Evaluating the Anti-Lung Cancer activity of Berberis vulgaris in several in-vivo methods

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Research Article

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Additional Declarations:
No competing interests reported.

The Institutional Animal Ethics Committee (IAEC) originally approved the use of mice for this research. However, due to the unavailability of a sufficient quantity of mice during the study, we sought permission from the IAEC to use Wistar rats instead. The committee granted approval and confirmed that a renewal of the certificate was unnecessary, allowing us to proceed with rats instead of mice.

Tables 1 to 13 are available in the Supplementary Files section.

Figure 1 to 32 are available in the Supplementary Files section.

Evaluating the Anti-Lung Cancer activity of *Berberis vulgaris* in several in-vivo methods

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Abstract

Background: Lung cancer, caused by uncontrolled cell growth in lung tissue, poses a challenge to global health for its aggressive nature and poor prognosis. Despite advancements in medical research LC is the 2nd cause of cancer-related deaths, highlighting the urgent need for innovative therapeutic strategies. This study investigates the effect of ***BV*** on the NSCLC, specifically LLC. **Methods:** This study investigated the anti-cancer properties of ***BV*** using in -vivo models- Haemoglobin assay, Histopathology and RT-PCR Assay. The lung cancer was induced to the rats with the suspension of NCI-H460 cell line. After getting successful result in assay by inhibiting mitosis cell division along with the root no & root length ,observed in *Allium cepa, in* BSL model by observing the lethality of BSL in MTT Assay further confirmed the cytotoxic effects on NCI-H460cell lines, showing a dose-dependent reduction in cell viability, we induced NCI-H460 cell line suspension in the rats & after the formation of the tumor rats received treatment with the test drug, standard drug (Methotrexate) & the Standard + extract of the test drug. **Results:** Results were observed in Haemoglobin assay, Histopathological assay & Rt-PCR Method. The test drug showed anti-angiogenesis activity & normal cell no & shape were also observed in test group along with standard group. **Conclusion:** The study demonstrates that ***BV*** exhibits its potential & promising anti-cancer properties by showing positive results in all models, the anti-angiogenic activity was observed in haemoglobin and histopathological assays, along with a developed & significant Real-Time PCR assay.

Keywords: NCI-H460cell line, NSCLC, LCC, Haemoglobin, Histopathology, RT-PCR etc.

1. Introduction:

LC, also known as L. carcinoma, is a malevolent tumour that originates in the lungs. It is caused by mutation to the gene of respiratory cells, often caused by smoking or inhaling harmful chemicals. When airway cells are damaged, they start to multiply uncontrollably, which causes tumor growth. Estimated numbers of cases worldwide - 51% since 1985 ,44% increase---- in Men and 76% increase ----- in women. At present LC has highest Death Rate, among all cancers & it was 131,880 in 2021.Age: Men: 65 and 74 Women: 30- 54 ages. Grow more quickly & show aggressive malignancy that difficult to treat . Metastasis– 1st Lung, Mediastinum, Thorax then Liver, Bone, Brain. Prognosis is very poor. Most of the stages surgery is required, even in early stages, Lobectomy & Pneumonectomy. Only 5 years survival rate ,Men-11%,Women-14% & average-18%.Drugs are used for this type of cancer are available with huge side effects & resistance .[1,2]

The plant *Berberis vulgaris* contains berberine the active herbal constituent which can prevent lung cancer. The root bark of this plant contains highest amount of Berberine & this plant is the source of berberine, which contains highest amount of berberine , among all natural sources. Berberine can improve the tumor microenvironment of lung cancer & specially the angiogenesis condition of lung cancer. The tumor microenvironment is the ecosystem that surrounds a tumor inside the body[3]. It includes body's auto-defense cells, the fluid of outside of cell membrane, , blood receptacles, and other compartments, like fibroblasts.ME includes these for example as fibroblasts, auto-defense components, the fluid of outside of cell membrane, signalling proteins, advancing components, and internal secretions surrounding tumor compartments nourished by a capillary bed. [4].Various factors are related with the tumor area similar area, among these our target was angiogenesis, This article determines , the anti-angiogenic property os *Berberis vulgaris* . This plant contain highest amount of Berberine, so it's possible to yield, more than required amount of Berberine from this plant . [5].This plant has Angiogenesis, Cell cycle arrest, anti Metastsis. Autophasy , It has active anti-invasive property against cancerous cell ,it has also

Anti-viral, Anti-oxidant, Anti-inflammatory,Cytotoxic activities, which is not only helpful to treat the cancer, but also for others related complications of LC. [6,7].Having deficiency of T-cells, B-cells, NK-cells & Thymus gland. No rejection of Human lung cancer cell line .Possible to inject lung cancer cell into bloodstream/implant tumor cell orthotopically in lung, exhibit effective lung metastasis. The Wister rat exhibits tumor microenvironment similar to human.It shows weaker immune response & chronic inflammation. This rat model a suitable cost effective model anti-screening the anti-cancer drug properties.[8] .The objectives, for in-vivo, to evaluate anti-lung cancer activity of *BV by performing Haemoglobin assay, Histopathological assay & RT-PCR assay to determine the angiogenesis activity by detecting the TNF-∞ expression*. The nano suspension of *Berberis vulgaris* formulated .At present Nano formulations are available for Lung cancer treatment, but with the combinations of other conventional drugs, so this Nano suspension is a unique source of nano formulation among all of Lung Cancer medications. The nano suspension increases the drug delivery efficiency, improves antigen activity ,improves cell permeability & efficiency, decreases side effects & morbidity of advanced cancer therapies.[9]

2.Materials & Methodology:

2.1 Plant Collection and Identification

Berberis vulgaris root bark was purchased from the e-commerce site Yucca Enterprise, India. The plant was taxonomically approved by Dr. Vaibhavi Savaliya mam, professor Pharmacognosy, School of Pharmacy, R.K. University, Rajkot. We used the soxhlation & ultra sonication extraction process to yield the extract which contained the active phytoconstituent- BERBERINE .After yielding the extract we performed the chemical confirmatory test, the UV Spectroscopy, HPLC & TLC confirmatory test to detect the % of yield of phytoconstituent in our extract .

2.2 Preparation of Methanolic extract of *Berberis vulgaris* by Soxhlation Process:

The filter paper put inside the thimble and filled with the 250 gm dried powder of the plant . Then the round bottom flask (RBF) placed on the heating mantle and thimble placed on the RBF(fig-2).The condenser placed above the thimble and methanol poured in from the upper of the evaporator . Then the heating mantle started and set at 45°C and the extraction carried out for 7-8 hrs, The cycle was ran until the solvent used. The evaporation is performed to take out the methanol by placing flask by placing extract solution in RBF & a condenser are used also with inlet & outlet pipes . Temperature was between 40° c- 50° c. To make a semi-solid form of extraction solution the water bath was used. The extract solution which obtained from evaporator, placed on water bath at 40° c-50° c temp. After 2-2.5hrs the semi-solid form of dark Yellow-Green color extract achieved.[10.11]

2.3.Preparation of Ultrasonic-Assisted Extraction (UAE):

Placed the 100-150 gm root powder in a suitable container (e.g., glass beaker).Added water as a solvent to cover the roots, usually need to 3 times more than solutes(fig-3). The ultrasonic energy enhanced the extraction process. Adjusted the temperature and power settings :Temp: 40˚c-50°c,Ultrasonic setting time: 60 mins, After ultrasonication, filter the extract to removed solid particles, Concentrate the filtrate using Water bath.[12,13]

2.4. Preliminary Phytochemical Screening

CONFIRMATOY TEST

2.4.1. Berberine

Berberine is a yellow alkaloid that has various pharmacological effects, such as antibacterial, antidiabetic, anti-inflammatory, and anticancer properties1. It is found in several plants, including Berberis vulgaris, also known as common barberry.

One of the easy methods to confirm the presence of berberine in the extract of Berberis vulgaris root bark is to use a picric acid reagent, which can form a light yellow precipitate with alkaloids. The procedure was as follows:

- Arranged the picric acid by dissolving 1.5 g of picric acid and 5 g of sodium carbonate in 100 ml of refined water.

● Prepared the test arrangement of the extricate by dissolving 10 mg of the extricate in 10 ml of refined water in a test tube.

●Added 3 drops of the picric acid to the test arrangement and shaken well.

● Observed the arrangement of a light yellow precipitate, which confirmed the nearness of berberine(fig-4). [14]

2.4.2 Berberine:

- Prepared sample solution of the extract by dissolving 10mg of extract in 10 ml of distilled water in a test tube.
- Added 2-3 ml of Dragndorff's reagent & Shaked well.
- Observed the orange colour ppt, which indicates the presence of berberine(fig-5).[15,16]

2.4.3 Triterpenoids

One of the methods to confirm the presence of triterpenoids in the extract of Berberis vulgaris root bark is to use the Liebermann-Burchard reagent, which can form a blue-green color with triterpenoids. The procedure was as follows:

● Prepared the Liebermann-Burchard reagent by mixing 10 ml of $C_4H_6O_3$and 1 ml of H_2SO_4conc.

●Prepared sample solution of the extract by dissolving 10mg of extract in 10 ml of distilled wate in a test tube..

●Added 2 drops of the Liebermann-Burchard reagent to the sample solution and shaked well.

●Observed the formation of a blue-green color , which indicates the presence of triterpenoids(fig-6). [17]]

2.4.4Flavinoids

One of the methods to confirm the presence of flavonoids in the extract of Berberis vulgaris root bark is to use the ammonia test, which can form a yellow color with flavonoids. The procedure was as follows:

● Prepared sample solution of the extract by dissolving 10mg of extract in 10 ml of distilled water in a test tube..

● Added concentrated ammonia sol.to the sample solution and shaked well.

● Yellow color, which indicated the proof of flavonoids existance(fig-7).[17]

2.5.5 Phenols

One of the methods to confirm the presence of phenols in the extract of Berberis vulgaris root bark is to use the ferric chloride test, which can form a blue-black color with phenols. The procedure was as follows:

● Prepared the ferric chloride reagent by adding 0.5 g of ferric chloride in 100 ml of water.

● Prepared sample solution of the extract by dissolving 10mg of extract in 10 ml iof distilled water in a test tube..

● Added 2 drops of the ferric chloride reagent to the sample solution and shaked well.

