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RESEARCH ARTICLE

ANTI-CARCINOGENIC ACTIVITY OF SHILAJIT REGARDING TO APOPTOSIS ASSAY IN CANCER CELLS: A SYSTEMATIC REVIEW OF IN-VITRO STUDIES

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Abstract

Shilajit, a natural mineral substance with a long history of use in traditional Ayurvedic medicine, has garnered attention for its potential therapeutic properties, including anti-oxidant, immunomodulatory and anti-inflammatory effects. Although its role in rejuvenation and healing is well-documented in ancient texts, its potential anti-cancer properties, particularly in inducing apoptosis in cancer cells, remain unexplored. This systematic review investigates the anti-carcinogenic activity of Shilajit through apoptosis assays in various cancer cell lines based on in-vitro studies. A comprehensive search was conducted across scientific databases to identify in-vitro studies evaluating the apoptotic and anti-proliferative effects of Shilajit on cancer cells.The reviewed studies reveal that Shilajitexhibitssignificant dose and time dependent cytotoxic effects in various human cancer cell lines, including breast, lung, liver, ovarian, cervical, and colorectal cancers. Apoptosis induction was observed in many cases, with increased cell death and reduced cell viability correlating with higher concentrations of Shilajit. The molecular mechanisms underlying these effects include the modulation of genetic expression from more than one pathway, reactive oxygen species production and suppression of key anti-apoptotic proteins. Notably, Shilajit was found to induce both apoptotic and anti-proliferative effects in cancer cells without significantly affecting normal human cells at lower concentrations.The findings from multiple studies suggest that Shilajit may act as a potential chemopreventive agent by inducing apoptosis and inhibiting the proliferation of cancer cells. Its ability to modulate gene expression, including the up-regulation of pro-apoptotic markers and down-regulation of anti-apoptotic proteins, further supports its anti-cancer

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potential. However, the high dosages recommended in Ayurvedic texts, particularly in the context of cancer treatment, warrant caution due to potential cytotoxicity at excessive levels along with minimal toxicity to normal cells at controlled dosages. While preliminary data supports its potential as an adjunct to conventional cancer therapies.

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Introduction:-

Shilajit 'one that conquers the mountains' or Shilaras 'an absolute essence of the mountains' promoted in various Ayurvedic texts for it's rejuvenation and therapeutic usage from ancient ages. Shilajit is naturally forming mineral found on the upper surface of mountains of specific higher altitude. It's obtained from Himalayan Mountain ranges, China, Nepal, Russia, Afghanistan and other native regions of Asian continent. It has been known by many local names, sold in markets with abundance in many forms. Traditionally, it is being a part of daily dietary regimen of Sherpas and other cultures who live on high altitude, and in extremely cold atmospheric conditions.

Indication of Shilajit in various disorders are mentioned in Sushruta Samhita, as in Diabetes, Skin disorders, Epilepsy, Toxicity caused by consuming heavy metals, Inflammation, Haemorrhoids, Anaemia, Tumours and Fever etc.[1] Perhaps, glorified in terms that there's no any disease that can not be cured by Shilajit.[2] Although, very high dosage advisory for oral consumption of Shilajit is seen in the same text that is 100pala (1pala=48gm approx.) or more.[3] Which seems near to impossible for a person to consume, giving rise to the questionable perception for the quotation. In this study, we have tried to investigate the relevant answers.

Cancer is a leading life threatening disease of today's era, millions of mortalities are reported every year worldwide.[4] In multiple stages of cancer, the formation of tumour and proliferative growth by over-mutation of cells is observed. What more brutal is the current immunotherapy treatments, the health risks they posses and the adverse effects they usually tend to cause to the patients. Immunotherapy and Target therapy like Radiation are invasive methods. Most anti-neoplastic agents could act as potent carcinogenics to Humans.[5][6] Leading us to have no other way to find an alternative medication, treatment plannings for this disastrous fallout of mankind in most possible ways.

Spermatogenetic activity of Shilajit is well known worldwide,[7][8] numerous others studies have been reported indicating anti-oxidant, anti-inflammatory, immunomodulatory results. Some studies with free radical scavenging activity of the same are reported as well, which could be possibly taken into consideration as the chemopreventive agent.[9] Indeed this study focus on the advanced stages of the tumour where anti-proliferation and apoptosis results are intended.

Very limited numbers of studies are available which shown apoptotic changes in cancer cell lines. This study collected data from various studies performed worldwide with respected assays, analysed, expressed systematically, critically reviewed, discussed and concluded the final results through authors' viewpoints.

Aim and Objectives:-

The main aim of this study is to find out whether Shilajit induces apoptosis in cancer cells by reviewing multiple in-vitro studies. Objective of the study is to point out the relevance of the results with such high dosage of Shilajit consumption for a patient has indicated in Ayurvedic treatments. An another objective is to discuss Gene modulation capabilities of Shilajit based on the data reported by respective studies.

Materials and Methods:-

Study Design:

Data collected from reports of the various studies of Shilajit indicating apoptosis in cancer cells which is available on the internet. We ran multiple Keyword searches related to the topic through many scientific journals and articles search engines. Selection of study articles was implied by preferable selection criteria. Obtained articles were arranged systematically, as per their year of publication. Observations, results and conclusions of the selected study articles were critically reviewed, discussed and published.

