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EVALUATION OF CYTOTOXICITY, ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITY OF *NARDOSTACHYS JATAMANSI* DC EXTRACT

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Abstract:

Cancer is the second leading cause of death worldwide. Current treatment packages available are unable to control the mortality of cancer. Alternate treatment cum management packages are to be explored in particular from medicinal plants. Jatamansi appears to be one of the prospective herbal single drugs to address inflammation, oxidative stress and may contain cancer cell lines. Study being done to explore efficacy of Jatamansi's cytotoxic, anti-oxidant and Anti- inflammatory activity on malignant cell lines.

Key Words: *Anti-inflammatory, Anti-Oxidant, Cytotoxic, Malignant Cell Lines, Nitric Oxide*

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INTRODUCTION:

Cancer is a major health problem in both developed and developing countries. Cancer refers to a group of diseases which share similar characteristics. Cancer can affect all living cells in the body, at all ages and in both genders. The causation is multi factorial.

Nardostachys jatamansi **[Family *Valerianaceae*][1]** is a perennial herb found in Alpine Himalayas. *N. jatamans*i used for long period in various chronic diseases therapeutically. It is a reputed Ayurvedic herb and used in various multiple formulations. *jatamansi* has been used in the treatment of many diseases, as per Ayurvedic texts Jatamansi is Medhya (Brain tonic), Rasayana (Rejuvenative to the mind), Nidhrajnana (Promotes sleep), Manasrogaghna (Alleviates mental diseases), Pachana (Digestive), Kasawasahara (Alleviates- coughs and breathing difficulties), Kushtaghna (Stops skin diseases and itching), Dahaprasha- mana (Stops burning sensations), Varnya (Benefits complexion) and Roma sanjanana (Promtes- hair growth). As on date not much study was done the effect of Jatamansi on cancer cell lines there by this study conducted to understand the anti-proliferative, anti-inflammatory and anti-oxident effect of Jatamansi root extract on different cancer cell lines evaluation by MTT Assay, Nitric Oxide (LPS induced) inhibition assay and oxidative stress enzymes determination [2].

NEED FOR THE STUDY:

Irrespective advanced research in the cancer treatment high death rate associated with cancer and serious side effects of chemotherapy and radiation therapy not changed much, thereby many cancer patients seek alternative and/or complementary methods of treatment.

Methods - 1. Cytotoxicity evaluation by MTT assay [3,4]

The effect of methanolic extract of Jatamansi, was studied on five different cancer cell lines (B16F10 (Melanoma), MCF-7 (Brest Adenocarcinoma), A549 (Lung Carcinoma), SK-Hep-1 (Adenocarcinoma Liver) and WRL-68 (Liver Cancer Hepatocyte)). All the cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India except MCF7 cells which were obtained as a gift sample from Dr. Lekha Dinesh Kumar, CSIR-CCMB, Hyderabad, India. B16F10, and MCF-7 cells were maintained in DMEM (Gibco, USA) media supplemented with 10% fetal bovine serum (FBS, Gibco, USA), and Penicillin-Streptomycin (100 IU/mL each; Sigma Aldrich, USA), A549 cells were maintained in RPMI-1640 (Sigma Aldrich, USA) and SK-HEP-1 and WRL-68 cells were maintained in MEM media (Gibco, USA) supplemented with FBS and antibiotic mixture. The cytotoxicity assay was performed as per MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) (Himedia, India).

The effect of Jatamansi extract was studied in 96 well plates based anticancer screening platform. Cells at a confluence of 70-80 % were trypsinized and used for further seeding into the plates. For cytotoxicity evaluation, 96 well plates (Nest, India) were seeded with approximately 5000 cells/well and incubated overnight at 37ºC and 5% CO_2 in a humidified incubator (Thermo Fisher, USA). The respective extracts were incubated with cells for 48 hours at eight gradient concentrations in triplicates (1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200 and 400 µg/mL) prepared in respective plain media without FBS and were filtered with sterile syringe filters (0.2 µ, Pall, USA) prior use. After incubation, drug solutions were rinsed and the cells were incubated with 100 µL MTT solution/per well (0.5 mg/mL, prepared in respective plain media) for 4 hours. Thereafter, the formed formazan crystals were dissolved by adding 200 µL DMSO/well, and kept on orbital shaker for 20 min. The OD was measured at 570 nm and results were quantified for the calculation of percentage viability using Graphpad Prism, version 5.0.