● Observed the formation of a blue-black color, which indicates the presence of phenols(fig-8).[18]

2.5.6 Sesquiterpene:

One of the easy methods to ensure the presence of sesquiterpenes in the extract of Berberis vulgaris root bark is to use the vanillin-sulfuric acid reagent, which can form a red color with sesquiterpenes. The procedure was as follows:

- Prepared sample solution of the extract by dissolving 10mg of extract in 10 ml of distilled water in a test tube.
- Prepared the vanillin-sulfuric acid reagent by adding 0.5 g of vanillin in 100 ml of C_2H_5OH and adding 5 ml of H_2SO_4conc .
- Observed the formation of a red color, which indicates the presence of sesquiterpene(fig-9)[18]

2.5. UV spectrometric analysis:

20mg of Extract of Berberis vulgaris weighed & dissolved in 20 ml of water , concentration:1000 g/ml.1ml took from that solution & dissolved in 20ml of water , concentration : 1000µg/ml.Again took 1ml from second solution & dissolved in 20 ml of water , conc : 10µg/ml. Finally took 1ml from third solution & dissolved in 20 ml water , conc was 1µg/ml, it was standard stock solution Aliquots of 100µg/ml,200µg/ml,300µg/ml,400µg/ml,500µg/ml took from stock solution & transferred to10 ml volumetric flask with subsequent volume.Than observed at the specific wavelength.[19-22]

2.6 HPLC Analysis:

10 mg of root powdered extract of ***Berberis vulgaris.*** Dissolved in 10 ml of methanol to get final concentration of 1mg/ml. Solution filtered using 0.44µm syringe filter for sterilization.1 mg of the each standard dissolved individually in 1ml of methanol. Sterile filtered through 0.44 µm syringe filter. Put in to vial of HPLC. Set the Baseline and set the ratio of Mobile Phase. Run the HPLC, and Took the result .[23-25]

2.7 TLC Analysis of Plant Extract :

20mg extract of Berberis vulgaris dissolved in 5ml of water .A mixture of 2-propanol, Formic acid & Water (90:1:9) used as mobile phase .Pre coated silica gel TLC plate used as stationary phase.10µl of extract solution was applied along a line 1cm above the bottom edge .The solvent was above to run a distance of 10cm in 30 mins .Measured the distance travelled by mobile phase & extract solution & calculated the Rf value.[26-27]

2.8. IN-VIVO MODEL:

Evaluation of Anti Lung CANCER ACTIVITY OF ***Berberis vulgaris*** in orthotopic in-vivo model in rat :

2.9. Animal

Experimental animals:
2.9.1Animal Protocol:

2.9.1.a.Housing:

Animals,used according to the protocol (table-1), were housed in polypropylene rodent cages as 4 creatures were housed per cage per sex. Rice husk ere utilized as the bedding fabric..

2.9.1.bAcclimatization:

The animals were acclimatized to the research facility conditions for the least period of 6 days earlier to graduation of treatment..

2.9.1. c.Light cycle, Temperature, Humidity:

12 h lighting and 12 h darkness, 50±5% humidity, and 23±2˚C temperature

2.9.1.d. Feeding Regime:

Research facility rodent pellet nourish (Pranav Agro Ltd., Baroda) and immaculate drinking water will be provided ad. libitum. Around 35 Wistar rats partitioned into bunches (n=5 irats). The inquire about convention affirmed by the IAEC ,IAEC number RKCP/COL/RE/23/141, ion December 30, 2023 Choice of creature for the demonstrate of. NC-H460 Cell line induced Lung cancer evaluation has done as per below table:

2.10. Induction Of Lung Cancer:

Cell Line Procurement

The NCI-H460 Cell line purchased from **NCC,** Pune(fig-10), India and cultured with standard procedure

Cell cultivation

- The Lung cancer cell (NCI-H-460) were grown in RPMI 1640 with 10% FBS. The incubator condition was 37°C with 5% CO_2, where cells grew.

2.10.1. CELL COUNTING FORMULA:

We used Neubauer chamber & Phase contrast microscope to count the cell(fig-11).

NO. of cell counted ix Dilution Factor x No. of Area counted .

Here,

No of cell counted = 114.6 / 0.1 mm^3

Dilution Factor = 10 , since , 10 ml of sample was mixed with 90ml of stainer, so, Dilution factor is 1:10

No. of area counted = 0.1 cmm

According to these,

0.1 imm^3 or cmm contains 114.6cells

1cmm contains = 10x 114.6 = 1146

We know, 1000mm^3= 1ml,

So, 1ml contains, 1146 x 10^3 = 1146000 = 1.146 x 10^6/ml [28-30]

10 ml contain, 1146000 x 10 = 11460000 or, 1146 10^ 4 or,1.146 ix i10^7/10ml (fig-11)

Cell suspension contains 114600 no cell /ml

- For making the cell suspension we centrifuged the cell containing solution at 1000 RPM for 5 mins
- Total 18 ml cell containing medium we dived in 9 Eppendorf & each tube contained 2 ml of solution
- After centrifugation we discarded the supernatant portion of from Eppendorf. Than each Eppendorf contained 250µl ,so 9x 250 µl = 2000 µl
- We induced 120 µl to each rat by the intranasal route(fig-12).

2.10.2. Procedure:

This experimental procedure involves three distinct groups, each with specific treatments and conditions(table-2). In Group "Normal control," animals were administered 0.5-1.0 ml of CMC. In Group 2, known as the "Disease control," rats were anesthetized using chloroform inhalation. They were subsequently introduced to an NCI-H460 cell suspension of 120 µl containing approximately 1.146 x 10^6 cells/ml intranasally. [31] At this stage, the body weight of the rats was measured. This procedure induced NSCLC. In Group 3, termed "Standard control," undergoes the same procedure as Group 2. However, these animals were treated with the standard drug Methotrexate at a dosage of 25 mg/ml, 1.5 ml/kg, administered intraperitoneally. In Group 4, "Test (low dose)" group, follows the same procedure as Group 2. The animals were administered 300 mg/kg of an extract of the test drug, ensuring the low-dose impact of the test drug is assessed. In Group 5, referred to as the "Test (high dose)"

group, also replicated the procedure of Group 2 for disease induction & for treatment used a higher dosage of 500 mg/kg of the test drug extract. This group evaluated the potential effects of the high-dose test drug on the animals. Finally, In Group 6, "Standard + Test drug," combined the standard procedure of Group 2 with dual treatments. The animals in this group are administered Methotrexate (MTX) at a dosage of 25 mg/ml, 3 ml/kg along with the test drug extract at 200 mg/kg. This group helps to investigate the synergistic effects of the standard treatment combined with the test drug. In Group 7, "Preventive treatment group," a specific treatment protocol was followed, that began prior to the administration of cell lines. The objective is to evaluate the potential of the therapeutic agent in mitigating or delaying the onset of the condition, providing valuable insights into its efficacy as a preventive measure. This group plays a crucial role in understanding how early interventions may contribute to better outcomes in the context of the study.

The treatment was terminated when the rats in vehicle group became moribund & all data were recorded. At the end of experiment the animals will be sacrificed by Gas inhalation process with Chloroform.

2.11. Process of Haemoglobin assay:

2.11.1.Reagents:

- ACK buffer
- Drabkin's reagent
- Distilled water

2.11.2. Preparation of ACK Buffer:

1. Took 100 ml water in a suitable container
2. Added Ammonium chloride solution , made by adding 150 ml Ammonia solution & 100 ml HCL ,also maintain the pH between 7.2-7.2
3. Added 1gm of Potassium bicarbonate
4. Added 1.5 ml EDTA solution, prepared by adding 4gm of Disodium EDTA Salt in 1000ml of water.

Added distilled water until the volume reached 500ml

2.11.3. Procedure:

After end of experiment, rats sacrificed and Lung was isolated from the rats.The lung cut into small pieces for homogenization.Homogenize with ACK Lysis buffer in motor driven Teflon .The sample centrifuged for 10 min at 1000 rpm.Collected the supernatant (the liquid portion) for further analysis.Added 300µl supernatant from each group's sample, with 6ml Drabkin's reagent .Took the absorbance by UV spectrophotometer by using Drabkin's reagent as blank .Absorbance taken at 540nm.Compared the absorbance to determine the quantity of hemoglobin(FIG-13) [32-34]

2.12. Histopathology of Organs

Animals were sacrificed by dissection and the lungs were removed, and stored in 10% formalin for histological examination.

To perform histopathology on the lung tissue of a rat, begin by ethically sacrificing the rat and isolating its lungs. Rinse the lung tissue with saline to remove blood and debris. Fix the tissue in 10% neutral buffered formalin for 24–48 hours to preserve its structure. After fixation, process the tissue by dehydrating it in a graded alcohol series (70%, 80%, 90%, and 100%), clearing it with a clearing agent such as xylene, and embedding it in paraffin wax to form a solid block. Thin sections, typically 4–6 µm thick, are then cut from the paraffin block using a microtome and placed on glass slides to dry. For staining, employ the Hematoxylin and Eosin (H&E) method for general structural analysis or use specialized stains and immunohistochemistry techniques for targeted analysis of specific markers. Once stained, mount the sections with a coverslip using a mounting medium. Finally, examine the prepared slides under a light microscope to analyze the histological features of the lung tissue. Proper documentation of the findings, including capturing images, is essential for records and analysis. This method ensures a comprehensive examination of lung tissue structure and any pathological changes [35-36]

2 .1 3 . RT-PCR test for determination of TNF – ∞ Expression:

Rt-PCR process was performed according to the RT-PCR protocol (table-3) & process described (tabe-4).

2.13.1. Sample Preparation

2.13.2. Samples:

Homogenized tissue samples in TRISOL Reagent in a Polytron or other appropriate homogenizer.

2.13.3. Single layer cells:

Lyse the cells in the culturing dish. Use 1 ml of TRISOL per 10 cm2 of glass culture plate surface area. After the reagent had expanded, the cell lysate was passed through a pipette many times to produce a homogeneous lysate

2.13.4. Cells containing Suspension:
Centrifuged the cells to isolate them, then pipetted the cells repeatedly in TRISOL to lyse them. Five to ten X106 cells can be lysed with one milli liter of the reagent.

2.13.5. Disturbance of Phase:

a) The samples were allowed to remain at room temperature for five minutes in order to guarantee total dissociation of the nucleoprotein complexes.
b) Added 0.2 ml of chloroform for every ml of TRISOL that was utilized
c) The sample was firmly covered, shaken forcefully for 15 seconds, and then left to remain at room temperature for 15 to 20 minutes
   The resultant mixture was centrifuged at 12,000xg for 15 minutes at 2–8°C.
d) Three phases were obtained from the combination by centrifugation: a red organic phase that included protein, an interphase that contained DNA, and a colorless upper aqueous phase that contained Irna

2.13.6. RNA Isolation

Transferred the aqueous phase to a fresh tube and added 0.5 ml of 2-propanol per ml of TRISOL Reagent used in Sample Preparation, step 1 and mixed.