Selection Criteria:
No any restrictions about the year of publication is set, but articles that are only in English language are taken into consideration. Recent studies have shown Shilajit actively contain Humic Substances and Fulvic Acid.[8] Humic substances are formed due to decomposing of organic matter over time. Although, Shilajit isn't formed out of every decomposing Humic Substances. There must be some geographical and mineralogical significance to it. Fulvic Acid and Humic Acids acting as carrier molecules for the more active, smaller compound of Mineral Pitch have been reported by Hartman.(2007) Hence, we are taking Shilajit as a whole or in the form of extract rather than solely considering HA or FA for the respected assay.

Inclusive Criteria:
Study articles those were published on the internet. Articles discovered from Google Scholar, PubMed, Elsevier, Cochrane. Articles published on any year, in English language. Only in-vitro study models were included. Assessment criteria of studies with Colony formation assay or MTT or TUNEL assay for cell viability or Fluorescent microscopy. Data from the studies with pictorial or graphical interpretations.

Exclusive Crtiteria:
Articles those were not published on the internet. Study articles those were other than in English language. Study design of In-vivo, In-silico and Clinical trials were clearly excluded from the study.

Observation and Results:-

Some Biological Activities and Safety of Mineral Pitch.
ThawatchaiPhaechamud et.al.(2008)
In this study Shilajit was found notably toxic to normal Human Lung fibroblast, Human Breast carcinoma cells, Human Lung carcinoma cells, Human Cervical carcinoma cells, Human Colorectal adenocarcinoma cells, Human Ovarian carcinoma cells and Human Hepato carcinoma cells.

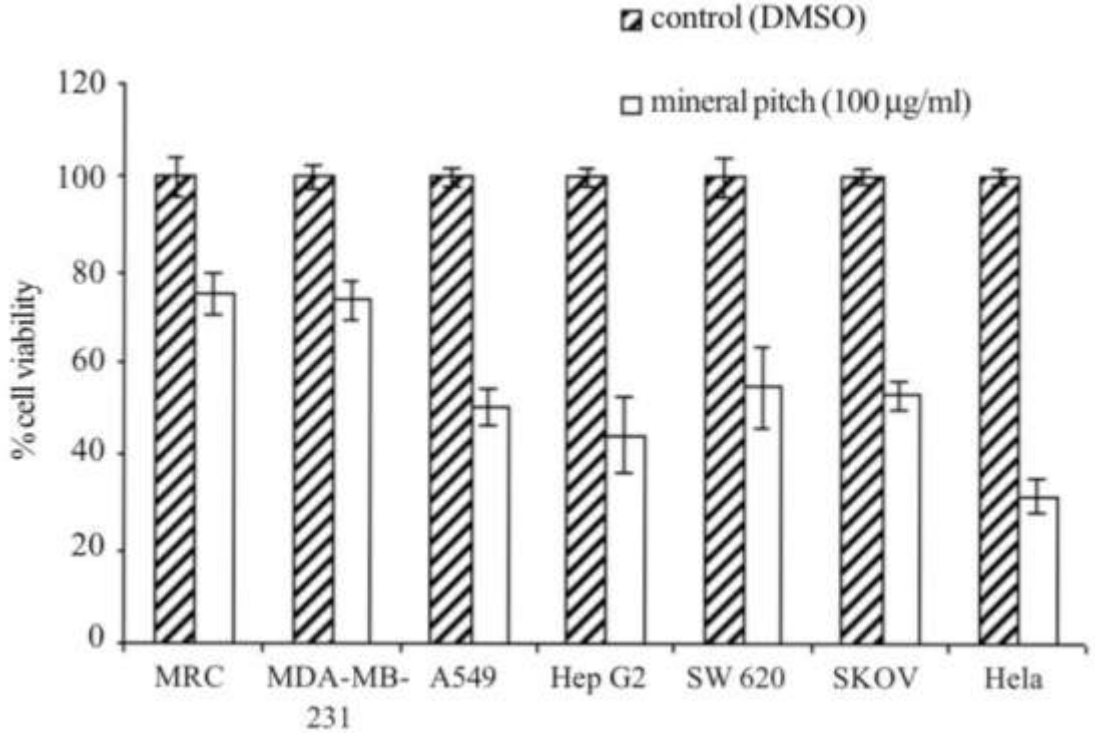

Figure 1:- Cytotoxicity of Mineral pitch on one Human Normal lung fibroblast cells line (MRC-5) and Human carcinoma cell lines from 6 various organs, Breast (MDA-MB-231), Lung (A549), Liver (HepG2), Colorectal (SW-620), Ovary (SKOV-3) and Cervix (HeLa). All cells were treated with 100µg/ml of the Mineral pitch for 24h and followed determined the viability by the MTT technique. Relative cell viability was expressed as % control cells at the same concentration of solvent. All values were mean±S.E.M; n=3, $p<0.01$ (*, **) comparing to the control, only solvent, and $p<0.01$ (**) comparing to MRC-5 (Normal human cells).

