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Anti-Cancer Activity of Liposomal Medical Leech Saliva Extract (LSE)

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Abstract **-** Leech could be a trendy therapeutic agent introduced by Food and Drug Administration (FDA) and contains totally different peptides and proteins as well as histamine, serotonin, steroid hormones and modulators, enzymes, proteinase inhibitors and anti-microbial agent. human breast carcinoma cell line (MCF-7) was utilized in this project. Medical Leech saliva (*Hirudo Medicinalis)* extracted with %8 ethanol in deionized water without leech scarification. Demonstrated the biological activity of Leech Saliva Extract (LSE) as Anti-Cancer agent in vitro, the MCF-7 cell line was cultured in RPMI that has 10% fetal bovine blood serum (FBS) at 37°C with 5% CO2. Cell line divided into 2 groups A and B. Type A simply treated with liposomal LSE and group B treated with leech saliva without liposomal formulation. For testing Anti-Cancer activity of liposomal LSE serial dilution of solutions were added to cell line. LDH test was utilized in this study to the determined cytotoxic activity of components and for showing anti-cancer activity Scratching test was used and Apoptotic bodies were determined by DAPI staining. Results demonstrated that Liposomal LSE as a targeting drug has the more practical action against LSE on MCF-7 cell line. SDS-PAGE of LSE showed that it contains about hundred proteins. Every of those proteins have own activity in body. Treatment Graphs verified that formulation of liposomal LSE has a lot of impact on Cancer cells.

Keywords**:** Leech Saliva Extract (LSE), Liposome, MCF-7, LDH, Hirudo Medicinalis.

1. Introduction

The uses of medical application of leeches can be traced back to the beginning of evolution. Traditionally, in many countries including medical leech had been used for many human body illnesses starting from bloodletting of the leech for conventional usage. Furthermore, many reports mentioned the uses of leech in skin sicknesses, nervous system irregularities like brain congestion, urinary and reproductive system difficulties. Also, optical inflammation, dental problems, and hemorrhoids were too treated by leech therapy[1-4].

By respect to cancer and metastasis therapy, many scientists defined the effective uses of leech saliva extract as an antimetastatic and Anti-Cancer agents[5]. Other exploration defined a successful synthetic hirudin preparation as an effective metastasis inhibitor of a wide range of malignant cancer cells, such as pulmonary carcinoma, osteosarcoma, breast carcinoma, leukemia, etc.[6]. Recent studies, exploring the compounds and therapeutic potential of leech saliva extract have recognized many peptides and proteins with multiple therapeutic properties containing anti-thrombotic, antimicrobial and anti-metastatic. In vitro anti-cancer effects were revealed in breast, prostate, and lung cell lines. In vivo anticancer activity of the LSE was shown in multiple breasts and prostate cancer[7]. hirudin extracted from H. medicinalis has also been demonstrated to have effective and metastatic activity in a wide range of malignant tumor cells, such as pulmonary carcinoma, osteosarcoma, breast carcinoma and leukemia[6].

2. Material and Method

2.1. Leech Saliva Extract

During the study, the leeches were maintained starve. The process of saliva extraction began with washing the leeches by distilled water, then slowly added the 8 % (v/v) ethanol solution. Whereas the leeches are sensitive to ethanol, vomiting as a reaction to this sensitivity after 15 min causes saliva secretion. To obtain pure saliva and remove other impurities

multistages of filtering and centrifuges were accomplished[8, 9]. Protein concentration was determined by spectrophotometer using absorption at 280 nm[10].

2.2. Preparation of Liposomal LSE

Liposome containing soybean lecithin and 5% cholesterol were prepared by mixing lipid component with an organic solvent of chloroform and methanol in the ratio of 1:1. Vacuum resulting by rotary evaporation was applied to remove organic solvents. In the subsequent step thin layer of lipid (film or cake) was hydrated, also LSE was added. Through the hydration process of lipids, the formation of large multilamellar liposomes was occurred[11]. Improving absorption may achieve by diminishing the size of Nanoparticles by extrusion, homogenization or sonication. The size of the liposomes was ranging from nanometres to several micrometres[12].