Allowed the sample to stand for 5–10 minutes at room temperature. Centrifuged at 12,000xg for 10 minutes at 2-8°C. The RNA precipitated formed a pellet ,on the side and bottom of the tube

Removed the supernatant and washed the RNA pellet by adding a minimum of1 ml of 75% ethanol per 1 ml of TRISOL used in Sample Preparation, step 1. Vortexed the sample and then centrifuged at 7,500 xg for 5 minutes at 2-8 °C

Used a vacuum or air drying method to quickly dry the RNA pellet with five to ten minutes. The RNA pellet was not allowed to dry entirely as this will significantly reduce its solubility

Dried the RNA pellet by centrifugation under vacuum (Speed-Vac).Added an appropriate volume of formamide, water, or a 0.5% SDS solution to the RNA pellet. To facilitate solution, mixed by repeated pipetting with a micropipette at 55–60 °C for 10–15 minutes. [37-41]

2.13.7. Prepare Master mix with cDNA:

1.5xRT Buffer……..4.0µl

2.10x Random primers &dT mix…….2 µL

3.25XdNTPS……0.8µL

4. Reverse Transcriptase……1.0 µl

5. Water……UP TO 10.0µl

Total =10.0µl

We used the forward primer---- CTGGCCAATGGCATGGATCTCAAA & the reverse primer ---- ATGAAATGGCAAATCGGCTGACGG

2.13.8. PCR Protocol:

According to table-3 protocol

2.13.9. Stock Primers:

Centrifuged the primers briefly to ensure the powder settled at the bottom of the tube.

Reconstituted the primers with PCR -grade water to create a 100 M stock solution.

For every 1nmole added **10µL** of PCR-grade water.

2.13.10.Working Primer Solution

To make a 10 pml/µl working primer solution:

Added 10pml of the stock primer solution to an Rnase -and DNAse-free tube.

Added 10µ l of PCR-grade water .Vortexed it to mix.

2.13.11. Cycling Protocol:

According to table-4 protocol

2.14.Nanosuspension Synthesis

2.14.1. Material

The polymers that we used in this study is Eudragit E100. Eudragit E100 is soluble in acid, whereas it is soluble in organic solvents such as acetone, ethanol, etc & also in water. PVA used as an emulsifying agent.

All materials obtained from commercial sources and Department's Lab : Eudragit E100, Pharmaceutics Lab, SOP, RK University, Rajkot, India), PVA (Chemdyes Corporation, Rajkot i) Acetone & Methanol (Pharmacological Lab, SOP, RK University, Rajkot, India).

2.14.2. Method :

An organic phase contains of polymer (Eudragit E100).The extract of BV(2.5 mg) took & mixed with 15ml acetone & methanol 3:1.

After that the solution was sonicated for 15mins.

In a beaker the 1.5 % (w/v) PVA in water took & the sonicated extract solution was injected in tufflon tube into the beaker containing PVA in water.

The extract solution was be added to PVA-Water by continuous stirring with 1000 RPM

Resultant Emulsion was diluted in 0.2%w/v)50ml PVA .

Continuously Stirring at 500 RPM for 6hrs

2.14.3. Evaluation

Particle morphology was observed using scanning electron microscopic method (SEM) & UV [47,52,53]

2.14.3.a. Statistical Analysis

PRISM version 5 software was used to do the statistical analysis. Dunnett's test was performed after one-way ANOVA was used to analyze the data for all parameters. The findings were given as mean ± SEM.

3. Result:

3.1. Percentage Yield of Berberine:

The root powder of ***Berberis vulgaris*** was extracted with methanol using a Soxhlet extraction method and the percentage yield was found to be 6% w/w.

& Was also extracted with water using Ultrasonication method & the percentage yield was found to be 9%w/w .

3.2. Preliminary Phytoconstituents Test

Confirmatory Tests for Phytoconstituents, mentioned in table-5

3.3 TLC Analysis:

$R_{f\,=}$ Distance travelled by the compound / Distance travelled by the Solvent

Here,

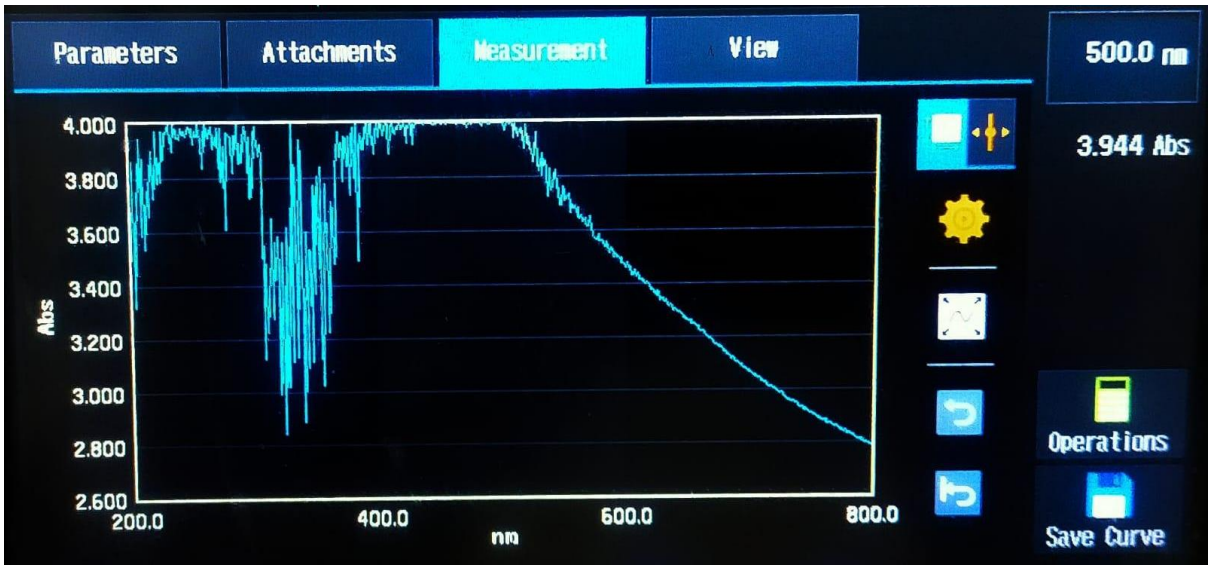
Distance travelled by the compound= 6 cm

Distance travelled by the solvent front= 8.5cm

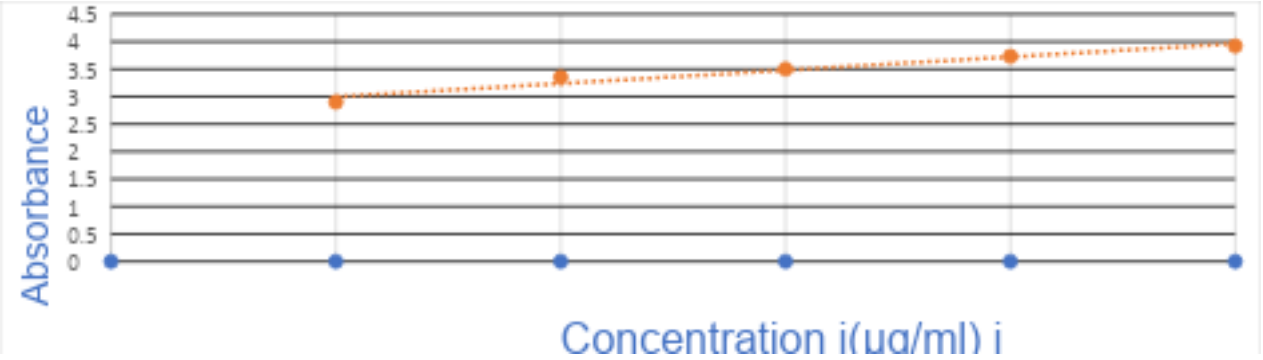
So, $R_{f\,=}$ 6cm / 8.5 cm= 0.70 cm

Silica gel TLC plate was used & according to the standard R_f value (0.6-0.7) for silica

3.4. UV Analysis:



Graph 1: Absorbance of Berberis vulgaris extract in UV



Graph 2:Abs vs conc graph of extract

3.5.1Result of Linearity:

Absorbance of Plant extract in different concentration,mentioned in table-6

3.5.2. Limit of Detection

The restrain of location (LOD or LoD) is the least flag, or the most reduced comparing amount to be decided (or extricated) from the flag, that can be watched with an adequate degree of certainty or factual centrality

LOD =3.3 (SD / S)

Where,

SD= the standard deviation of the response

S= the slope of the calibration curve

3.5.3. Calculation of SD (Standard of Deviation):

1. Absorbance Data points:

2.9 + 3.35 + 3.5 + 3.73 + 3.92 = 2.78

2.Squared Differences:

$(2.9– 2.78)^2 + (3.35- 2.78)^{2i}+(3.5 -2.78)^2 + i(3.73- 2.78)^2 + (3.98- 2.78)^2$

= 0.0144 + 0.3249 + 0.5184 + 0.9025 + 1.44

= 3.2002

3.5.4. Divide by No of Data Points :

3.2002/ 5 = 0.64004

3.5.5. SOD,

0.64004 = 0.800025

LOD = 3.3 (SD / S) = 3.3 (0.800025 / 0.242) = 10.909

3.5.6. Limit of Quantitation

The constraint of Measurement (LOQ) is the least analyte concentration that can be quantitatively identified With expressed Precision and accuracy. LOQ = 10 (SD / S) Where, SD = the standard deviation of the reaction

Where, SD the standard deviation of the response
S = The slope of the calibration curve
SO, LOQ= 10 (SD/S)
= 10 (0.800025/0.242)= 33.0589

3.5.7. Result of Concentration:

According to Beer-Lambert's Law :
A=abc
Here, A, absorbance= 3.94
b , path length = 1cm
a, molar extraction coefficient = 4209 L/mol /cm
c = A/ab = 3.94/ 4209 = 0.00094 µg/ml= 9.36 mg/ml

3.5.8.Result of Assay:

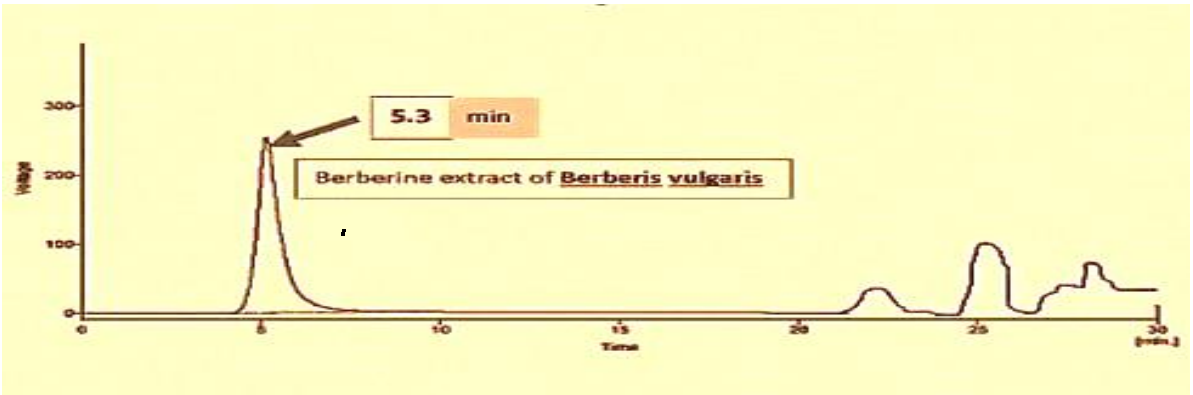
Amount of the found Plant extract,mentioned in table-7

3.5.9.Result of Validation parameters :

Results Of UV analysis ,mentioned in table -8

3.10. HPLC Analysis

HPLC analysis was performed to quantify the phytoconstituents. For this, a water extract was obtained using the Ultrasonication extraction method. Berberine was quantified in the water extract using HPLC, employing a 250×4.6 mm i.e. Symmetry-C18 5 µm column with a mobile phase consisting of Water:Methanol:Acetonitrile (60:30:38, v/v/v). The flow rate was set at 1.0 ml/min at room temperature, with an injection volume of 10 µL. Detection was done at 262 nm, and the retention time (tR) for Berberine was 5.3 minutes, using a Shimadzu LC-2010 RP-HPLC system for qualification.



