LUPINE PUBLISHERS

Lupine Online Journal of Pharmacology & Clinical Research

DOI: 10.32474/LOJPCR.2022.03.000152

ISSN: 2644-1373

Research Article

Is Shilajit a Safe and Non-Toxic Substance to Study the Healing Effect on Oral Ulcers?

Fatemeh Lavaee[1], Fateme Zarei[2], Maryam Shahrokhi Sardo[3]* and Vida Massahi[4]

[1]*Assistant Professor of Oral and Dental Disease Research Center, Oral and Maxillofacial Disease Department*

[2]*School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran*

[3]*Graduated students, school of dentistry, Shiraz University of medical sciences, Shiraz University of Medical Sciences, Ghasr-dasht Street, Shiraz, Iran*

[4]*BA of English translation, Graduated of Narjes university of Rafsanjan, Iran*

***Corresponding author:** Maryam Shahrokhi Sardo, Graduated students, school of dentistry, Shiraz University of medical sciences, Shiraz University of Medical Sciences, Ghasr-dasht Street, Shiraz, Iran

Received: December 06, 2021 **Published:** January 04, 2022

Abstract

Aim: Although many studies have described the therapeutic effects of Shilajit (mumijo), a large number of its therapeutic effects are still mysterious. This substance is used because it is considered to be able to be effective in the improvement of oral ulcers. In this study we aimed to determine the cytotoxicity of shilajit.

Method and material: This is an in-vitro assessment to determine the cytotoxicity of shilajit on Standard HeLa mucosal epithelial cells. The mumijo used in this project was prepared from the mountains of Sardoyeh region of Jiroft city. Different concentrations of mumijo have been evaluated for cell cytotoxicity by MTT colorimetric method. The initial concentration was (50 mg)/ (2 ml). It is serially diluted, and each concentration is half of the previous one. Optical density was registered for each concentration. Cell viability and cytotoxicity have been calculated for each concentration.

Results: The concentrations less than 50×2-6(mg/ml) had acceptable cell viability, and concentrations more than 50×2-6(mg/ml) were cytotoxic.

Conclusion: Shilajit is a natural safe biocompatible substance which can be used in the clinical studies for its therapeutic effect on oral ulcers.

Keywords: Cost-Sharing Research; Conjoint Analysis; Economic Axiomatization; Inconsistency; Value Assessment; Choice Models

Introduction

There is a global effort for introducing new treatments for oral ulcers with different pathogenesis. The treatment effect is considered during the prescription in addition to the safety and side effects of these treatments. These properties should be according to the routine gold standard treatment modality of oral diseases. One of the approaches is natural compounds, including herbs, which has been taken into the consideration of numerous studies. Using herbs in the ancient medicine has been common in many regions of the world [1]. One of this popular herbal medicine is mumijo. There are many studies about mumijo and its therapeutic effects on the different diseases like brain edema, intracranial pressure [2] and bone pains and fractures [3-5]. Memory improving, neuroprotective effect, anti-inflammatory, antioxidant, anti-allergic effects have also been reported in literatures [6]. Shilajit has also an antiviral activity against a panel of viruses, exhibited a dose-dependent inhibitory activity against herpes simplex types 1 and 2(HSV1, HSV2), human cytomegalovirus (HCMV), and human respiratory syncytial virus (RSV) infectivity in vitro but it was inactive against human rotavirus (HRV) and vesicular stomatitis virus (VSV) [7].

The biological effect of Shilajit has been attributed to di- benzo-alpha-pyrone, humic acid, fulvic acid, a bioactive compound and folic acid contents. [8,9] Shilajit is a light brownish material obtained from rocks, such as the Himalaya Mountain between India and Nepal, as well as mountains in Russia, Tibet, Afghanistan, and Norwa [8].

Asian Shilajit (Mumiju) includes 20% minerals, 15% protein, 5% lipids, 5% steroids and some carbohydrates, alkaloids and amino acids [10]. With regard to the many beneficial effects mentioned for mumijo (Shilajit), we hypothesized that the administration of this substance can be effective in the improvement of oral ulcers. To set up a new therapeutic formulation of Shilajit for oral ulcer treatment, it is necessary to assess the epithelial cytotoxicity of Shilajit. HeLa cell line is cervical epithelioid carcinoma cells with fast growth. Within 24 hours, the initial cell count becomes double, and this allows them to be used in cell cytotoxicity evaluations of different medication, toxins and Radiation [11,12]. In many studies, HeLa cells are used for toxicity assessment of different medicine [13,14]. In the present study, different concentrations of Shilajit were used to determine the cytotoxicity of HeLa cells as epithelial-like cells.

Material and Methods

This study is an in-vitro evaluation on shialjit (mumijo) which is collected and prepared from the Sardoyeh Mountains of Jiroft city in Kerman province of Iran. 25 mg of Shilajit was disinfected and dissolved in 10 ml of methanol and boiled for70 min. The product was centrifuged at 2500 rpm for 10 min. The supernatant fluid was used for further evaluations (Figure 1).