2. Nitric oxide inhibition assay [5]

Estimation of nitric oxide under the influence of lipopolysaccharide (LPS) on Raw 264.1 macrophages was carried out by using Griess reagent (Sigma Aldrich, USA). Optimally grown macrophages were seeded into 96 well plate at a seeding density of 5000 cells/well. After incubation for 24 hours at 37ºC and 5% CO_2, cells were treated with different concentration of respective extracts (1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200 and 400 µg/mL) for 24 hours. All the dilutions were prepared in plain media containing 2 µg/mL LPS without FBS and were filtered with sterile syringe filters (0.2 µ, Pall, USA) prior use. Respective controls were kept for data interpretation. After the incubation time period was over, 100 µL of respective supernatant was mixed with 100 µL of Griess reagent, kept in dark for 15 minutes and the OD was measured at 540 nm using spectrophotometer (Spectramax, Molecular Devices, USA). The results were compared with respective controls.

3. Intracellular reactive oxygen species determination using DCFDA assay [6]

Intracellular ROS generation was measured by DCFDA method as reported previous literature. The Raw 264.1 macrophage cells were seeded into a 96 well plate. The cells were allowed to grow for 24 h.

After treatment with respective extracts at the selected doses (1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200 and 400 µg/mL) for 24 h in presence of LPS (2 µg/mL), the treatment solutions were removed, the cells were incubated with 10 µM DCFDA (100 µL/well) at 37ºC for 20 min followed by two times washing with sterile PBS. The intracellular reactive oxygen species (ROS) mediated oxidation of DCFDA to the fluorescent compound 2,7-dichlorofluorescein (DCF) was photographically measured using a fluorescent microscope (Nikon, Japan) at excitation wavelength of 498 nm and emission cut off at 530 nm.

RESULTS AND DISCUSSION:

1. Effect of Jatamansi extract on cancer cell proliferation

Cell viability of five different cancer cell lines was performed by using MTT assay. The primary stock of respective crude extract was prepared in DMSO (4 mg/mL) and stored at -20ºC for further experiments. The sub-cultured cells were plated in cell culture grade 96-well plates. After 24 hours incubation, the respective extracts were incubated for 48 h with cells at the concentration range of 1.5-400 µg/mL. The results of MTT assay suggest safety of Jatamansi extract studied as even up to a dose as high as 400 µg/mL more than 80% cells were alive except in B16F10 melanoma cells though not that significant.

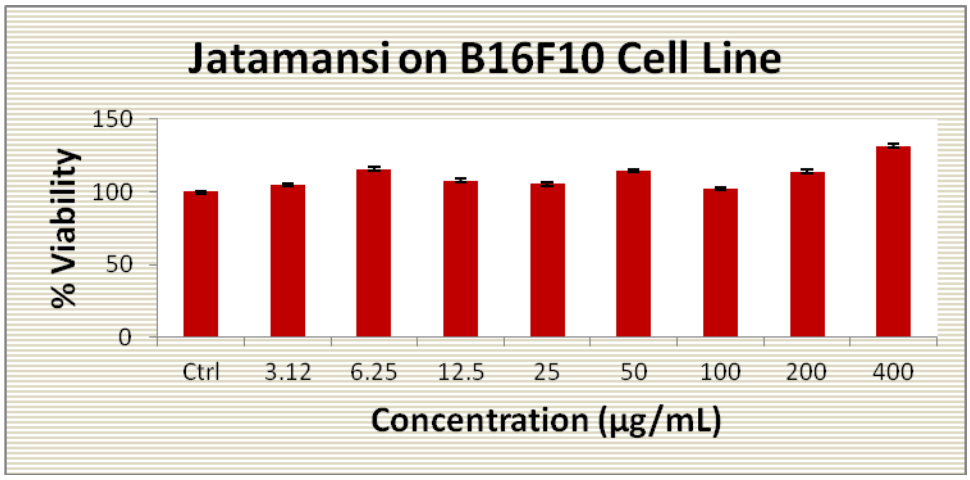
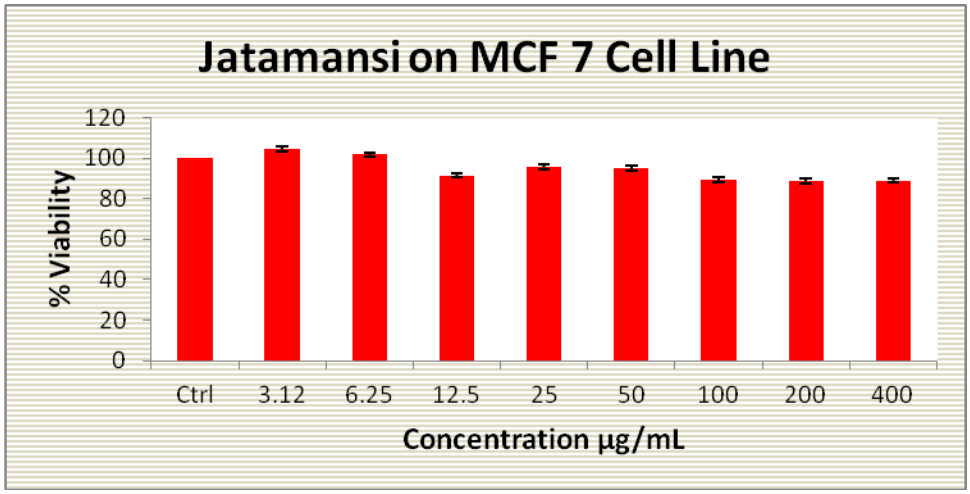