Shilajit notably decreased the viability in Cervical carcinoma cells which exhibited 31.5+/-3.85% compared to the control. While rest of Human carcinoma cells were affected quite similarly around 44.5-54.9%. Shilajit have been reported to repress the growth of Lung carcinoma cells higher than Normal lung cells. All cultured cell lines were treated equally with 100ug/ml Shilajit concentration.

Mineral Pitch Induces Apoptosis and Inhibits Proliferation via Modulating Reactive Oxygen Species in Hepatic Cancer Cells.
Kishor Pant et.al.(2016)
Differential concentrations of Shilajit were tested against Human Hepatic cancer cells and were analysed for apoptosis, by colony formation assay. Apoptosis in cultured cells was observed increased by the increase in concentration of Shilajit after introduction. Based on the data observed in the study, apoptotic cells were found 21.7, 63, 77.3 and 80.1% with concentrations of 100, 200, 500, 1000ug/ml respectively as compared to untreated group.

This study concluded potent anti-proliferative and anti-apoptotic properties of Shilajit via modulating the expression levels of miRNA-21 and miRNA-22 in Hepatic cancer cells. The study reported Shilajit induces anti-cancer activity by modulating more than one pathway.

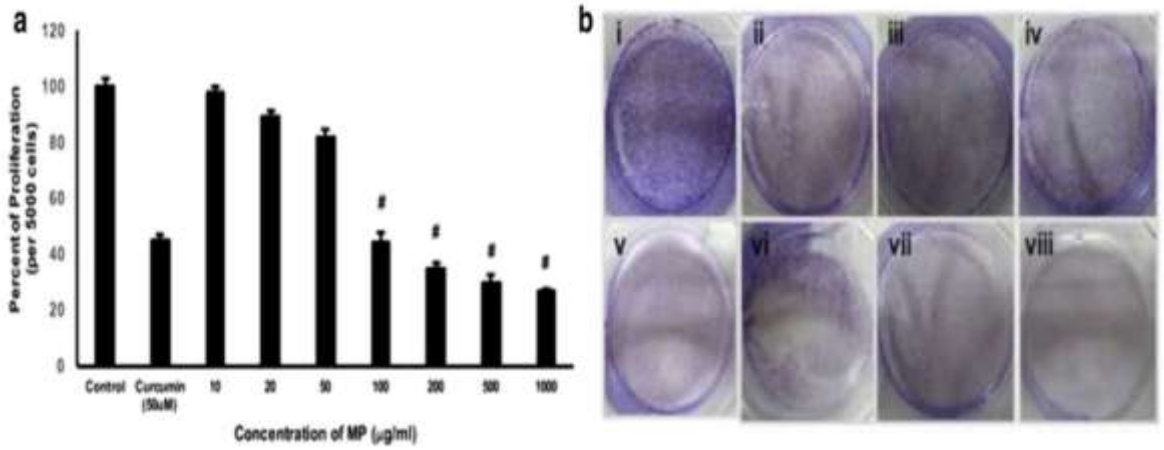

Figure 2:- a.Huh-7 cells (5,000 cells per well of 96-well plates) were cultured with different concentrations of Mineral pitch for 24h under serum-free conditions. MTT assay was performed and the percent of proliferation was calculated. In parallel experiments, Curcumin (50µM) was used as a positive control (n=3; #p<0.05). b.The colony formation assay was performed after plating cells in 60mm dishes and incubating them with different concentrations of Mineral pitch for 6 days. After that the cells were stained with 0.5% crystal violet and the colonies were visually observed. The representative pictures show, untreated cells (i); cells treated with different concentrations of Mineral pitch, 10µg/ml (ii), 20µg/ml (iii), 50µg/ml (iv), 100µg/ml (v), 200µg/ml (vi), 500µg/ml (vii), and (viii) 1000µg/ml

Antioxidant, Cytotoxic and Hyperalgesia-Suppressing Activity of a Native Shilajit Obtained from Bahr Aseman Mountains.
MandanaJafari et.al.(2019)
In order to analyse cytotoxicity of Shilajit against Human Breast cancer cell line and Human Lung cancer cell line, it was tested in both extracts, water and DMSO. This study also noted that, the viability of applied cell lines was significantly decreased by increasing of extract concentration.

The half-maximal inhibitory concentration (IC50) observed was 727.5±1.9µg/mL and 1103±3.2µg/mL for Breast Cancercell line and Human Lung cancer cell line in aqueous extract respectively. However, the DMSO extract of Shilajit produced the same effect (i.e. 50% cell death) at the concentration of 2363.4±3.8µg/mL and 2020.8±1.7µg/mL for Breast Cancer cell line and Human Lung cancer cell line respectively.

