.
81. Ullah SM, Gerzabek, M.H. Influence of fulvic and humic acids on Cu-toxicity and V-toxicity to Zea mays. Bodenkultur. 1991;42:123-34.
82. Bunluesin S, Pokethitiyook P, Lanza GR, Julian FT, Maleeya K, Baoshan X, Suchart U. Influences of Cadmium and Zinc Interaction and Humic Acid on Metal Accumulation in Ceratophyllum Demersum. Water Air Soil Pollut. 2007;180:225-35.
83. Kezhong G, Jack P, Jeniffer J, Colin T, Colin T. Interaction between peat, humic acid and aqueous metal ions. Environmental Geochemistry and Health. 1999;21:13-26.
84. Xue H, Sigg L. Comparison of the Complexation of Cu and Cd by Humic or Fulvic Acids and by Ligands Observed in Lake Waters. Aquatic Geochemistry. 1999;5:313-35.
85. Winner RW. Bioaccumulation and toxicity of copper as affected by interactions between humic acid and water hardness. Water Res. 1985;19:449-55.
86. Sanmanee N, Areekijseree M. The Effects of Fulvic Acid on Copper Bioavailability to Porcine Oviductal Epithelial Cells. Biol Trace Elem Res. 2010;135:162-73.
87. Chen Y, Mao X, Zhu D, Synthesis of macroporous Humic acid resins and their Chelating properties for heavy metal ions. Polymer communications. 1984:159-68.
88. Christl I, Metzger A, Heidmann I, Kretzschmar R . Effect of Humic and Fulvic Acid Concentrations and Ionic Strength on Copper and Lead Binding. Environmental Science & Technology. 2005;39:5319-26.
89. Coates RJ et al . Cancer Risk in Relation to Serum Copper Levels Cancer res. 1989;49:4353-6.
90. Malcova R, Gryndler M, Hrselova H, Vosatka, M. The Effect of Fulvic Acids on the Toxicity of Lead and Manganese to Arbuscular Mycorrhizal Fungus Glomus intraradices. Folia Microbio. 2002;47(5):521-6.
91. Hayes RB. The carcinogenicity of metals in humans. Cancer causes and control. 1997;8:371-85.
92. Järup L. Hazards of heavy metal contamination. British Medical Bulletin 2003;68:167-82.
93. Mcelroy JA, Shafer MM, Gangnon RE, Crouch LA, Newcomb P.A . Urinary Lead Exposure and Breast Cancer Risk in a Population-Based Case-Control Study. Cancer Epidemiol Biomarkers Prev. 2008;17:2311-7.
94. Gallagher CM, Chen, JJ , Kovach JS. Environmental cadmium and breast cancer risk. aging. 2010;1:804-14.
95. Su C-c, Lin,Yo-Yu., Chang,Tsun-Kuo., Chiang, Chi-Ting., Chung, Jian-An., Hsu, Yun-Ying., Lian, Ie-Bin.,. Incidence of oral cancer in relation to nickel and arsenic concentrations in farm soils of patients ' residential areas in Taiwan. BMC Public Health. 2010;10:1-10.
96. Gardner JL, Al-Hamdani S H. Interactive effects of aluminum and humic substances on Salvania. Journal of Aquatic Plant Management. 1997;35:30-4.
97. Romundstad P, Haldorsen T. Andersen A . Lung and bladder cancer among workers in a Norwegian aluminium reduction plant. Occupational and Environmental Medicine. 2000;57:495-9.
98. Exley C, Charles L M, Barr L, Martin C, Polwart A, Darbre PD. Aluminium in human breast tissue. Journal of Inorganic Biochemistry. 2007;101:1344-36.
99. Krone CA, Harms LH . Zinc Supplement Use and Risk of Prostate Cancer. Journal of the National Cancer Institute 2003;95:95-6.
100. Gallus Sea. Dietary Zinc and Prostate Cancer Risk : A Case-Control Study from Italy. european urology. 2007;52:1052-7.

101. Weng L, Williem H, Van R, Tjisse H. Effects of Fulvic and Humic Acids on Arsenate Adsorption to Goethite: Experiments and Modeling. Environ Sci Technol. 2009;43:7198-204.
102. Palmer NE, Wandruszka RV. Humic acids as reducing agents: the involvement of quinoid moieties in arsenate reduction. Environ Sci Pollut Res. 2010;17:1362-70.
103. Buschmann J, Kappeler A, Lindauer U, Kistler D, Berg M, Sigg L Arsenite and Arsenate Binding to Dissolved Humic Acids: Influence of pH, Type of Humic Acid , and Aluminum. Environmental Science & Technology. 2006;40:6015-20.
104. Mazumder DNG. Chronic arsenic toxicity & human health. Indian Journal Of Medical Research. 2008;128:436-47.
105. Huang C, Ke Q, Costa M, Shi X Molecular mechanisms of arsenic carcinogenesis. Molecular and Cellular Biochemistry. 2004;255:56-66.
106. Moore LE, et al. Arsenic-Related Chromosomal Alterations in Bladder Cancer. Journal of the National Cancer Institute. 2002;94:1688-96.
107. Thirtha SSS. The Ayurveda Enccyclopedia. Bayville, NY: Ayurveda Holstic Center Press; 1998.
108. 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 Pacific Journal of Tropical Biomedicine. 2012:210-114