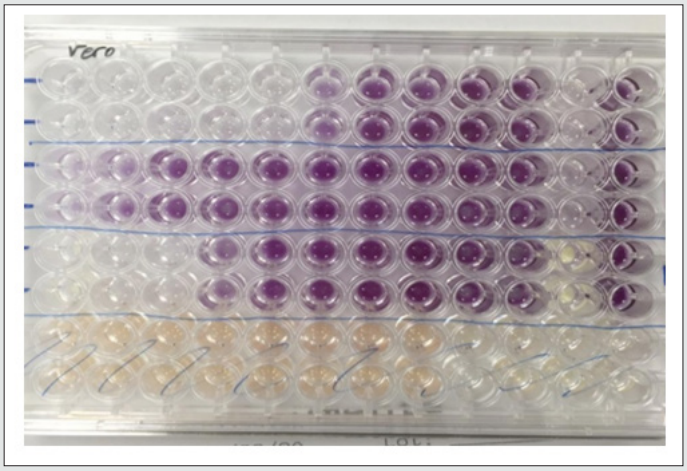

Figure 1: Hela cell culture wells after MTT staining process.

Cell culture

The cytotoxicity of shilajit has been assessed on standard HeLa mucosal epithelial cells. Hela cells were grown on DMEM media containing 10% GIBCO (fetal serum bovine), 100 ug/ml penicillin and 100 ug/ml streptomycin. Then, they were piped into 96-well microliter plates and incubated for a day at 37°C and under 5% pressure of carbon dioxide [15]. In order to dilute the solutions, we consider 14 wells for each dilution. In the first 4 wells, the pure form of shilajit solution was added. In the other wells DMEM 2% was inserted and serial dilution was done by multi-channel method from the first column to the next columns in 96-well microliter plates. Then, they were incubated for 24-72h.

MTT staining assessment

The shilajit solution was then removed from the wells by phosphate buffered saline (PBS) wash and 1X MTT was added in each well. The plates were kept in 37°C for 3 h and shaken every 30 minutes. DMSO or isopropanol (isopropanol is better) was added in all the wells for one to two minutes, the plates were placed on a shaker to dissolve the paint deposit in isopropanol [16]. The absorbance of each well was read at 630-410 nm. The optical density of each well containing hela cells and shilajit solutions and control group including only hela cells in normal culture medium without any other solution were registered to compare. Each test was repeated three times and cell loss in the medium was measured (Table 1).

Table 1: Optical density of Shilajit for different concentrations.

Shilajit concentration(mg/ml)	Optical Density	Cell Viability	Cytotoxicity
50×2^{-1}(mg/ml)	0.093	26.49%	73.5%
50×2^{-2}(mg/ml)	0.101	28.77%	71.23%
50×2^{-3}(mg/ml)	0.115	32.76%	67.24%
50×2^{-4}(mg/ml)	0.138	39.31%	60.69%
50×2^{-5}(mg/ml)	0.145	41.31%	58.68%

50×2^{-6}(mg/ml)	0.208	59.25%	40.74%
50×2^{-7}(mg/ml)	0.223	63.53%	36.47%
50×2^{-8}(mg/ml)	0.241	68.66%	31.34%
50×2^{-9}(mg/ml)	0.252	71.79%	28.21%
50×2^{-10}(mg/ml)	0258	73.50	26.50%
50×2^{-11}(mg/ml)	0.295	84.04%	15,95%
Control	0.351	100%	0%

Determining the percentage of cytotoxicity and cell viability

In this study, different concentrations of Shilajit were added to the wells containing Hela cells and after 72 hours, MTT staining was done in order to stain the remaining viable cells. Each well was stained proportionally to the amount of surviving cells. The results were spectroscopically measured by spectrophotometer as optical density (OD). The cell viability percentage and cytotoxicity percentage were measured by using the following formula: the data were analyzed by Spss version 18.

Cell viability percentage :(OD shilajit/ OD control) × 100

Cytotoxicity percentage = 100 × (OD shilajit/ OD control – 1)

The cytotoxicity of each Shilajit concentration was obtained relatively and compared with the control well (just hela cells) [17].

Results

In this study, the initial concentration of Shilajit was (50 mg)/ (2 ml). This concentration is serially diluted and each concentration is half of the previous one. The lowest and the highest Shilajit concentration were 50×2^{-11}(mg/ml) and 50×2^{-1}(mg/ml), respectively. The reported OD for control well (hela cells without shilajit) with optical density of 0.351 is considered as zero cytotoxicity. Lower OD is related to the less viable cell concentration. As the concentration of the extracts increased, the amount of cell viability decreased. More diluted concentrations were less cytotoxic.

The concentrations less than 50×2^{-6}(mg/ml) had acceptable cell viability, and concentrations more than 50×2^{-6}(mg/ml) were cytotoxic.