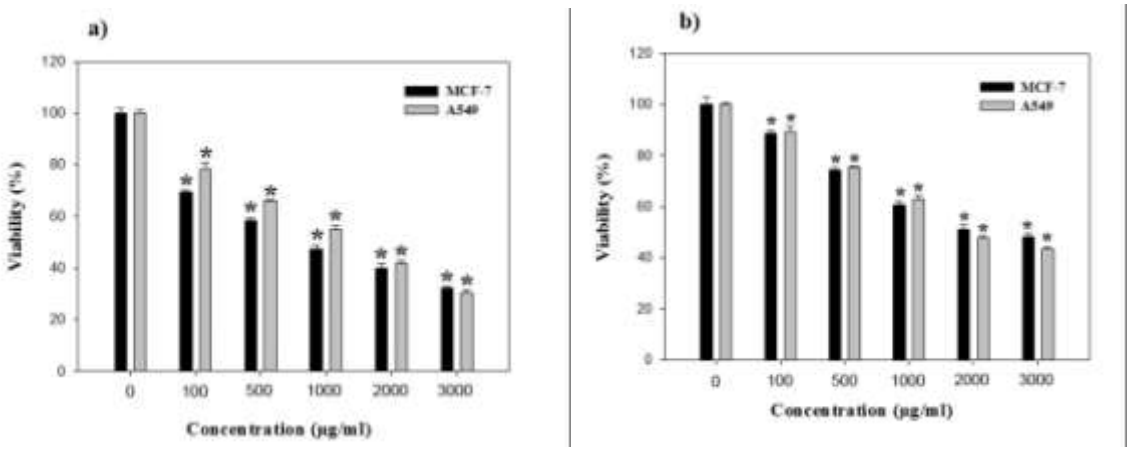

Figure 3:- Effect of a) aqueous and b) DMSO extract of Shilajit on cell viability of MCF-7 (Human Breast cancer cell line) and A-549 (Human Lung cancer cell line) cell lines examined by MTT assay. Each value is represented as mean±SD of the independent experiments.

Mummy Induces Apoptosis Through Inhibiting of Epithelial-Mesenchymal Transition (EMT) in Human Breast Cancer Cells.

SolmazBarouji et.al.(2020)

To evaluated the anti-tumour effects of the Shilajit, included a very metastatic variant of Breast Cancercell-line and another with a low metastatic condition along with a Normal Mammary epithelial cell line. A dose-dependent cell-growth inhibition was observed in Shilajit treated Breast Cancer cells. Besides, the inhibitory effect of the Shilajit at the same dose on normal cells were increased by an increase in the incubation time was observed. The percentage of apoptotic cells increased in Shilajittreated MDA-MB-231 compared to Shilajit treated MCF-7cells (67.3±2.17 vs. 59.83±1.57%)

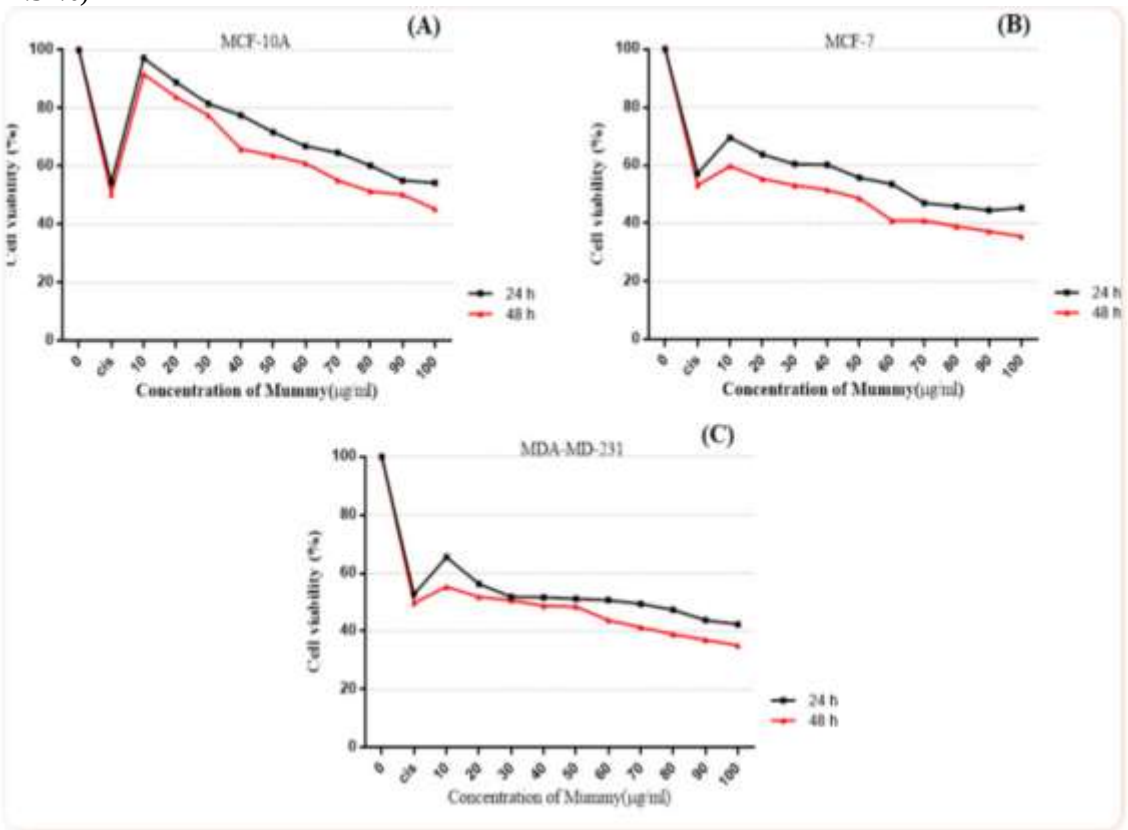

Figure 4:- Cytotoxicity effect of various concentrations of the Shilajit in human breast cancer and normal cell lines.