Dynamic light scattering (DLS) was used to monitor the formation of the nanoscale complexes [13, 14]. To prepare the sample, LSE liposomal complex was diluted 10 times with deionized water. Size of nanoparticles was measured by a ZetaPlus instrument (Zeta Sizer, ZS, UK) at 25C°.

2.3. Cancer Cell Line and Cell Culture

Human breast adenocarcinoma cell line (MCF-7) was obtained from the American Type Cell Collection ATCC. Cells were cultured at an initial inoculum cell concentration of 104 cells/cm2 in 15 ml Roswell Park Memorial Institute medium (RPMI) with 10% FBS (v/v) strep in Corning® 75 cm2 cell culture flask. The cultured cells were incubated at 37°C in 5 % CO2 humidified atmosphere[5].

2.4. Result

The presence of proteins in leech extracted saliva was evaluated by SDS-PAGE analysis. More than 25 kinds of peptides and proteins with molecular weight ranging from 10 to 170 kDa were stained with Coomassie blue and silver nitrate (Figure1).

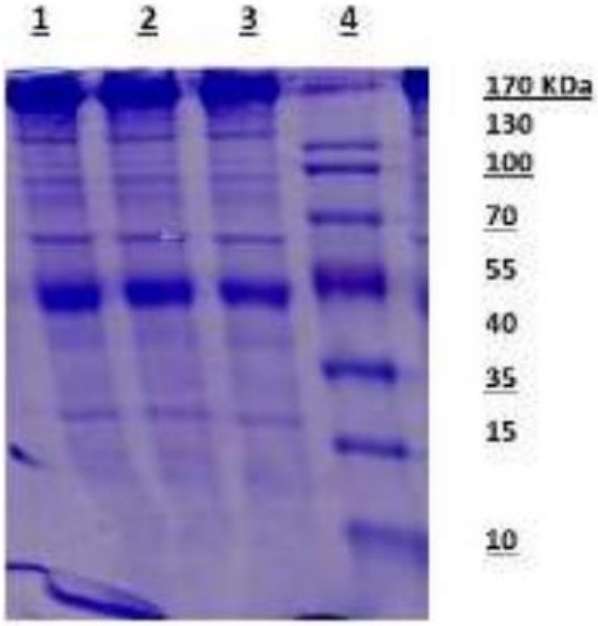


Fig. 1: SDS-PAGE analysis of leech saliva extract. (Coomassie blue staining). Lanes 1-3: medical leech saliva extract. Lane 4: Molecular Weight Size marker.

3. Liposomal LSE Production

As respect to low skin absorption of LSE gel, the Nano scale-liposome was used to resolve this drawback. The size of liposomal LSE particles was determined by DLS after20 minutes homogenizing and 10 minutes' sonication. Results of DLS (Figure.2) showed that Z- Average was 193.8, PDI was 0.144 nm.