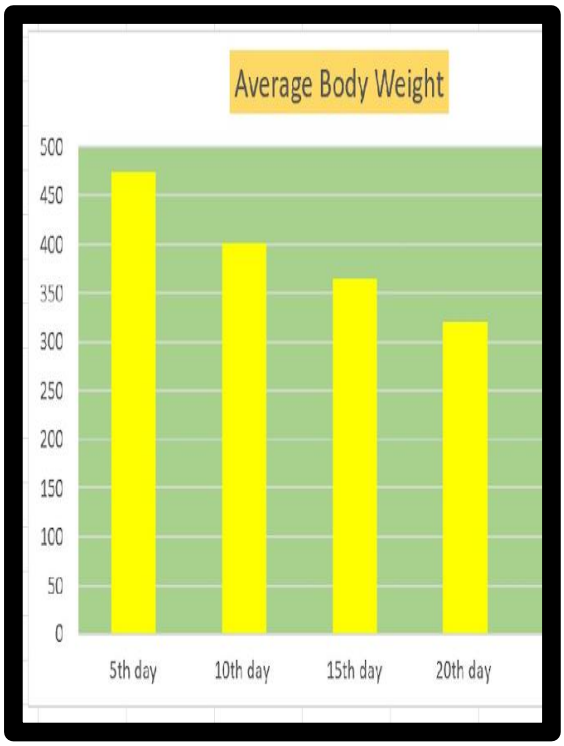
Graph3:HPLC Graph of Plant ***extract***

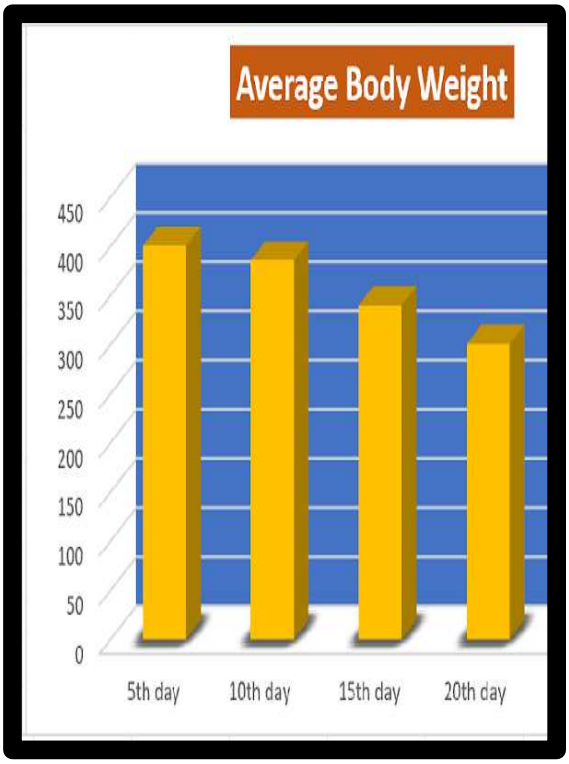
3.11. Formation of Lung Cancer:

We observed the lung cancer in rat in 1st rat (fig-15)on 7th day after inducing of lung cancer cell line suspension, in 2nd rat (fig-15)on 13th day, in 3rd rat(fig-15,16) & 4th rat (fig-16,17)on 18th & 20th day respectively ,in 5t rat (fig-17,18)& 6th rat(fig-18) on 22th & 24th day respectively.

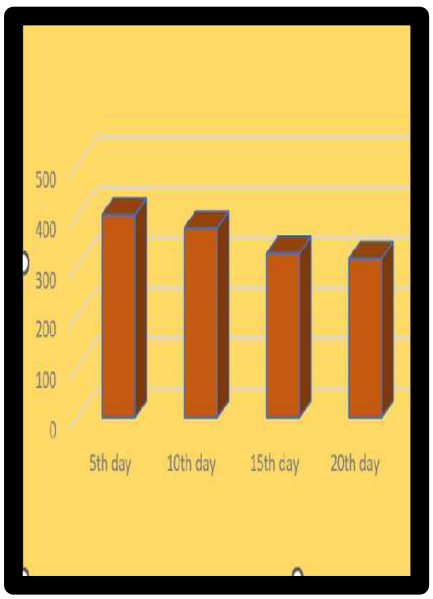
3.12.Symptom of Lung cancer:

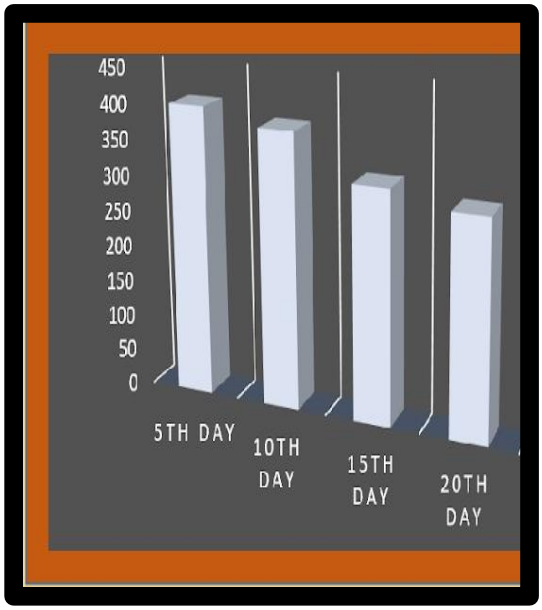
3.12.1 BODY WEIGHT:





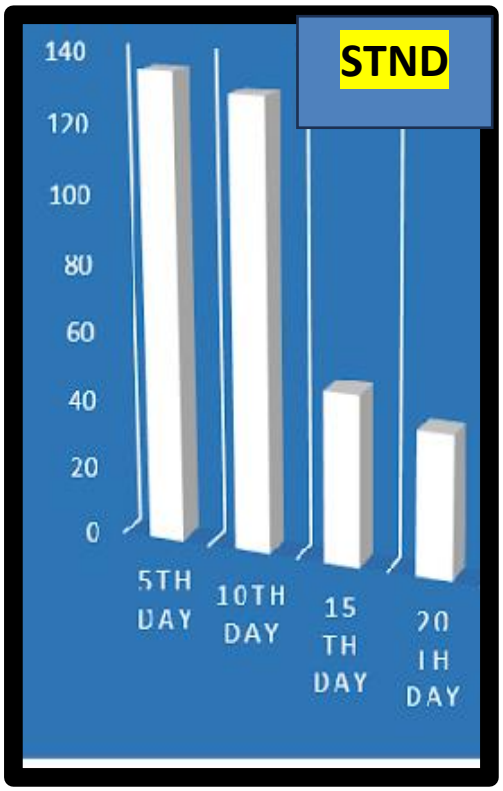
Graph 4::Cumulative decrease of body weight in Std group *Graph 5::Cumulative decrease of body weight in T1 group*

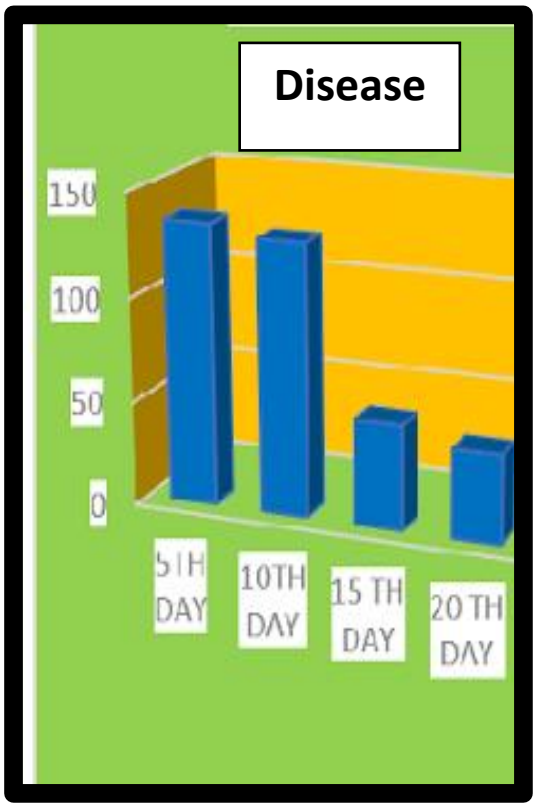




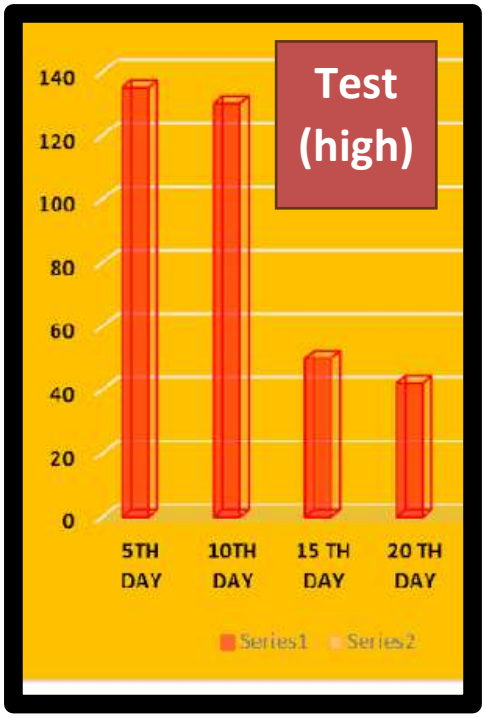
Graph 6 :Cumulative decrease of body weight in T2 group Graph :Cumulative decrease of body weight in DC group

