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Shilajit attenuates behavioral symptoms of chronic fatigue syndrome by modulating the hypothalamic–pituitary–adrenal axis and mitochondrial bioenergetics in rats

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ABSTRACT

Ethnopharmacological relevance: Shilajit has been used as a rejuvenator for ages in Indian ancient traditional medicine and has been validated for a number of pharmacological activities.
Aim of the study: The effect of processed shilajit which was standardized to dibenzo-α-pyrones (DBPs;0.43% w/w), DBP-chromoproteins (DCPs; 20.45% w/w) and fulvic acids (56.75% w/w) was evaluated in a rat model of chronic fatigue syndrome (CFS). The mitochondrial bioenergetics and the activity of hypothalamus–pituitary–adrenal (HPA) axis were evaluated for the plausible mechanism of action of shilajit.
Materials and Methods: CFS was induced by forcing the rats to swim for 15 mins for 21 consecutive days. The rats were treated with shilajit (25, 50 and 100 mg/kg) for 21 days before exposure to stress procedure. The behavioral consequence of CFS was measured in terms of immobility and the climbing period. The post-CFS anxiety level was assessed by elevated plus maze (EPM) test. Plasma corticosterone and adrenal gland weight were estimated as indices of HPA axis activity. Analysis of mitochondrial complex chain enzymes (Complex I, II, IV and V) and mitochondrial membrane potential (MMP) in prefrontal cortex (PFC) were performed to evaluate the mitochondrial bioenergetics and integrity respectively.
Results: Shilajit reversed the CFS-induced increase in immobility period and decrease in climbing behavior as well as attenuated anxiety in the EPM test. Shilajit reversed CFS-induced decrease in plasma corticosterone level and loss of adrenal gland weight indicating modulation of HPA axis. Shilajit prevented CFS-induced mitochondrial dysfunction by stabilizing the complex enzyme activities and the loss of MMP. Shilajit reversed CFS-induced mitochondrial oxidative stress in terms of NO concentration and, LPO, SOD and catalase activities.
Conclusion: The results indicate that shilajit mitigates the effects of CFS in this model possibly through the modulation of HPA axis and preservation of mitochondrial function and integrity. The reversal of CFS-induced behavioral symptoms and mitochondrial bioenergetics by shilajit indicates mitochondria as a potential target for treatment of CFS.

1. Introduction

Fatigue can be clinically defined as a feeling of lack of energy resulting not exclusively from exertion. If fatigue is disabling and is accompanied by other constitutional and neuropsychiatric symptoms and lasts more than 6 months, a diagnosis of chronic fatigue syndrome (CFS) should be considered (Fukuda et al., 1994). The absence of concrete etiopathology makes CFS diagnosis difficult (Van Houdenhove and Luyten, 2007). Disturbances in the stress sensitive hypothalamic–pituitary–adrenal (HPA) axis as well as in brain neurotransmitter balances, particularly serotonin and norepinephrine have been reported due to exposure of chronic stress (Jerjes et al., 2007). The brain fMRI and morphometric studies have shown fatigue-related abnormalities in the frontal lobe in patients with CFS (Tanaka et al., 2006; Cook et al., 2007). Selective serotonin reuptake inhibitors have been widely prescribed, however chronic administration failed to show clinically significant effects in treatment of CFS (Maquet et al., 2006). Hence, there has been interest in alternative medicines for

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treatment of CFS. A clinical study based on biochemistry of the illness observed a remarkable correlation between the degree of mitochondrial dysfunction and the severity of CFS (Myhill et al., 2009). Of particular interest is the class of adaptogenic plants such as *Panax ginseng* and *Nardostachys Jatamansi* which have been reported to attenuate symptoms of CFS (Lylea et al., 2009). Shilajit has been used in traditional medicine for over 3000 years as a rejuvenator and an adaptogen (Sharma, 1978). Hence, we presume that shilajit may have beneficial effects in the treatment of CFS.

Shilajit is blackish brown exudate of variable consistency obtained from the rocky layers of mountain ranges (Kong et al., 1987). Shilajit comprises of 60–80% humus along with other organic components such as benzoic acid, hippuric acid, fatty acid, ichthyol, ellagic acid, resin, triterpenes, sterol, aromatic carboxylic acid, 3,4-benzocoumarins, amino acids and phenolic lipids (Srivastava et al., 1988). The major physiological action of shilajit has been reported to be due to the presence of bioactive dibenzo alpha pyrones along with humic and fulvic acids as carrier molecules for the active ingredients (Ghosal, 1990). Dibenzo alpha pyrones have been shown to protect mitochondrial function in hypoxic rats (Bhattacharyya et al., 2009a). Natural organic matter such as humic acid and fulvic acid acting as carrier molecules for the active ingredients can enhance the intestinal absorption and blood brain barrier penetration (Mirza et al., 2011). Processed shilajit has been reported to significantly modulate the central nervous system thereby showing learning augmentation, antistress activity, memory enhancement and anxiolytic activity (Agarwal et al., 2007). *Withania somnifera* (WS) has been used as prototype anti-stress agent (Bhattacharya and Muruganandam, 2003). Another factor in choosing WS, apart from its reported anti-CFS effect (Singh et al., 2002) is its effect on the mitochondrial function. Standardized extract WS dose-dependently attenuated ATP-depletion and other energy related indices during short and long-term FST (Bhattacharyya et al., 2009b).