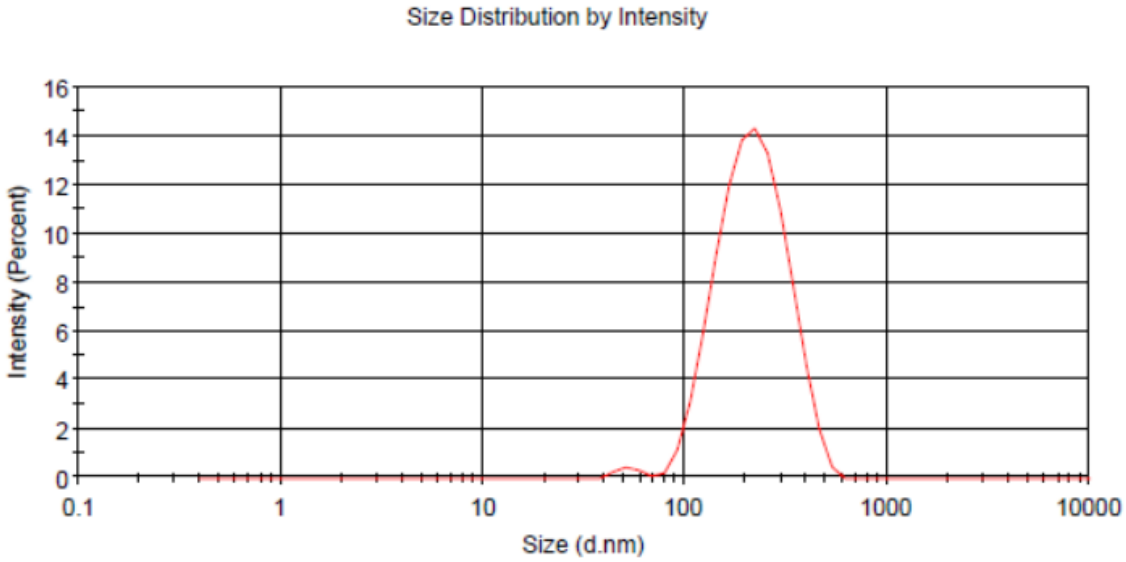
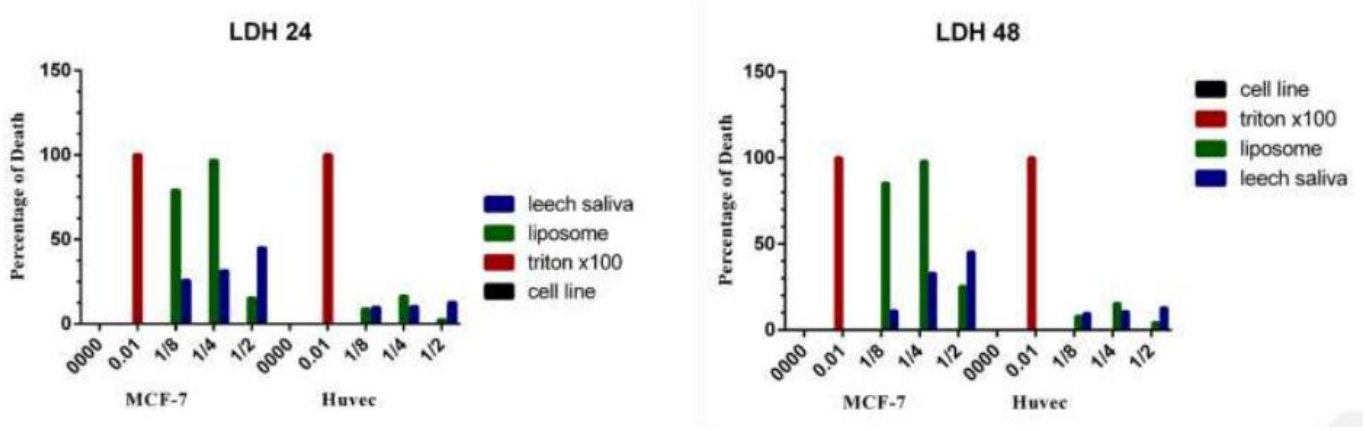


Fig. 2: Dynamic light scattering measurement of the average diameter of the liposomes. Z-Average (d. nm) is about 193.8, PDI is 0.144 and result quality is good.

4. Cell Line Treatment

Results demonstrated that LSE had a significant anti-proliferation activity against human breast adenocarcinoma cell line (MCF-7). The concentration of the total protein of leech saliva extract that inhibits the growth of 50% of the treated cells after 24 and 48 hours of incubation was 80.52 µg/ml. On the other hand, the anti-proliferation effect of Nano liposomal LSE was compared with Leech saliva extract effect, and all of them compared with Triton X 100 that kills cells. It was found that the Nano liposome LSE kills % 97 of cancer cells but Leech saliva was about % 50. Beside that Leech saliva and liposomal LSE had about % 10 anti-proliferation activity on HUVEC cell line as a normal cell.



A B

Fig. 3: LDH Test for MCF-7 as breast cancer cells and HUVEC Cell line as control group. A: Treatment after 24 hours. B: Treatment after 48 hours. In tow times confirmed that %97 of Cancer cells destroyed with Nano Liposomal LSE and %50 of Cancer cells were destroyed only with LSE and on HUVEC cell line had no effect.