Figure 1



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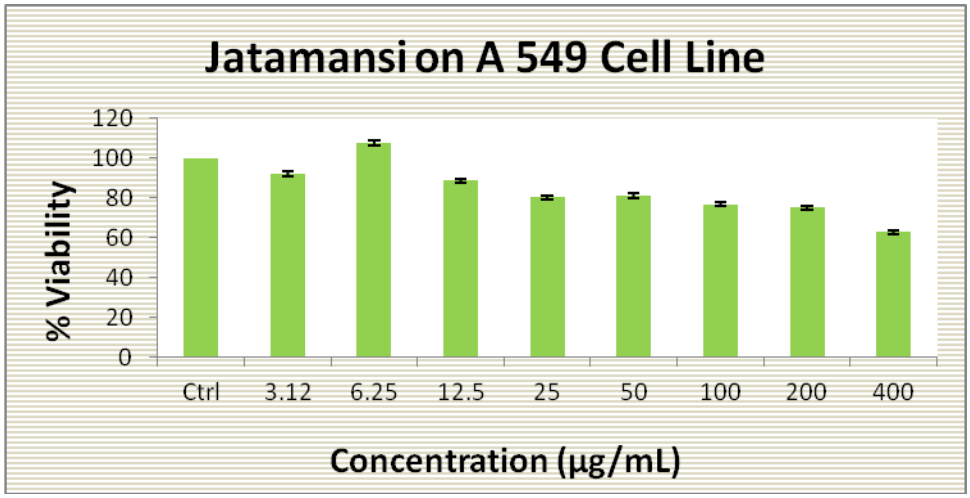

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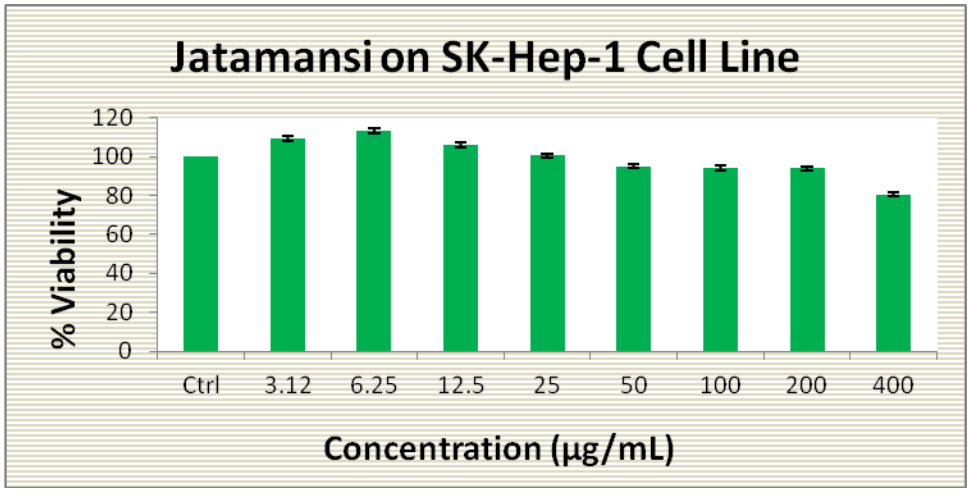