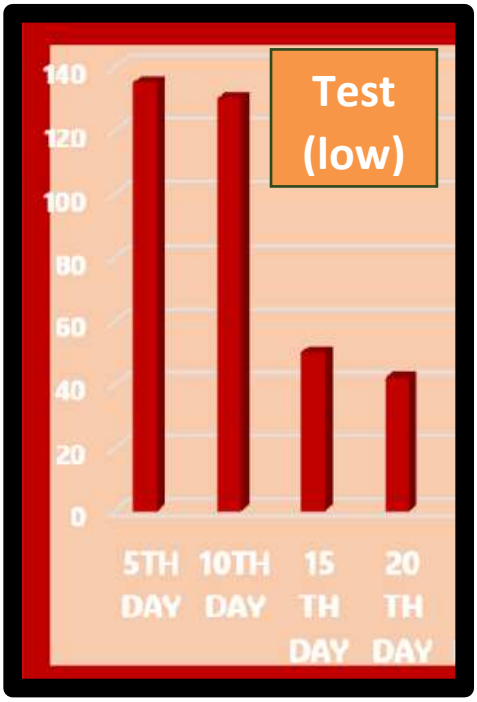
3.12.2.FOOD INTAKE





Graph 8:Cumulative decrease of food intake in stnd group Graph9:Cumulative decrease of food intake in DC group





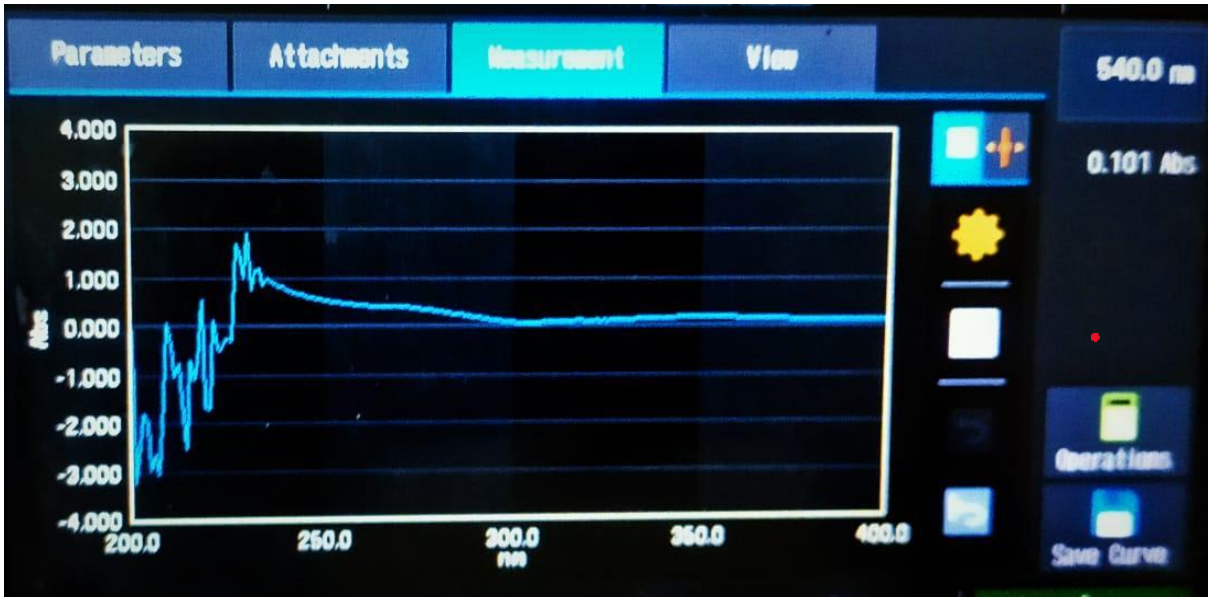
Graph 10:Cumulative decrease of food intake in low dose group Graph 11::Cumulative decrease of food intake high dose group

3.12.3.SKIN PROBLEM

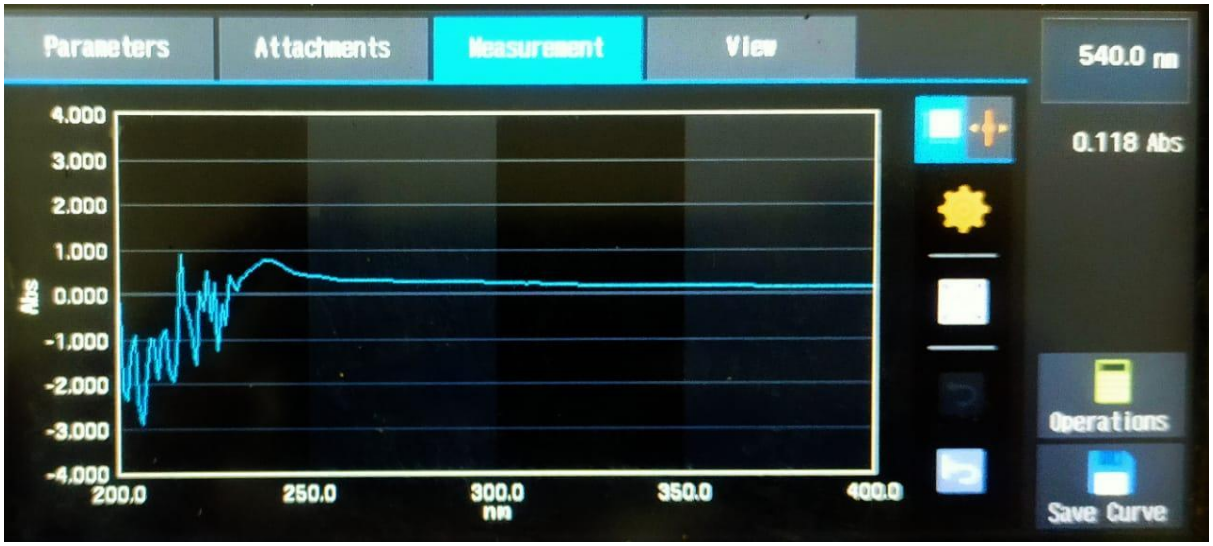
After 12 hrs Inflammation of RATS PENIS & Scrotum

Skin Redness & Itchy skin have observed after 3-4 days, are one of the important symptoms of lung cancer(fig-19,20)

3.13 Calculation of Haemoglobin concentration:



Graph 12 : Absorbance of sol. Of Test (high dose) group in uv for hemoglobin assay

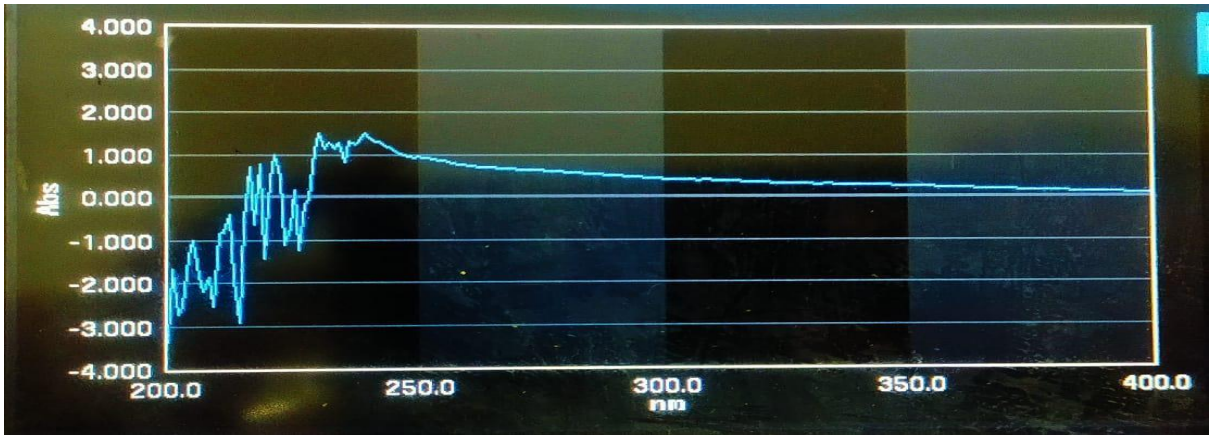


Graph 13 : Absorbance of sol. Of Test (low dose) group in uv for hemoglobin assay

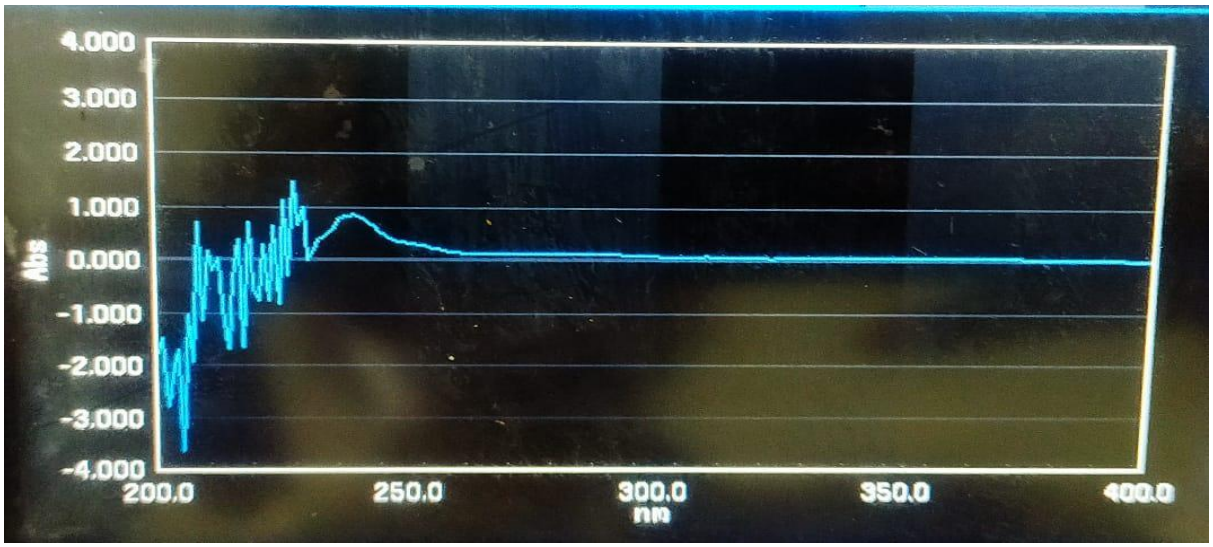
Graph 14 : Absorbance of sol. Of standard group in uv for hemoglobin assay

