Anti-Inflammatory Insights: MTT Assay of Vedi Annabedhi Chendhooram on LPS-Induced ELT-3 Cell Lines

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Abstract:

Siddha medicine,a traditional healing practice rooted inTamil culture, recognizes an categorizes over 4,448diseases. Among its formulations, *Vedi Annabedhi Chendhooram* (VABC),a herbo-mineral medicine,is not able for its potent effects on ailments such as jaundice, anemia, dysentery, fever, dropsy, ascites, and generalized anasarca.Traditional Siddha practitioners also use VABC for uterine disorders, including dysmenorrhea and leiomyomas (uterine fibroids,) where inflammation plays a crucial role in disease progression. This study investigates the anti-inflammatory effects of VABC on uterine leiomyomas. Methods: An invitro studywas conducted using lipopolysaccharide(LPS)-induced Eker LeiomyomaTumor-3(ELT-3)cells to assess the anti-inflammatory effects of *Vedi Annabedhi Chendhooram* (VABC). Cytotoxicity was measured using the MTT assay, with LPS applied at1 µg/ml to induced cell damage, resulting in 36% cell viability.VABCwas administered at doses up to 100 µg/ml, and cell viability was measured after 24 hours. Results: The MTT assay revealed that VABC increased cell viability in LPS-induced ELT-3 cells in a dose-dependent manner up to 50 µg/ml, suggesting effective cell recovery at lower doses. However, at 100 µg/ml, VABC showed moderate toxicity, resulting in decreased cell viability. These findings indicate VABC's potential for reducing inflammation-induced damage in ELT-3 cells at specific doses.Discussion: The observed anti-inflammatory properties of VABCin ELT-3 cell lines highlight its therapeuticpotential inmanaging uterineleiomyomas and other inflammation-linked disorders. Further studies are warranted to explore the underlying mechanisms of VABC's effects and to evaluate its clinical applications. Conclusion: This study underscores the potential of VABC as an anti inflammatory agent, particularly in the context of uterine leiomyomas. Its application in Siddha medicine may offer a natural therapeutic approach to managing inflammation - driven conditions.

Key words: *Vedi Annabedhi Chendhooram,* Anti-inflammatory activity, uterine leiomyomas, Eker Leiomyoma Tumor -3.

Introduction

Many traditional healing practices are still actively followed by people across diverse regions, with Siddha medicine being one of the most prominent. Firmly embedded in the culture of the Tamil people, Siddha is often referred to as "Tamil medicine." In this ancient tradition, a remarkable 4,448 diseases are recognized and categorized. The Siddha system comprises a vast array of formulations categorized into *Agamarunthu* (internal medicines) and *Puramarunthu* (external medicines), derived from plant, mineral, metal, and animal sources.

Significantly, the system places special emphasis on higher-order medicines derived from metals and minerals, which are considered particularly potent in treating various ailments. Among these, *Vedi Annabedhi Chendhooram* stands out as a herbo-mineral medicine used internally.It is acclaimed for its effectiveness in treating a range of health conditions, including jaundice, anemia, dysentery, fever, dropsy, ascites, and generalized anasarca. In addition to its recognized uses, traditional Siddha practitioners also employ *Vedi Annabedhi Chendhooram* (VABC) for managing uterine disorders, such as dysmenorrhea and leiomyomas.

One of the most common causes of jaundice, hepatitis refers to inflammation of the liver, often caused by viral infections (such as hepatitis A, B, or C), alcohol, drugs, or toxins. This inflammation impairs the liver's ability to process bilirubin, leading to its accumulation in the bloodstream and subsequent jaundice of Liver inflammation.[1]

A common anemia type associated with chronic diseases and inflammatory states. In chronic inflammation, elevated hepcidin levels, cytokine activity, increased RBC turnover, and reduced nutrient absorption all combine to limit the availability and production of red blood cells. This type of anemia often persists until the underlying inflammation is managed or resolved.[2]

Dysentery is commonly caused by infections, typically either bacterial (e.g., *Shigella* species, *Escherichia coli*) or amoebic (e.g., *Entamoeba histolytica*). When pathogens invade the intestinal lining, the immune system responds by releasing inflammatory mediators such as cytokines (e.g.,IL25,IL-35) and prostaglandins to combat the infection. This immune response leads to localized inflammation in the colon, which is essential for fighting the infection but also results in symptoms like pain, tissue damage, and diarrhea.[3]

When the body encounters pathogens (like bacteria or viruses), damaged cells, or harmful stimuli, the immune system responds with inflammation to contain and eliminate the threat. During inflammation, immune cells (such as macrophages and neutrophils) release signaling molecules called cytokines — notably interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). These cytokines reach the hypothalamus, the brain's temperature-regulation center, and signal it to raise the body's core temperature, resulting in fever. This response is thought to help inhibit pathogen replication and enhance immune function.[4]