The Shilajit treated MCF-10A group, the cell viability was only decreased from 95.1 to 82.53%, indicating the Mummy inhibition efficiency on the growth of Breast cancer cells with low cytotoxic effects on Normal breast cells. The results of quantitative apoptosis analysis where the maximum rate of cell death (nuclear deformation and loss of cell wall integrity) was in the cells treated with mummy in MDA-MB-231 Cancercell line.

The Anti-Cancer Property of Mumie as Natural Product on Human Cervical Cancer Cell Line. (HeLa). AzinTavassoli et.al.(2021)

In this study, Shilajit significantly seemed to decrease cell viability of cultured Human Cervical cancer cells even in very low concentration and in short time.

Data revealing, 19, 31, 38, 43, 48 and 68% reduction in cell proliferation when treated with 100, 200, 300, 400, 500 and 1000µg/ml concentration of Shilajit respectively after 24h. While the cell cultures showed 35, 45, 62 and 92% reduction in cell viability when introduced with 300, 400, 500 and 1000µg/m concentration after 48h.

The study too could significantly express the dose dependant efficacy of Shilajit. However, Shilajit did not significantly shown decrease in viability when tested for Normal Human Fibroblast for rest of other concentrations except 1000, which indicated 49 and 70% reduction after 24h and 48h respectively.

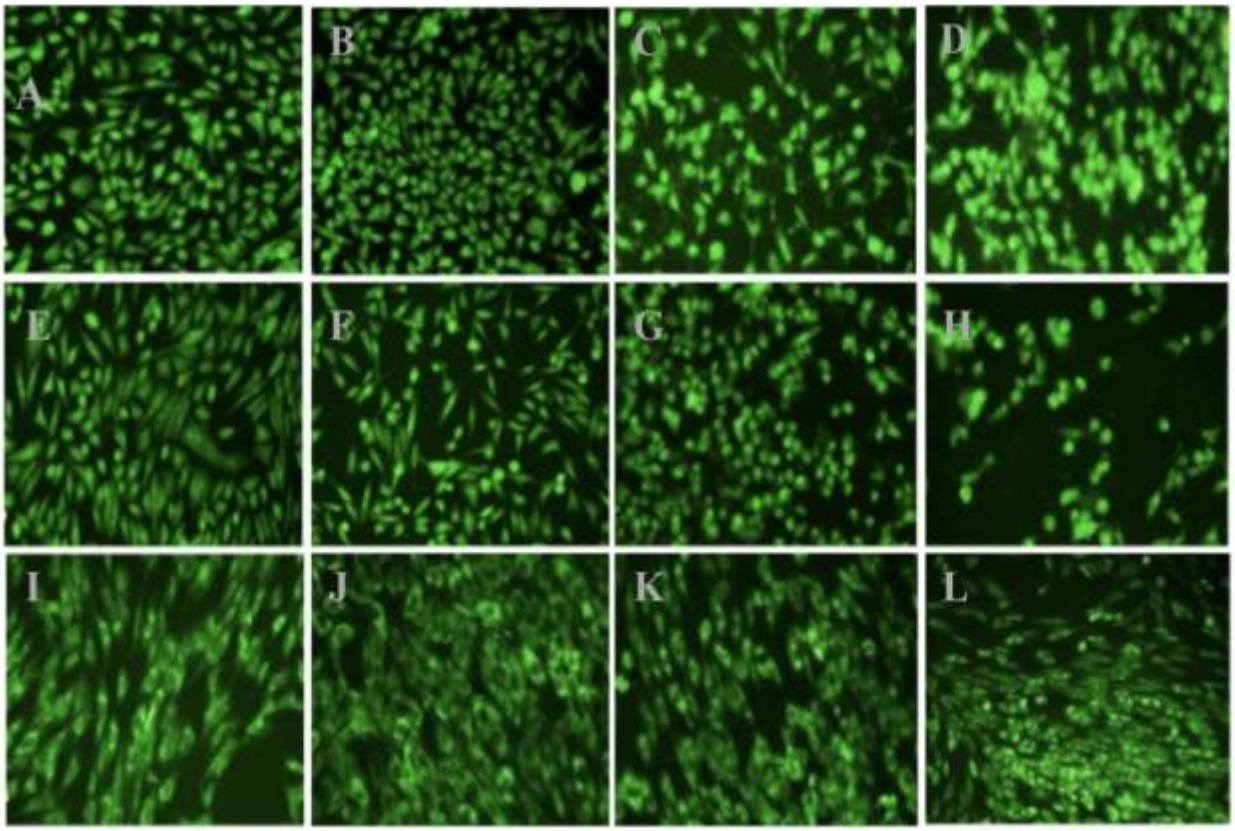

Figure 5:- The effects of Shilajit on HeLa and NIH cells cultures at different concentrations of control, examined after AO/EB staining 200, 400 and 1000µg/ml respectively. (A-D) showed HeLa cells cultured after 24h, (E-H) showed HeLa cell cultures after 48h. (I-K) showed NIH cell cultures after 24h and (L) after 48h. The cells were observed using a fluorescence microscope (Nikon Eclipse-E600) and photographs were taken at ×100 magnification using a digital camera (Nikon, Japan).