Graph 15 : Absorbance of sol. Of)stnd + test group in uv for hemoglobin assay

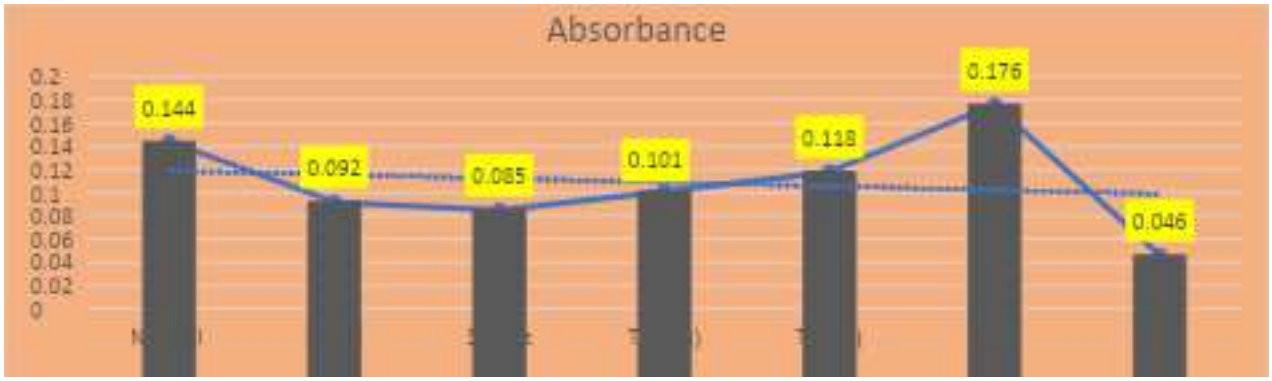
Graph 16 : Absorbance of sol. Of Preventive treament group in uv for hemoglobin assay



Graph 17 : Absorbance of sol. Of normal group in uv for hemoglobin assay



Graph 18 : Absorbance of sol. Of Disease control group in uv for hemoglobin assay



Graph 19: Graph of UV absorbance of different groups in haemoglobin assay

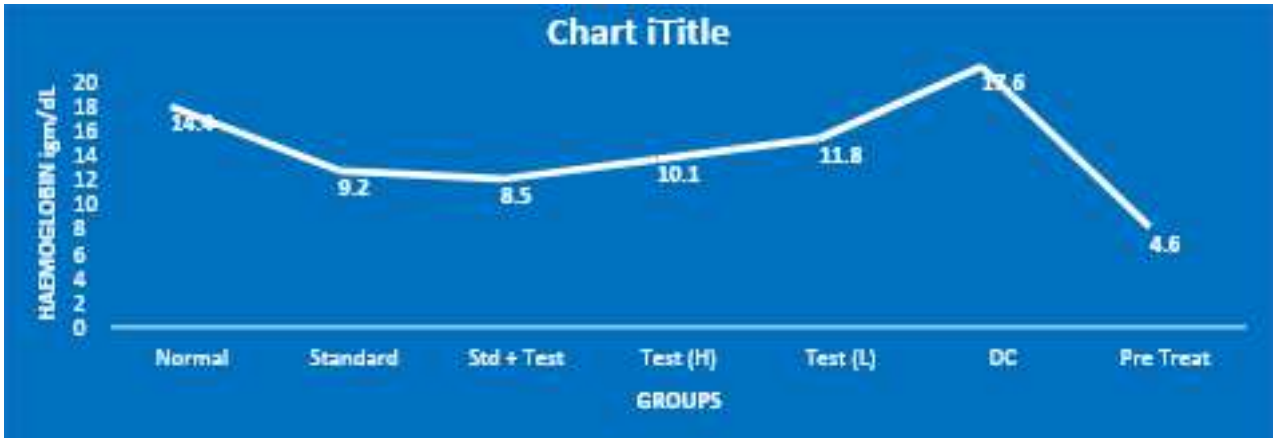
3.13.1.Calculation of Haemoglobin Concentration :

OD Of test/ OD of Stand x Conc. Of Standard

According to this equation ,

The conc. Of Haemoglobin in all group is :

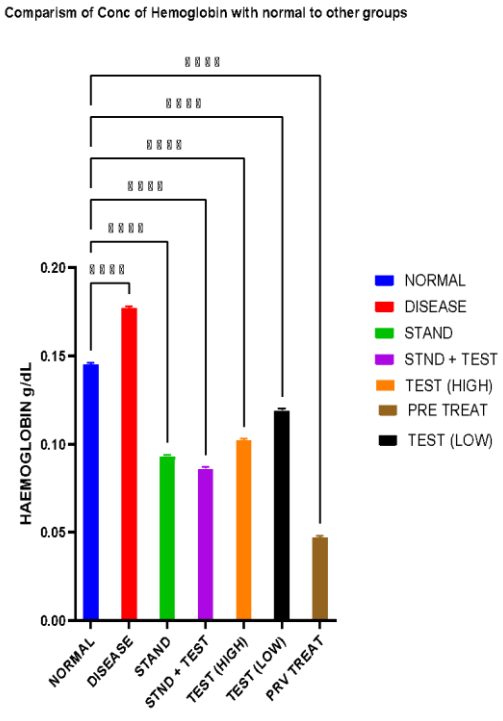
Concentration of Haemoglobin of different groups,mentioned in table-10



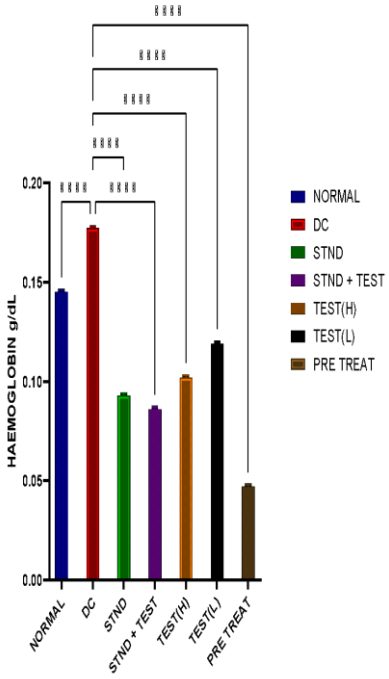
Graph 20: Graph OF haemoglobin conc. Of different groups

3.13.2.Haemoglobin Estimation i:

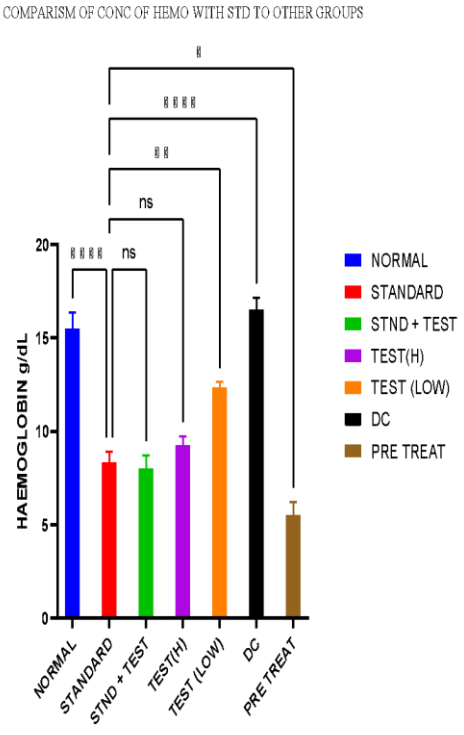
Statical chart for Haemoglobin assay, mentioned in table-11



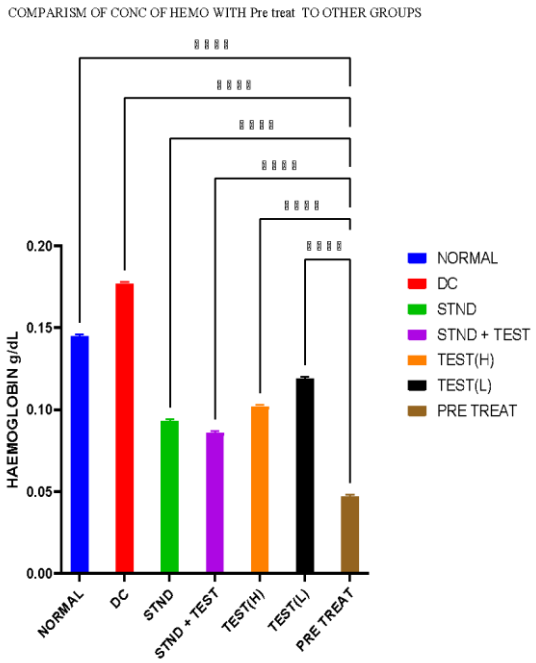
Graph21: Comparison of normal to other groups in Hemo assay



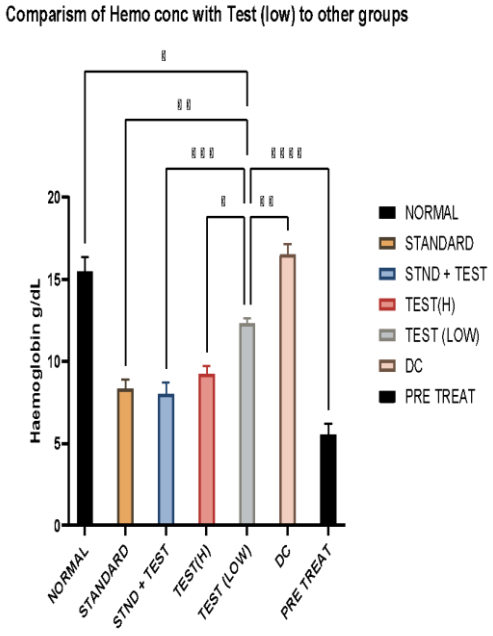
Graph 22:Comparison of DC to other groups in Hemo assay

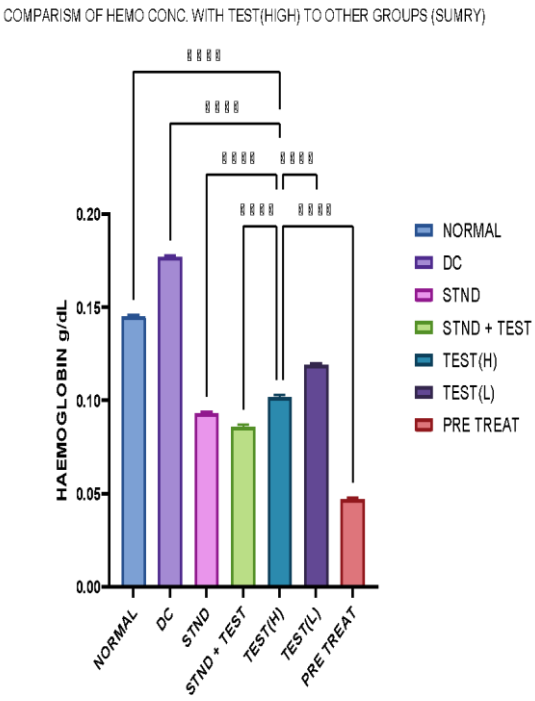


Graph 23: Comparison of Stnd to other groups in Hemo assay

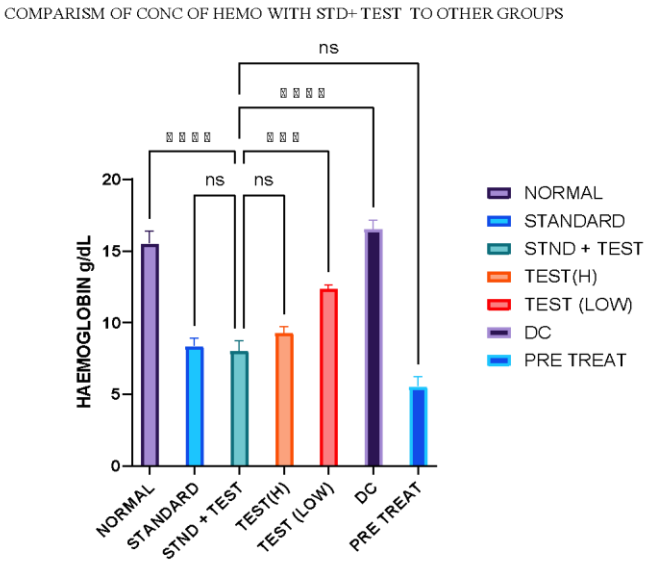


Graph 24:Comparison of Pre treat to other groups in Hemo assay





Graph 25:Comparison of t(low) to other groups in hemo assay Graph 26: Comparison of t(high) to other groups in haemo Assay