Discussion

According to the results, shilajit is biocompatible. Shilajit was more biocompatible at concentrations less than 50×2^{-6}(mg/ml). In some studies, the cell cytotoxicity of different types of shilajit with different geographic origin have been assessed [18,19]. In an in-vitro MTT based colorimetric evaluation, more diluted shilajit extraction showed more viable L929 mouse fibroblast line cells. They evaluated the concentration between 100µg/ml to 7-8 µg/ml [19]. In some animal studies, the safety of different shilajit concentrations have been assessed. In a study, systemic use of shilajit at the dose of 200mg/kg and 100mg/ml were found to be safe even for liver, kidney, heart, blood cells, endocrine and nervous system. Also, mumijo was not teratogen for pregnant rats and mice [20,21]. These results have been confirmed [22]. Evaluations in another assessment insisted on no genotoxicity effect of shilajit [22]. In an in vivo study on human, no adverse effect on liver and kidney have been observed [22]. In a double-blind study, the prescription of 2000mg of processed shilajit for 45 days have increased HDL, vitamin C, vitamin E and decreased LDL, VLDL and triglycerides [23]. Although shilajit has been used since many years ago, relatively few clinical usages have been considered for it. Animal and human researches confirmed the safety of shilajit. Shilajit is composed of four main ingredients containing gold, silver, iron and copper [24]. Silver, lead and their irons are other ingredients of shilajit [25,26]. Different geographical origins for shilajit in addition to its color can determine its active ingredients and their amounts. The shilajit diverse therapeutic use in the ancient medicine is directly related to its prominent compositions [19]. Wide proper medical effects, its safety and biocompatibility make shilajit a remarkable herbal composition for different medical aims. This natural substance can be evaluated for treatment of many diseases.

Conclusion

Shilajit is a natural biocompatible substance with more cell viability at concentrations less than 50×2^{-6} mg/ml.

References

1. Arrow KJ (2014) Conflict of values: a decision view Proceedings. American Philosophical society 158: 25-30.
2. Arrow KJ Auerbach A, Bertko J, Brownlee S, Casalino LP, et al. (2009) Toward a 21st century health care system: Recommendation for health care reform. An Intern Med 150: 493-495.
3. Basoglu N, Tugru lU, Topcoan DU (2012) Determining patient preference for remote monitoring J Med Syst 36: 1389-1401.
4. Danner M, Vennedey v Hiligsmann M, Faiser S, Gross C, Stock S (2017) "Comparing Analytic Hierarchy Process and Discrete Choice Experiments to elicit patient preferences for treatment characteristics in age-related maculardegeneration". ValueinHealth 20(8): 1166-1173.
5. Evans R (1974) Supplier-Induced Demand. Some empirical evidence and implications. McMillan, London, UK.
6. Friedman M, Savage LJ (1952) The expected utility hypothesis and the measurability of utility. J Political Economy 60: 463-474.
7. Huttin CC (2016) A random utility models on physicians 'choice sets and health care financing systems, communication to 84th workshop of the MCDA-Euro working group, Wien.
8. Huttin CC (2018) Consistency issues and conjoint models in health care: an application on economics and physicians' choices. Journal of Pharmaceutics and Drug Research-JDPR 2(1): 36-42.

9. Huttin CC (2021) Analysis of Medical Markets and use of mechanism design-Physician-¨Patient interactions and role of Organizations. Science. Technology and Public Policy 5(1): 29-39.

10. Huttin CC (2017) Clinical judgement research on economic topics: role of congruence of tasks in clinical practice. Technology and Health care 25(2): 353-365.

11. Huttin CC (2017) A joint estimation using stated and revealed preference models for healthcare budgets; paper 85th meeting of Euro Working group on MCDA.

12. Hamalainen RP, Jones R, Saarinen E (1979) Being better better Aalto University Publications, Helsinki, Finland.

13. Hauser JR, Urban GL (1979) Assessment of attribute importance and consumer utility functions: Von Neumann -Morgenstern Theory applied to consumer behavior. J Consumer Res 5: 251-262.

14. Hauser JR, Shugan SM (1980) Intensity measures of consumer preferences Operation research.

15. Hauser JT, Ding M, Gaskin SP (2009) Non-compensatory (and compensatory models) models of consideration-set decisions. Sawtooth conference proceedings.

16. Marshak J (1973) Rational Behavior, Uncertain prospects and measurable utility Econometrica 18: 111-141.

17. Reed Johnson F, Mathews KE (2001) Sources and effects of utility theory inconsistency in stated-preference studies. Amer J Agr Econ pp: 1328-1333.

18. Saaty RW (1987) The Analytic Hierarchy Process, what is it and how it is used, Mathematical Modeling 9(3-5): 161-176.

19. Sina Shih YC, Tai Seale M (2012) Physicians 'perceptions of demand induced supply in the information age: a latent class model. Health Econ 21 (3): 252-269.

20. Von Neumann (1947) J-Morgenstern O The theory of Games and Economic Behaviors, ed 2 Princeton University Press, Princeton, NJ.

21. Timoshenko A, Hauser J (2019) Mining and organizing User-Generated Content to identify attributes and attribute levels, MIT proceedings of Sawtooth conference.

22. Wang K (2017) Using online self-assessment tool to improve conjoint analysis, applications in choices of wildlife excursions.

23. Wigton B (2015) Physicians versus nurses'judgement policies, Brunswik Newsletter.

DOI: 10.32474/LOJPCR.2022.03.000152