In inflammatory conditions, immune cells release cytokines like TNF-α, IL-1, and IL-6, which cause blood vessel dilation and increased permeability. This allows fluids, proteins, and immune cells to leak out into surrounding tissues and, in the case of ascites, into the abdominal cavity. This fluid leakage, along with increased production of lymph in response to inflammation, contributes to the accumulation of fluid in ascites. Ascites can also result from inflammation due to peritonitis (inflammation of the peritoneum, the membrane lining the abdominal cavity), often caused by bacterial infection. In peritonitis, the peritoneal lining becomes inflamed and porous, allowing fluid and immune cells to accumulate in the abdomen. This infection-related inflammation is common in people with pre-existing ascites, where the stagnant fluid serves as a medium for bacterial growth (a condition called spontaneous bacterial peritonitis).[5]

During inflammation, immune cells (like macrophages and neutrophils) release signalling molecules called cytokines (e.g., IL-1, IL-6, and TNF-α) and histamines. These molecules increase the permeability of blood vessel walls. This increased permeability allows fluids, proteins, and white blood cells to move out of blood vessels and into the surrounding tissue. This leakage of fluid leads to localized edema in the area of inflammation, which can be seen as swelling, warmth, and redness.[6]

Numerous cytokines, including tumor necrosis factor (TNF-α) and interleukins (IL-1 and IL-6), along with various chemokines and their receptors (such as MIP-1α, MIP-1β, RANTES, eotaxin, eotaxin-2, IL-8, CCR1, CCR3, CCR5, CXCR1, and CXCR2), play a role in the progression of leiomyomas (LM).[7]

1. Material and Methods:

1.1 Preparation of VABC

The detoxification process of *Vediuppu* (KNO3) and *Annabedhi* (FE2SO4) was carried out following the procedures outlined in an authorized Siddha textbook. Fresh lemon juice (100 ml) was used in the process. *Annabedhi* and *Vediuppu* were mixed into a *kalvam* along with the lemon juice and ground thoroughly until a wax-like consistency was achieved.The mixture was then shaped into small circular cakes and left to dry. Once dried, the cakes were placed in a clay vessel, which was covered with another clay vessel and wrapped in seven layers of cloth

(*Seelaimann*). This setup was then subjected to calcination using 70 cow dung cakes. The resulting product was further processed, yielding a powdered substance after three *pudams*.[8]

VABC was investigated for its potential to scavenge or protective ability of cell proliferation and cyto-toxicity by in-vitro (ELT-3 cell line) chemical models of anti-inflammatory activity.

1.2 Rationale of the Study

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-coloured water-soluble tetrazoliumdye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm. [9,10]

1.3 In Vitro study of Anti-inflammatory activity – MTT assay on LPS induced ELT-3 cell lines

The anti-inflammatory activity of the test drug sample VABC was determined using the MTT (3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyl tetrazolium bromide) assay.100-105 Seed 200µl cell suspension in a 96-well plate at required cell density (20,000c ells per well),without the test agent. Allow the cells to grow for about 24 hours. Induce the inflammation to cells by adding 1µg/ml of LPS followed by adding appropriate concentrations of the test agents Vedi Annabedhi Chendhooram (VABC) (6.25µg, 12.5µg, 25µg, 50µg and 100µg) was noted 24 hrs incubation period at 370C in a CO2 atmosphere. After the incubation period, take out the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/ml of total volume. Wrap the plate with aluminium foil to avoid exposure to light. Return the plates to the incubator and incubate for 3 hours. Remove the MTTreagent and then add 100µl of solubilisation solution (DMSO). Gentle stirring in a gyratory shaker enhanced dissolution. And read the absorbance on an ELISA reader at 570nm wavelength.[11,12] % Cell viability is calculated using below formula:

% cell viability = [Mean abs of treated cells / Mean abs of Untreated cells] x 100

1.3.1 Assay controls

(i) Medium control (medium without cells) - Blank

(ii) Negative control (medium with cells but without the experimental drug/compound)

(iii) (iii)Positive control (medium with cells treated by 1µg/ml of LPS)

Note: Extracellular reducing components such as ascorbic acid, cholesterol, alpha-tocopherol, dithiothreitol present in the culture media may reduce the MTT to formazan. To account for this reduction, it is important to use the same medium in control as well as test wells.[13]