Graph 27: Comparison of standard + test group to other groups In haemo assay

3.13.3.Statistical comparison:

Each group (n=3), each value represents Mean ± SEM. One-way Anova followed by Dunnett's test was performed. ns denotes non-significant, *denotes P<0.05 **denotes P<0.01, **denotes P<0.001 and **** denotes P<0.0001

Here,

- when the normal group is compared with other groups(graph-35), in this case graph shows, significant differences **** denotes (P<0.0001)
- The comparison of the Disease Group with other groups also shows significant differences(graph-33), **** denotes (P<0.0001)
- When the Standard group is compared with other groups("graph-29), the graph shows, significant differences, **** denotes (P<0.0001) with the normal group & also with the disease group, nonsignificant difference with the standard + test combined treated group & also with test (high dose) group, a significant difference **denotes P<0.01 with test (low) ,significant difference with preventive treatment group significant difference, *denotes P<0.05
- When the Preventive treatment group is compared with other groups(graph-30), the graph shows, significant differences , **** denotes P<0.0001,with all other groups
- When the test(low dose) group is compared with other groups(graph-31), the graph shows, a significant difference, *denotes P<0.05 between the normal & test (high dose) groups; significant difference,**denotes P<0.01 with disease control & standard group, Significant difference***denotes P<0.001 with stand+ test group & **** denotes (P<0.0001) with the preventive group.
- The graph was obtained by comparing the test (high dose group) with other groups"(graph-34). This graph shows, a significant difference **** denotes (P<0.0001) with all other groups
- The comparison graph of Standard + test group(graph-32) mentions non significant difference with standard ,test(high dose) group & preventive treatment group. Significant difference (p<0.001)with test (low dose) group. Along with shows significant difference **** denotes (P<0.0001) with disease group.

3.14 Histopathological Analysis

In fig A, B & C (fig-21,22,23)**:**These A,B,C figures are normal group show healthy lung regions without any signs of Abnormal cell size, shape & no. All of the main parts are mentioned here, which appear normal with no abnormality.

In fig D,E & F(fig-24,25,26) show the disease group lung histopathological views .In fig D ,Thickened Alveolar septa, Inflammatory cell filtrate & Hyperplasia are observed. Fig E –mentions the real feature of Large cell carcinoma, here the no of the cell increased & the larger abnormal cells are also observed. In fig F- Fibrotic area & Cancerous area have observed also. But after treatment with standard drug the cell no & size are normal ,which is the main characteristics of LCC this has already treated.

In fig G(fig-27) : In figure G, Normal alveolar has observed with irregular large alveolar also .But after treatment with standard drug the cell no & size are normal ,which S the main characteristics of LCC ,this has already treated.

In fig H(fig-28)- The alveolar size & shape are normal & the cell no & size are also in normal condition ,though the cell no has decreased more in standard group than test (high dose) group.

In fig I(fig-29)- All alveolar have observed with normal shape & size & all of the cells size & shape are also in exactly normal condition after treatment

In fig J(fig-30)- he abnormal size & shape of Alveoli were observed but the cell no & shape were in normal condition, which is the main feature of LCC was treated by low dose of test drug in this group also.

In image K(fig-31)- The alveolar shape & size are in normal condition along with normal size & shape of cell though some necrosis condition has observed.

3.15. RT-PCR TEST FOR THE DETERMINATION of TNF−∞ expression:

Consolidated Report

Experiment Name: FAST SYBER_20240618_171157
Experiment Type: Absolute
User Name: admin
File Name: C:\Himedia Laboratories\Insta Q96\experiment\18 06 2024 CHINMYEE RESEARCH.fqd
Run Time: 2024/06/18 17:19:53 - 2024/06/18 18:15:09
Gain: F1:10

Run Program

Initial Denaturation

Target	Incubation Time	Rate	Sampling
95	120	4	☐

PCR Stage Cycles:40

Target	Incubation Time	Rate	2nd Temp.	Step Size	Step Delay	Grad Temp	Grad Range	Sampling
95	10	4						☐
60	30	4						☑

Detectors

Detector	Reporter	Color	Master Mix	Primer	Probe	Supplies	Batch Number
SYBR GREEN	FAM						

Plot Plate

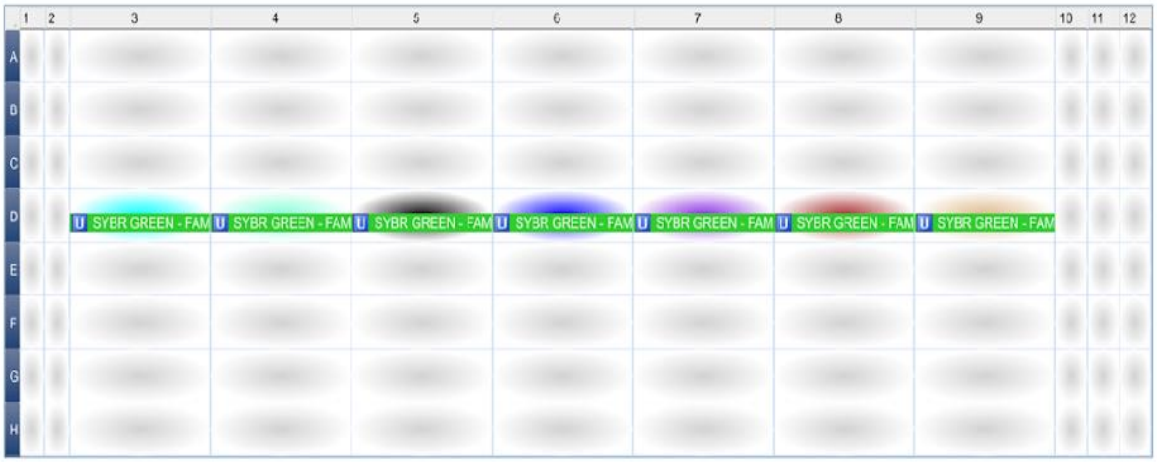


Table Plate

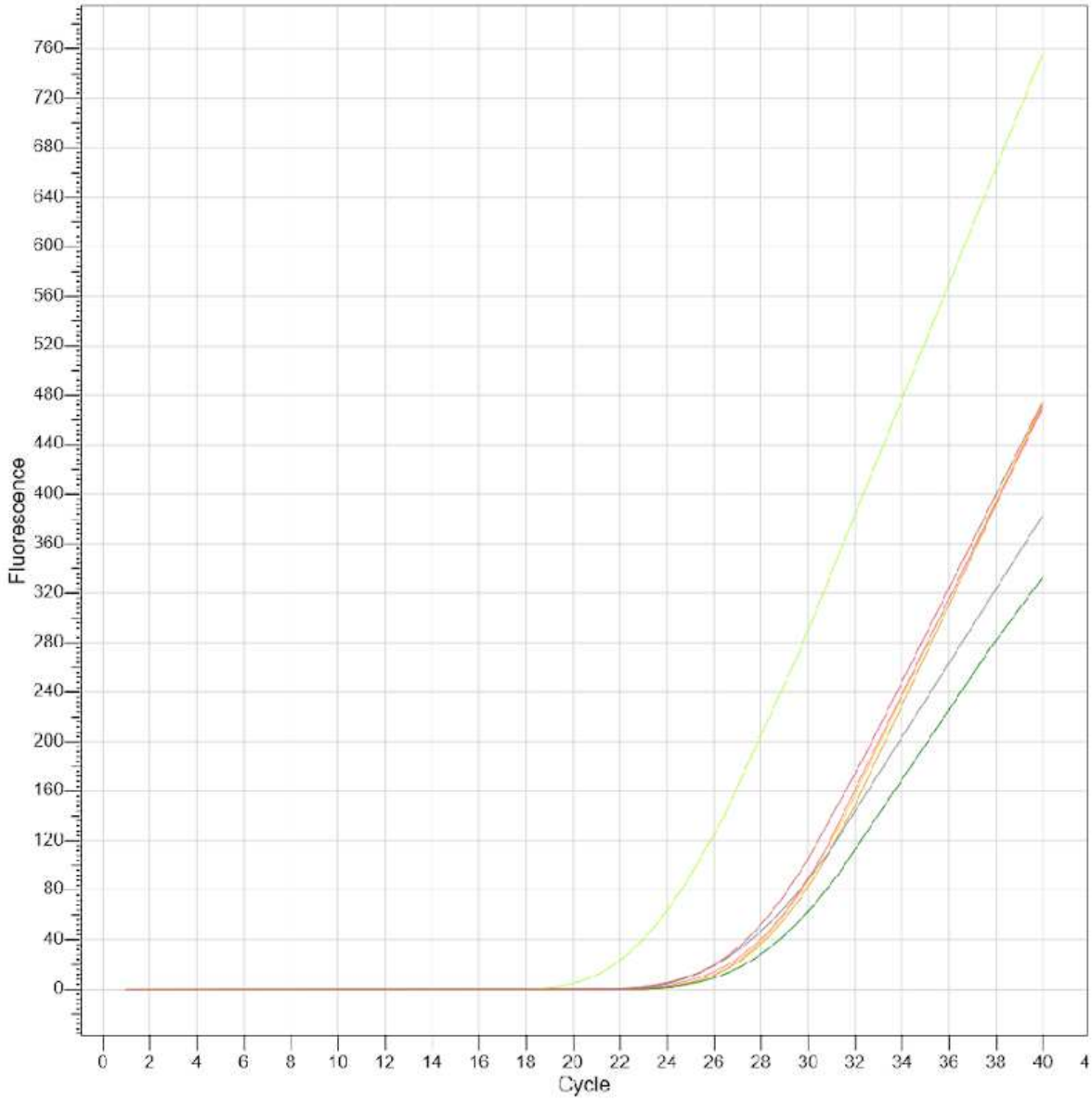
#	Well	Assay Item	Property	Dye	Std. Con.	Sample ID
39	D03	SYBR GREEN	Unknown	FAM		1
40	D04	SYBR GREEN	Unknown	FAM		2
41	D05	SYBR GREEN	Unknown	FAM		3
42	D06	SYBR GREEN	Unknown	FAM		4
43	D07	SYBR GREEN	Unknown	FAM		5
44	D08	SYBR GREEN	Unknown	FAM		6
45	D09	SYBR GREEN	Unknown	FAM		7