Figure 4

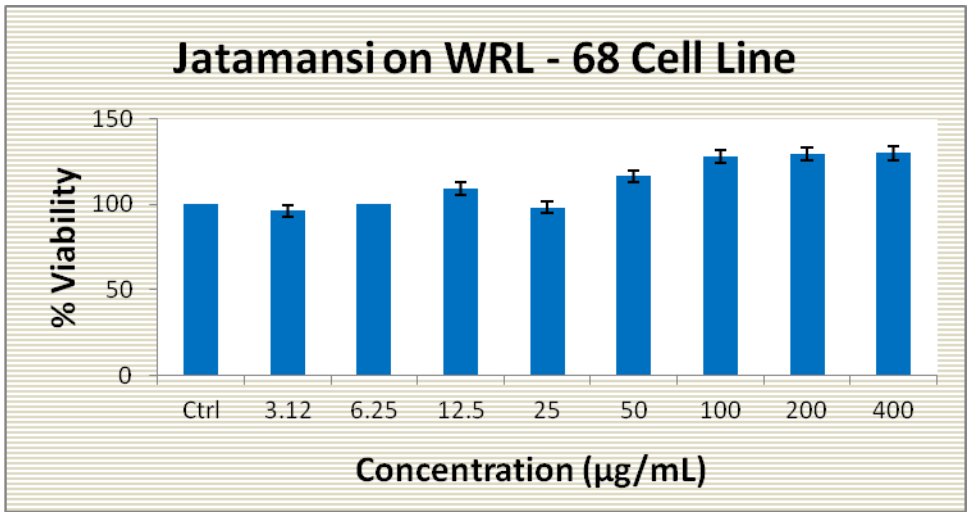

Figure 5

Figure (1-5). Cytotoxicity evaluation by MTT Assay: Anti-proliferative activity of jatamansi extract on relative percentage viability of cells at various concentrations (3.1 – 400 µg/mL) on different cancer cell lines (Figure: 1-5). The cell lines were incubated for 48 hours. There was no significant change in cell viability upon treatment with Jatamansi compared to untreated control cell lines was observed in treated cell lines. Values represented mean ± SEM (n = 3).

2. Effect of Jatamansi extract on LPS induced nitric oxide in Raw 264.1 macrophage cells

The Griess test is a simple biochemical assay which can detect the presence of nitric oxide indirectly through nitrite ion in cell culture supernatants. Raw 264.1 macrophages were seeded into 96 well plates and were incubated for 24 h in a cell cultureincubator. The cells were pre-treated with different concentrations of respective extracts (1.5-400 µg/mL, prepared in plain media) in the presence of LPS (2 µg/mL) for 24 hrs. Thereafter, the cell culture supernatant was taken (100 µL) and mixed with equal volume of Griess reagent (100 µL), incubated for 10 min and OD was measured at 540 nm. The nitrite concentration was significantly increased in LPS treatment group as compared to the untreated control group. Jatamansi extract showed most prominent nitric oxide inhibitory activity and we observed a dose dependant decrease in the levels of reactive nitrogen species (RNS) with increasing doses of Jatamansi extract (78 % inhibition at 200 µg/mL). The results indicate excellent anti-inflammatory and antioxidant activity of Jatamansi extract. Observations to date suggest that oxidative stress, chronic inflammation, and cancer are closely linked.

Table 1: Dose dependent effect of Jatamansi extract (JE) on nitric oxide % release in Raw 264.7 macrophages compared to LPS control

Concentration (µg/mL)	Control	LPS Ctrl (2 µg/mL)	JE 3.12	JE 6.25	JE 12.5	JE 25	JE 50	JE 100	JE 200	JE 400
Jatamansi extract (% NO release)	16.30	100	99.15	96.21	92.45	88.74	73.56	30.16	22.69	20.32