5. Conclusion

In each traditional and trendy therapeutic approaches, leeches have been found to be promising within the treatment of varied diseases. Except for the recognized anti-metastatic activity of leech saliva, we reported here for the primary time that the liposomal LSE achieved from the Hirudo medicinalis, had an anti-proliferative activity against human breast adenocarcinoma cell line (MCF-7). DAPI staining of cells proved that liposomal LSE had an anti-tumour activity and Scratching test demonstrated that drug had an anti-proliferative activity. Hence, additional studies still are required on this issue to isolate and determine the active principle, to check the mechanism of action, to judge its result on different cell line varieties.

References

[1] B. Porshinsky, S. Saha, M. Grossman, P. Beery Ii, and S. Stawicki, "Clinical uses of the medicinal leech:A practical review," *Journal of postgraduate medicine*, vol. 57, no. 1, p. 65, 2011.

[2] A. Srivastava and R. Sharma, "A brief review on applications of leech therapy," *Arch Appl Sci Res*, vol. 2, no. 2, pp. 271-4, 2010.

[3] I. S. Whitaker, J. Rao, D. Izadi, and P. Butler, "Historical Article: Hirudo medicinalis: ancient origins of,and trends in the use of medicinal leeches throughout history," *British Journal of Oral and MaxillofacialSurgery*, vol. 42, no. 2, pp. 133-137, 2004.

[4] E. P. Cherniack and E. Cherniack, "Bugs as drugs, part two: worms, leeches, scorpions, snails, ticks,centipedes, and spiders," *Altern Med Rev*, vol. 16, no. 1, pp. 50-8, 2011.

[5] A. Merzouk, A. Mohammad Ghawi, A. M Abdualkader, A. D. Abdullahi, and M. Alaama, "Anticancereffects of medical Malaysian leech saliva extract (LSE)," *Pharmaceutica Analytica Acta*, vol. 815, 2012.

[6] V. Nair, K. P. Das, N. Saha, "Hirudotherapy: Will The Glorious Past Return?."

[7] A. Ammar et al., "Mechanism of anticancer activity of BPS-001 (lyophilized leech saliva extract)," ed:AACR, 2017.

[8] C. Powelljones, R. Munro, and R. Sawyer, "Calin-A Platelet-Adhesion Inhibitor From The Saliva Of TheMedicinal Leech," *In Thrombosis And Haemostasis*, Fk SchattauerVerlag Gmbh Po Box 10 45 45, Lenzhalde 3, D-70040 Stuttgart, Germany, vol. 65, no. 6, pp. 1114-1114,1991.

[9] A. Shakouri et al., "Effectiveness of topical gel of medical leech (Hirudo medicinalis) saliva extract onpatients with knee osteoarthritis: A randomized clinical trial," *Complementary Therapies in Clinica lPractice*, 2017.

[10] M. Alaama, M. AlNajjar, A. Abdualkader, A. Mohammad, and A. Merzouk, "Isolation and analyticalcharacterization of local Malaysian leech saliva," *IIUM Engineering Journal*, vol. 12, no. 4, 2011.

[11] L. E. Stearne, R. M. Schiffelers, E. Smouter, I. A. Bakker-Woudenberg, and I. C. Gyssens, "Biodistributionof long-circulating PEG-liposomes in a murine model of established subcutaneous abscesses," *Biochimicaet Biophysica Acta (BBA)-Biomembranes*, vol. 1561, no. 1, pp. 91-97, 2002.

[12] R. Dwiastuti, M. Radifar, M. Marchaban, S. Noegrohati, and E. P. Istyastono, "Molecular DynamicsSimulations and Empirical Observations on Soy Lecithin Liposome Preparation," *Indonesian Journal of Chemistry*, vol. 16, no. 2, pp. 222-228, 2016.

[13] O. M. Merkel et al., "Stability of siRNA polyplexes from poly(ethylenimine) and poly(ethylenimine)-gpoly(ethyleneglycol) under in vivo conditions: effects on pharmacokinetics and biodistribution measuredby Fluorescence Fluctuation Spectroscopy and Single Photon Emission Computed Tomography (SPECT)imaging," *J Control Release*, vol. 138, no. 2, pp. 148-59, 2009.

[14] P. Qi et al., "The biological activity of cationic liposomes in drug delivery and toxicity test in animalmodels," *Environ Toxicol Pharmacol*, vol. 47, pp. 159-164, 2016.