1.3.2 Maintenance of cell lines

The ELT-3 (Rat Eker LeiomyomaTumor-3cellline) was purchased from ATCC, USA. The cells were maintained in D8 media supplemented with 20% FBS a2 5µg/ml Insulin along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at 37^0C temperature in the CO_2 incubator and sub-cultured for every 2-3 days. ELT-3 cells at passage number 21 were used for the present study.[14]

1.3.3 Drug concentrations details

In this study, given test compound was evaluated to analyse the cytotoxicity effect on LPS induced ELT-3 cells. The concentrations of the test compound used to treat the cells as follows:

Table1: Details of VABC treatment to ELT-3cells

Sl. No	Culture condition	Cell line	Concentrations treated to cells
1	Untreated	LPS induced ELT-3cells	No treatment
2	LPS		1ug/ml
3	VABC		6.25, 12.5, 25, 50, 100ug/ml
4	Blank	-	Only Media without cells

2. OBSERVATIONS & RESULTS

Table2: % cell viability values of LPS induced ELT-3 cells treated bydifferent concentrations of VABC after the treatment period of 24hrs.

Culture condition	%Cell viability
Untreated	100.00
LPS-1ug/ml	36.02
LPS+VABC-6.25ug	53.25
LPS+VABC-12.5ug	78.65
LPS+VABC-25ug	86.41
LPS+VABC-50ug	94.96
LPS+VABC-100ug	85.48

The results of the cytotoxicity study performed by MTT assay suggest that the test compound, VABC was satisfactorily recovered the LPS induced ELT3cells with increased % cell viability values in dose dependent fashion till the maximum dose of 50ug/ml and at 100 µg/ml dose the molecule was moderately toxic with decreased viability value after the incubation period of 24hours. LPS with 1 µg/ml was caused effective toxicity with 36% cell viability.

Figure 1: Graph of % cell viability values of LPS induced ELT-3cells treated by different concentrations of VABC after the incubation period of 24hrs

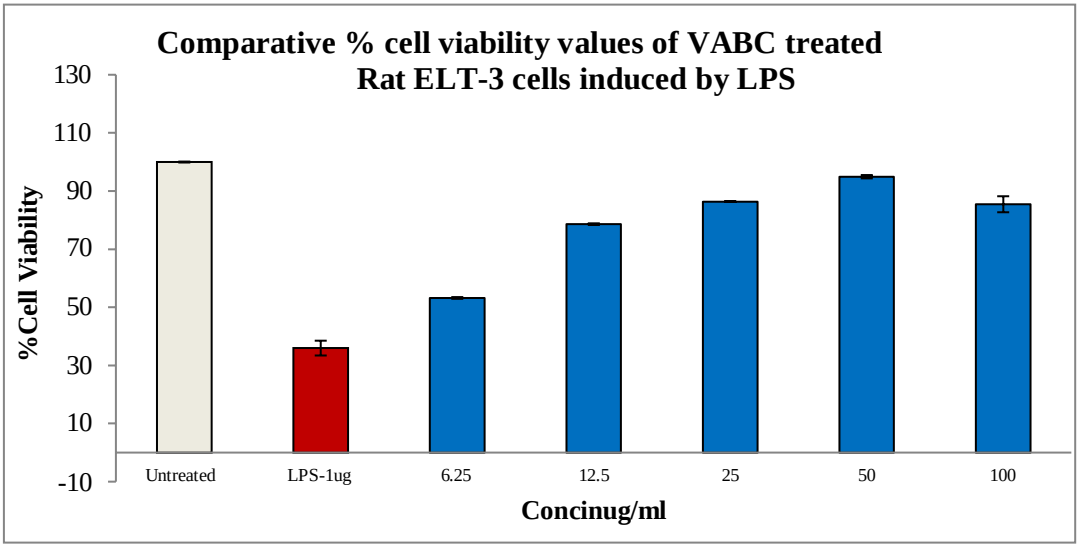
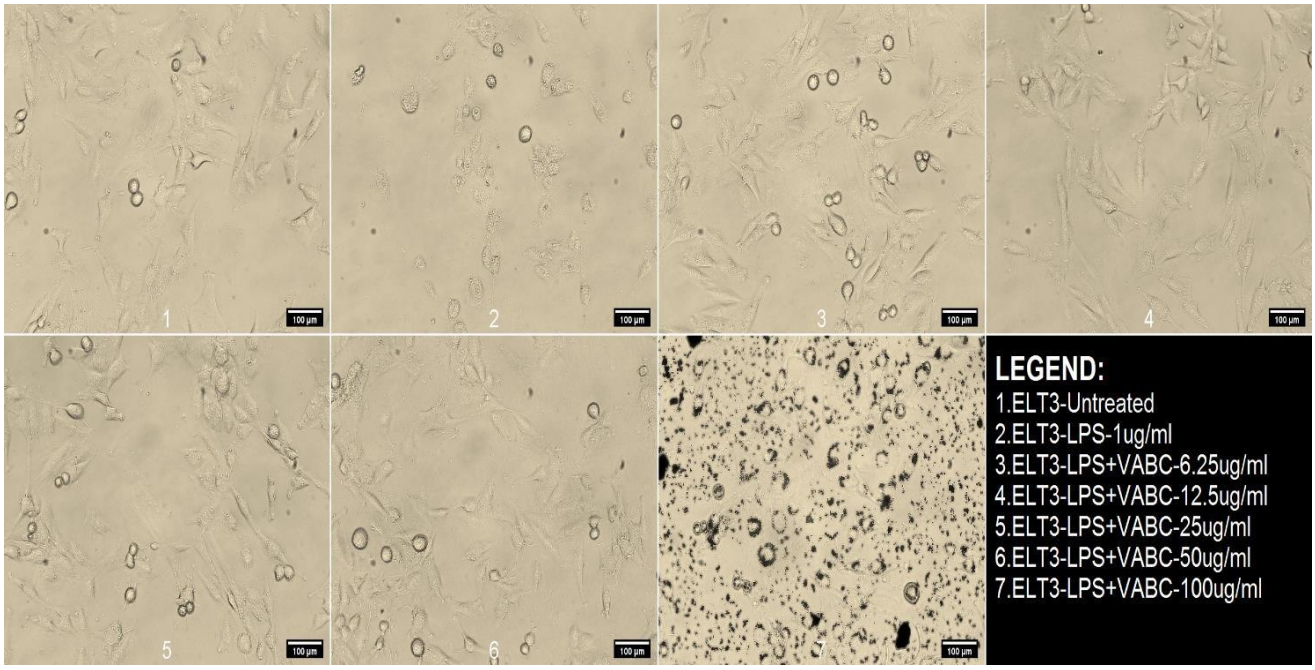