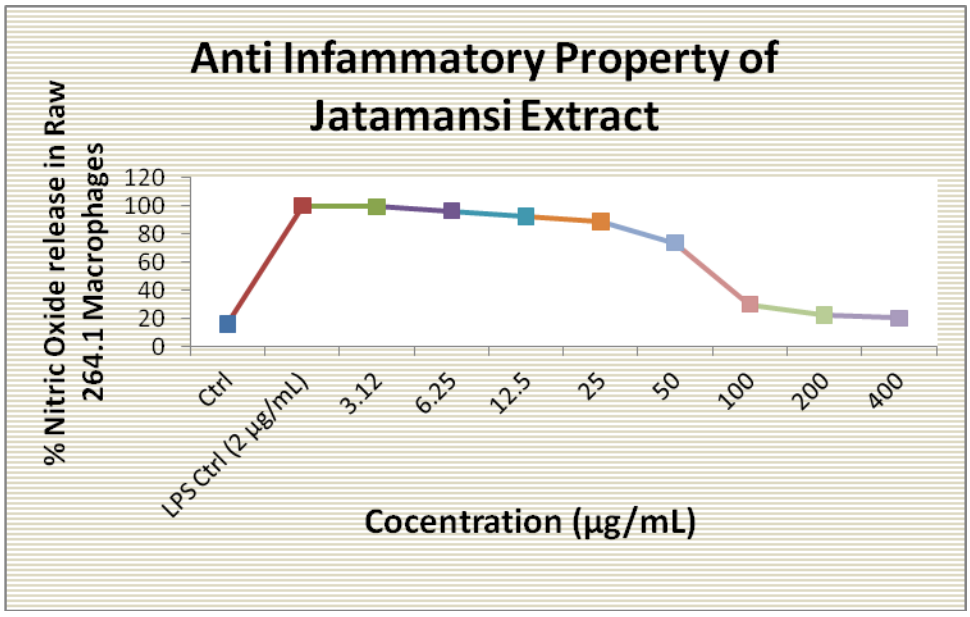

Fig 6: Dose dependent effect of Jatamansi extract on percentage nitric oxide release in Raw 264.1 macrophages compared to LPS control

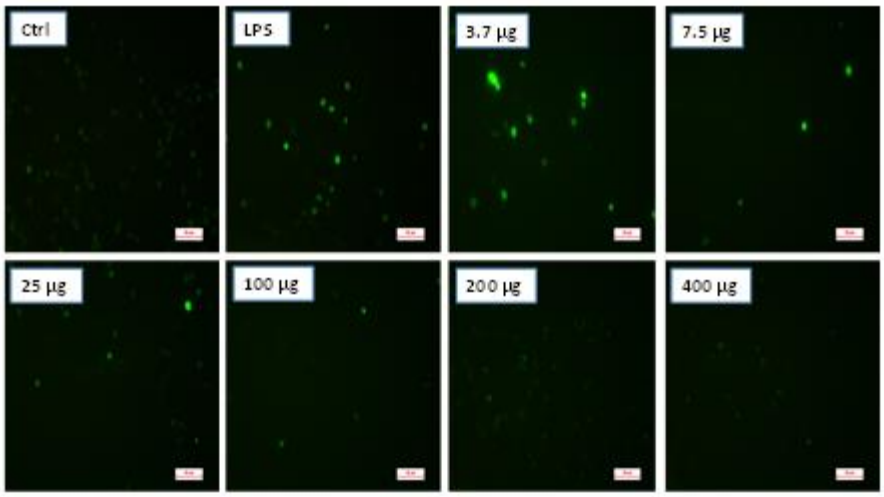

Fig 7: Reactive oxygen species detection by DCFDA assay: Effect of Jatamansi extract on reactive oxygen species generation induced by 2µg/mL LPS was detected by using DCFDA assay after treatment at concentrations of 3.7, 7.5, 25, 100, 200 and 400 µg/mL for 24 h on Raw 264.1 macrophage cells. The images were obtained using an inverted fluorescent microscope (Nikon, Japan).

Effect of Jatamansi extract on LPS induced intracellular reactive oxygen species induction in Raw 264.1 macrophage cells

To further explore the antioxidant potential of these extracts, we investigated their role in inhibition of intracellular ROS generation by using DCFDA dye based fluorescent assay. **Jatamansi shown its remarkable ability to inhibit intracellular ROS induced by LPS.** The representative fluorescent images are shown in figure7.

CONCLUSION:

To sum up the study – Though Jatmansi showed not much of anti-proliferative/cytotoxic activity, it showed significant anti – inflammatory activity and anti – oxidant activity, there by Jatamansi root extract may be useful in the management of cancer treatment for its anti-inflammatory and anti-oxidant properties. Future investigation of Jatamansi extract may aid in better understanding the molecular mechanism associated with the observed protection.

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