Mumio (Shilajit) as a Potent Chemotherapeutic for Urinary Bladder cancer treatment.
T. Kloskowsky et.al.(2021)

Dose and time dependant morphological changes in cancer cells were seen after introducing Shilajit. Cell shrinkage, lower number and rounding were observed with increase in concentration of Shilajit. An increased number of round, detached cells was observed with 500µg/ml and higher concentrations in Urinary bladder cancer cells. At 1000µg/ml

after 72h incubation most of the cells lost their Normal morphology, and a considerable amount of cell debris was observed.

Considering cell viability assay, Cancer cells were significantly lesser affected at 200 and 500µg/ml Mumio after 48h of incubation, while the opposite was observed at 1000µg/ml. A significant increase in apoptotic cells was noticed after Mumio exposure at LC50 and LC90 in T24 cells. There were no significant changes seen the lower concentrations.

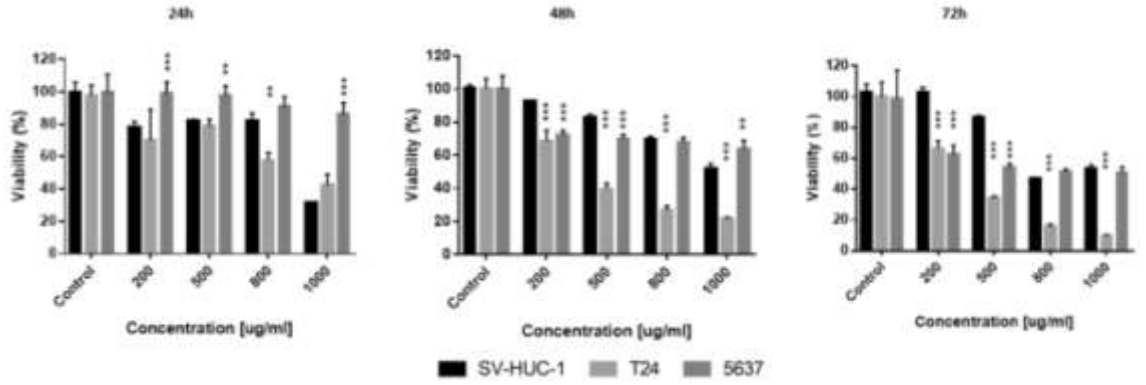

Figure 5:- Cytotoxic properties of Shilajit on SV-HUC1, 5637 and T24 cells. Results were obtained via MTT assay. Viability of SV-HUC-1, T24 and 5637 cells was affected in a time and concentration dependent manner by Mumio. *p<0.05; **p<0.01; ***p<0.001 (T24 and 5637 compared to SV-HUC-1). SV-HUC-1-Normal Urothelial cellline, T24-TransitionalUrinary bladder carcinoma cell line, 5637-Grade II Urinary bladder carcinoma cell line.

Modulation of IKK/NF-κB Signaling: A Therapeutic Mechanism of Shilajit in Breast Cancer Cells. ZeinabKorrdestani et.al.(2021)

In the study viability of Breast Cancer cultured cells after exposure to Shilajit was seen reduced in a dose or time dependant manner. A promising anti-tumour and anti-inflammatory activity was noted through inhibition of NF-kB signalling pathway. Cytotoxic effect was seen concentration dependant with approx. 20, 37, 57, 63, 69% reduction in metabolic activity after treating with 100, 200, 400, 600, and 800µg/ml of Shilajit. On the basis of findings implied that this Ayurvedic substance could exert a long term and dose dependant cytotoxic effect on the studied cells.

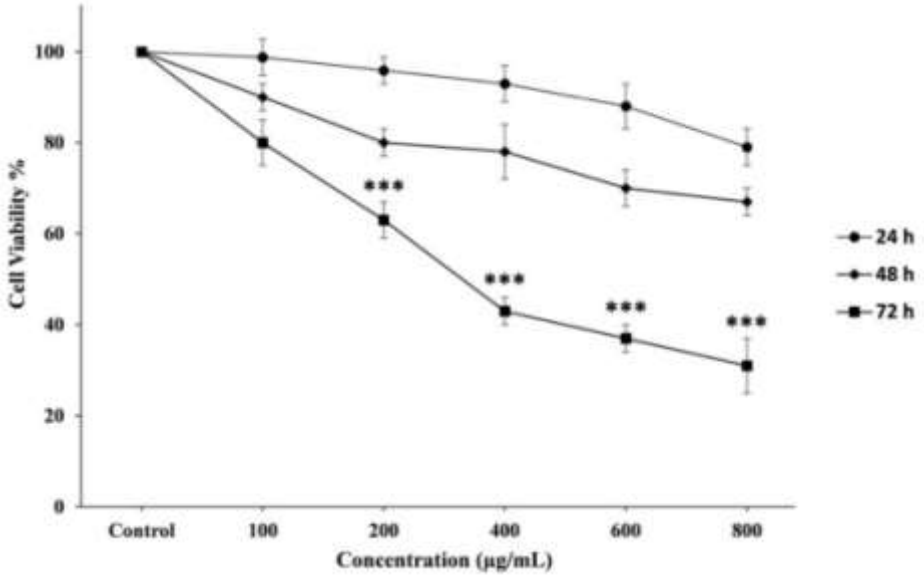

Figure 7:- Shilajit decreases the viability of the studied cells. The cells were treated for 24, 48, and 72h with different concentrations of Shilajit, and then an MTT assay was applied. After 72h exposure, the viability was reduced significantly (compared to the control value) in a dose-dependent manner. (IC50=280µg/mL).