In summary, the present study assesses the efficacy of shilajit in a stress-induced rat model of CFS. The effect of shilajit on the HPA axis was evaluated by estimating plasma corticosterone. Further, the PFC mitochondrial function and integrity was evaluated by measuring the activity of mitochondrial respiratory complex enzyme systems and mitochondrial membrane potential (MMP) respectively.

2. Materials and methods

2.1. Drugs and standardization

Processed and standardized shilajit was obtained from Natreon Inc, India. Standardization of shilajit with respect to bioactive contents (dibenzo-α-pyrones (DBPs), DBP-chromoproteins (DCPs) and fulvic acids) was done as reported earlier (Biswas et al., 2009). Briefly, high performance liquid chromatography (HPLC) was carried out in a WATERS (USA) HPLC system with PDA detector and isocratic mobile phase consisting of acetonitrile:orthophsophoric acid:water (32:1:67) with a flow rate of 0.6 ml/min using C-18 Novapak reverse phase column attached with a guard column for separation. The injection volume was 20 μl in water. The photodiode array detector wavelength was set at 240 nm. Quantification was done using the authentic markers. The shilajit contained DBPs (0.43% w/w); DCPs (20.45% w/w) and Fulvic acids (56.75% w/w).

Withania somnifera (WS) extract was obtained from Indian Herbs Ltd., India. WS was standardized with respect to their bioactives viz., withanolide glycosides, withaferin A and oligosaccharides content according to the published procedure (Bhattacharyya et al., 2009b). Briefly, HPLC analysis of withanolide glycosides and withaferin-A were performed using Waters HPLC system with PDA detector and Empower software with a Merck-HibarR pre-packed column (RT 250-4, LiChrosorbR RP-18, particle size 5 μm, 4×250 mm cartridge column) fitted with a reverse phase guard column and acetonitrile:water-1:1 (v/v) as the mobile phase, with a run time of 20 min and flow rate 0.6 ml/min in an isocratic mode, using withaferin A (isolated by multiple column chromatography) as an external standard. Oligosaccharides were determined using Waters HPLC system with a RI detector and Empower software with a carbohydrate analysis column [Waters] 300×3.9 mm; acetonitrile:water-80:20 (v/v) was used as the mobile phase; the run time was 10 min and flow rate was 2 ml/min in an isocratic mode. *Withania somnifera* extract was found to contain 14.56% w/w withanolide glycosides, 0.36% w/w Withaferin A and oligosaccharides 39.03% w/w.

2.2. Chemicals

Tetra methyl rhodamine methyl ester (TMRM) and Griess reagent were procured from Sigma Aldrich (St. Louis, MO, USA). Sodium succinate, sodium azide, Phenazine methane sulphonate (PMS) and Nitro blue tetrazolium were purchased from Merck (Daidrmstadt, Germany). All other chemicals and reagents were procured from local suppliers and were of analytical grade.

2.3. Animals

Charles Foster albino rats (140–150 g) were obtained from the Central Animal House, Institute of Medical Sciences; Banaras Hindu University (B.H.U). The animals were housed in polypropylene cages at an ambient temperature of 25 °C ± 1 °C and 45–55% RH, with a 12:12 h light/dark cycle. They had free access to commercial food pellets (Amrut Laboratory Animal feed, Sangli, India) and water. The experimental procedures were approved by Institutional animal ethical committee, B.H.U. The animals were cared in accordance with the Guide to the Care and Use of Experimental Animals (Vol. 1, 2nd ed., 1993, and Vol. 2, 1984). Animals were divided into six sets of 6 animals each. The sets represented the control group, stress control group, positive control (WS 100 mg/kg) and three doses of Shilajit (25, 50 and 100 mg/kg). The test and standard drugs were administered orally in 0.2% carboxy methyl cellulose (CMC) suspension. The dosing was done 1 h before the induction of stress procedure for 21 days. The stress control group received vehicle (0.2% CMC solution). On the 21st day, 30 mins after chronic swim stress the rats were tested in the elevated plus maze (EPM). The rats were then immediately decapitated and the prefrontal cortex was micro-dissected and stored immediately at −80 °C until further experimentation.

2.4. Chronic fatigue induced by forced swimming

Animals were forced to swim for a 15 min session every day for 21 days, in a fabricated metal cylinder (d=35 cm; h=45 cm) containing water up to 30-cm height at room temperature (24–28 °C). The total duration of immobility period and climbing period was measured in seconds using ANY-maze behavioral tracking system, USA (version—4.72) for the periods of 0–5, 5–10 and 10–15 min every day for 21 days (Porsolt, 1981; Lylea et al., 2009). Fig. 1.

2.5. Evaluation of anxiety

Elevated plus maze was used to analyze the anxiety in rats. The fabricated maze consists of two opposite open arms, 50 cm × 10 cm with 40-cm high walls and elevated to a height