Figure 2:Images of LPS induced ELT-3cells legend



3. Discussion

The MTT assay results suggest that the given test compound, VABC was protected the ELT3 cells from LPS treatment after the 24hours of incubation. LPS was used as a inflammation inducing agent which showed the 36% cell viability at1µg/ml dose after 24 hours of incubation. The combination of LPS followed by VABC with different doses effectively increased the % cell viability values and VABC proved to anti-inflammatory agent by effectively recovering the cells from LPS treatment on dose dependent fashion.

However, further studies need to be conducted to determine the molecular mechanism behind anti-inflammatory properties of the test compound at in vitro level with studies like Apoptosis/Necrosis study, Cell Cycle study, ROS and apoptotic proteins expression studies etc by choosing maximum non-toxic concentration of compound (50µg/ml) to find the molecular mechanism of action behind the anti-inflammatory effect in Rat ELT3 cells in LPS induced model. The MTT assay results suggest that VABC proved to anti-inflammatory agent by effectively recovering the cell from LPS treatment on dose dependent fashion.

4. Summary

This study investigates the anti-inflammatory potential of Vedi Annabedhi Chendhooram (VABC) in an in vitro model using LPS-induced Rat ELT-3 cells. LPS, known to induce inflammation, significantly reduced cell viability.The MTTassay results suggest that VABC, when applied in combination with LPS, effectively enhanced cell viability in a dose-dependent manner, indicating its anti-inflammatory effects. The study highlights VABC's potential in mitigating the inflammatory damage caused by LPS. However, further research is necessary to elucidate the molecular mechanisms underlying the anti-inflammatory properties of VABC, including studies on apoptosis/necrosis, cell cycle, ROS generation, and expression of apoptotic proteins.

5. Conclusion

VABC demonstrates promising anti-inflammatory activity by protecting ELT-3cells from LPS-induced damage. The results from the MTT assay confirm its potential to recover cells from inflammation in a dose-dependent manner. While this study provides evidence ofVABC anti-inflammatory effects, future investigations are required to explore the detailed molecular

mechanisms involved. These studies could include apoptosis/necrosis, cell cycle analysis, and evaluation of ROS and apoptotic protein expression, focusing on non-toxic concentrations of VABC to better understand its role in inflammatory processes at the cellular level.

6. Acknowledgement

The authors express their heart felt gratitude to the esteemed faculties at theDepartmentof Siddha, The Tamil Nadu Dr.M.G.R. Medical University, Guindy, Chennai and Averin Biotech Labs, Bangalore. The authors are truly grateful for the opportunity toconduct this article under such auspicious guidance, and their sincere thanks go to all those who have contributed to this scholarly endeavour.

7. Conflictof Interest

Wedeclarethatwehavenoconflict of interest.

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