Shilajit (Mumio) Elicits Apoptosis and Suppresses Cell Migration in Oral Cancer Cells through Targeting Urokinase-type Plasminogen Activator and Receptor and ChemokinaseSignalling Pathway.
Abdullah Alqarniet.al.(2024)
Shilajit significantly found reducing viability of Oral Cancer cells compared to control group in 24h and 48h of time intervals. The percentage of cell viability gradually decreased significantly with increase in concentration. Lower concentrations of Shilajit did not exhibit significant effects.

Overall percentage of apoptotic cells were observed as increased in Shilajittreated cancer cell line compared to untreated with 32.37%. Whereas less than 13% cells showed necrotic signs in Shilajit treated cell line and control. This finding underscores the inhibitory efficiency of Shilajit on growth of Oral Cancer Cells, demonstrating minimum cytotoxicity to Normal cells.

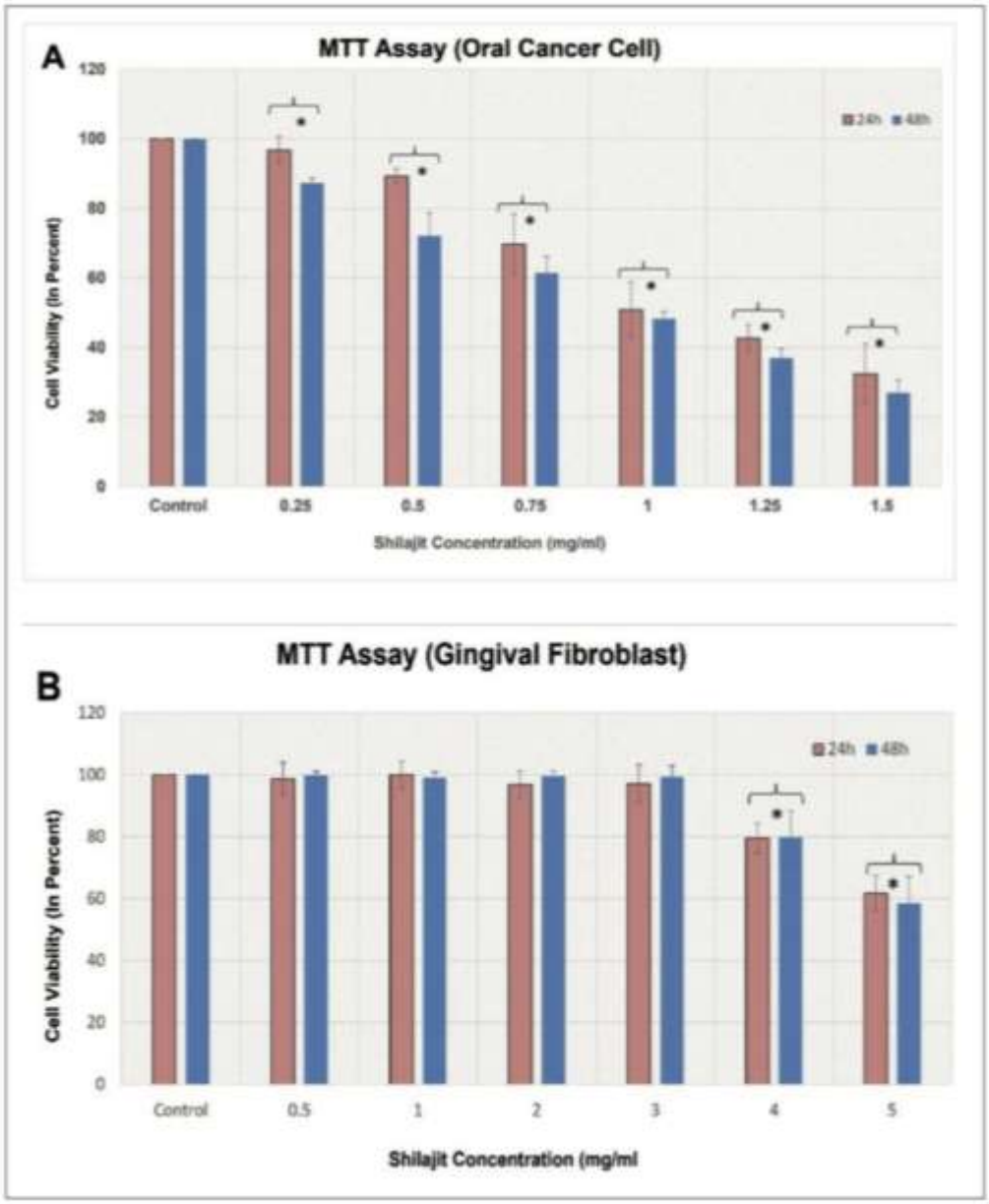

Figure 2:- The Cytotoxic effects of Shilajit on Oral Cancer (KB-1) and Human Gingival Fibroblast (hGF) Cells. (A) For cell viability assay, KB-1 cells were treated with Shilajit (0.25–1.5mg/mL) for 24 and 48h. (B) The cytotoxic effects of Shilajit on hGF cell. hGF cells were treated with Shilajit (0.5–6mg/mL) for 24h and 48h, and cell viability was measured by MTT Assay. The experiments were performed in triplicates. The results are shown as Mean±SD(n=3).

The main finding of the study showed that Shilajit had a greater cytotoxic effect on Oral Cancer cells than Normal cells (same organ) without harming healthy Oral Fibroblast cells. Also, modulation in Urokinase-type plasminogen activator (uPA), its receptor uPAR and Chemokinase genes expressions was significantly noted.

Discussion:-

Various studies concluded the anti-oxidant, anti-inflammatory, immunomodulatory, anti-viral, anti-microbial etc. assays of Shilajit, were already reported. Also differential reports of free radical scavenging activities of Shilajit has been already noted concluding that Shilajit may act as preventive care for cancer. There are very limited studies reporting the apoptosis or anti-proliferative activity of Shilajit in progressive stages of cancer cells have been conducted by this day. Above considered studies were selected, critically reviewed and discussed.

The main aim of our study was not only to expose the apoptosis induction activity of Shilajit but also to highly focus upon the dosage for consumption of Shilajit mentioned in Ayurvedic scriptures.

The lowest dosage for consumption of Shilajit for a person as mentioned in Charaka Samhita, is around 1tola (1tola=10gm approx.) per day for seven days. The medium dosage lies around 2tola per day for three weeks, whereas the highest dose which has advised is 4tola per day for seven weeks.[10] Considering Sushruta Samhita, the dosage mentioned for Shilajit could be equivalent to 4.5kg or more for several months seems ironically higher for a patient to consume in today's era.

The data based on the studies above, prospering a certain pattern, that the time and dose dependant outcomes showing significant efficacy of Shilajit for apoptotic and anti-proliferation changes in cancer cell lines were observed.

Phaechamud et.al.(2008); Shilajit found to be notably toxic to many Human Cancer cell lines along with normal Human cell lines.

Pant et.al.(2016); Apoptosis in cancer cells was observed increased by the increase in concentration of Shilajit. Jafari et.al.(2019); observed the viability of applied cell lines was significantly decreased by increasing of extract concentration. Similarly, was seen in previous study, Borouji et.al.(2020).

Tavassoli et.al.(2021); expressions in Cancer cells were seen likewise as mentioned previously. Perhaps ,Shilajit did not reduce the viability of normal cells except in higher concentrations.

Kloskowsky et.al.(2021); Cell shrinkage, lower in numbers of carcinogenic cells was observed with increase in concentration of Shilajit. Moreover, respected cells lost their normal morphological structure and a significant amount of cell debris was noted when introduced with higher concentrations. Normal cells on other hands, when treated at lower concentrations shown rise in viability but shown significant reduction in viability at higher concentrations. Considering, for cancer cells it hardly increased their viability in lower concentrations but reduction in viability at higher concentration was seen which was comparably much more than that of normal cells.

Alqarni et.al.(2024); Cell viability gradually decreased significantly with increase in concentration. Lower concentrations of Shilajit did not exhibit significant effects. Findings of this study shown inhibitory efficiency of Shilajit on growth of Oral Cancer Cells, demonstrating minimum cytotoxicity to normal cells.

Kordestani et.al.(2021); clarifying that this Ayurvedic substance could exert long term and dose-dependant cytotoxic effect on the studied cells.

Pant et.al.(2016); Shilajit found to induce both ROS and NO as well as modulating miRNA21 and miRNA22. Significant induction in miRNA22 expression and inhibition of miRNA21 was observed. Study concluded Shilajit might induce anti-cancer activity by more than one pathway.

Barouji et.al.(2020); Very important study findings regarding to gene expression modulation were denoted. The gene expression profile demonstrated a significant decrease in TGF- β1, TGF-βR1, TWIST1, NOTCH1, CTNNB1, SRC along with an increase in E-cadherin mRNA levels in Shilajit treated cells compared to the untreated control

group. There could be some negligible error of the graph with tittle label 'MDA-MD-231' in Figure 4.(C) can be considered as 'MDA-MB-231' instead.
Considering the topic, previously conducted study, Kordestani et.al.(2021) shown IC50 dose of Shilajit could reduce the mRNA expression levels of p50, RelB, and IKKα/β in the cells. Shilajit could induce anti-tumour and anti-inflammatory activity by inhibiting Nuclear factor kappa B signalling pathway.

Alqarni et.al.(2021); Shilajit shown a substantial rise in the expression of proapoptotic proteins, including p53, Bax, and caspase-3, in oral cancer cells. Indeed, it down-regulated the expression of the anti-apoptotic protein, Bcl-2.

Conclusions:-

1. Shilajit could promote to carry out the cell death in human cells.
2. Consumption of Shilajit may show progressive dose dependant efficacy.
3. Shilajit could possess some unknown genetic modulatory attributes, although more supportive research data with respected subject in the fields of molecular biology, genomics and genetic engineering is vividly required.

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