Consolidated Report

Samples

Sample Id	Color	Sample Name	Sampling Time	Submitting Date
1		STANDARD CONTROL	2024/06/18	2024/06/18
2		PREVENTIVE GROUP	2024/06/18	2024/06/18
3		LOW DOSE	2024/06/18	2024/06/18
4		STANDARD TEST	2024/06/18	2024/06/18
5		DISEASE CONTROL	2024/06/18	2024/06/18
6		HIGH DOSE	2024/06/18	2024/06/18
7		NORMAL	2024/06/18	2024/06/18

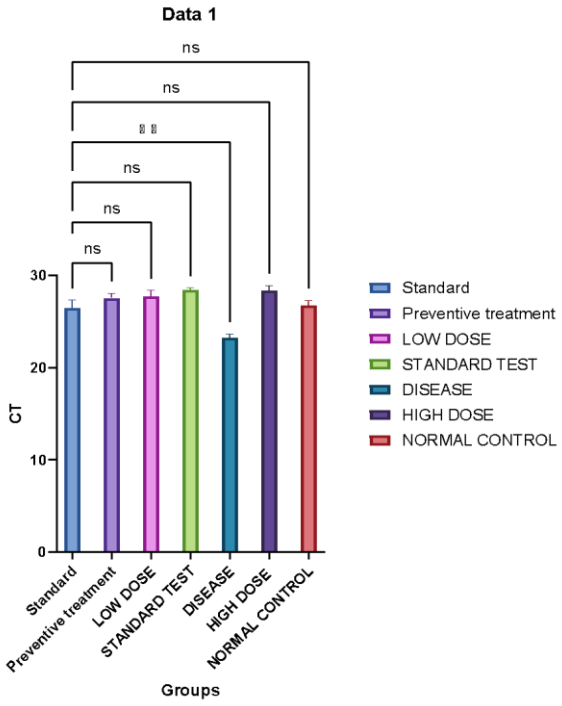
Amplification Curve



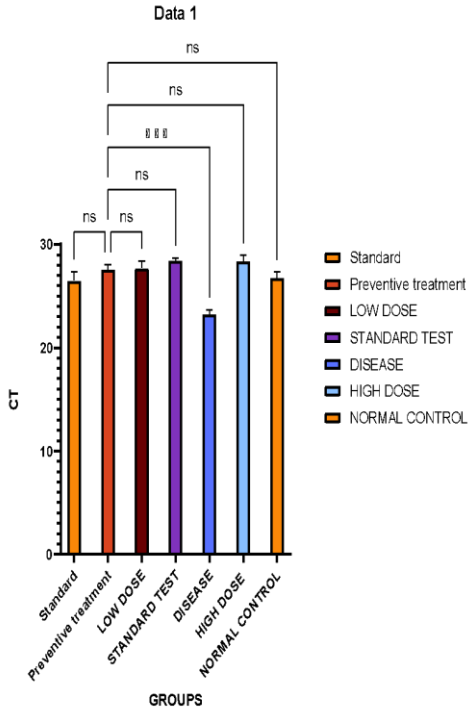
Graph 28 : Fluorescence vs cycle time graph in RT-PCR

3.15.1 CT value Estimation:

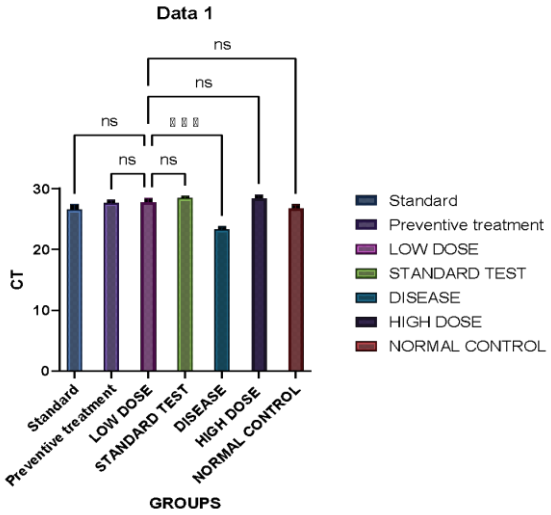
CT value estimation, mentioned in table-13



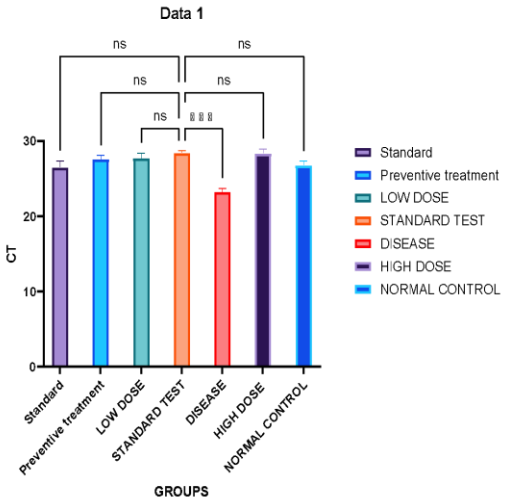
Graph 29: Comparison CT value with std to other groups to other groups



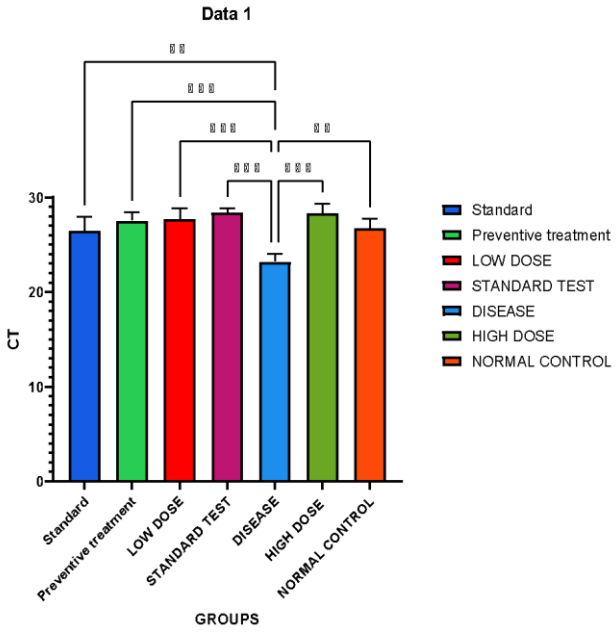
Graph 30: Comparison CT value with peventive

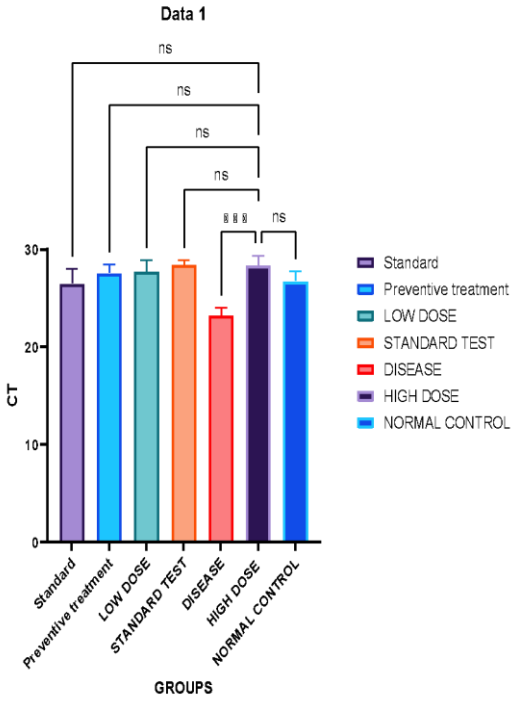


Graph 31: Comparison CT value with t(low) to other groups to other groups

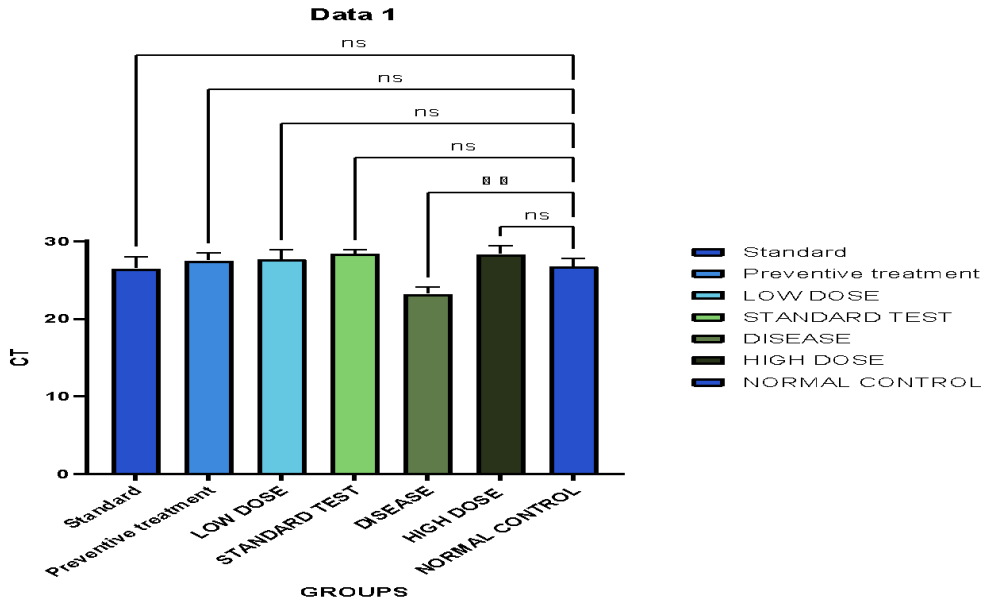


Graph 32: Comparison CT value with stnd+test





Graph 33: Comparison CT value with disease to other groups Graph 34: Comparison CT value with t(high) to other groups



Graph 35: Comparison CT value with normal to other groups

3.15.3.Statistical comparison:

In each group (n=3), each value represents Mean ± SEM. One-way Anova followed by Dunnett's test was performed. ns denotes non-significant, *denotes P<0.05 ,**denotes P<0.01, ***denotes P<0.001 and **** denotes P<0.0001

Here,

In first graph the standard group (graph-29) is compared with other groups, with all groups the graph shows non-significant differences, only with disease group it shows a significant difference **denotes P<0.01.

In the Second graph the preventive treatment (graph-30)group is compared with another group, where the graph shows non-significant difference with all groups, except the disease group it shows a difference

***denotes P<0.001.

The third graph the test Low dose(graph-31) group is compared with all other groups, in this group the low dose group shows the non-significant difference, and only with the disease group it shows a significant difference ,***denotes P<0.001.

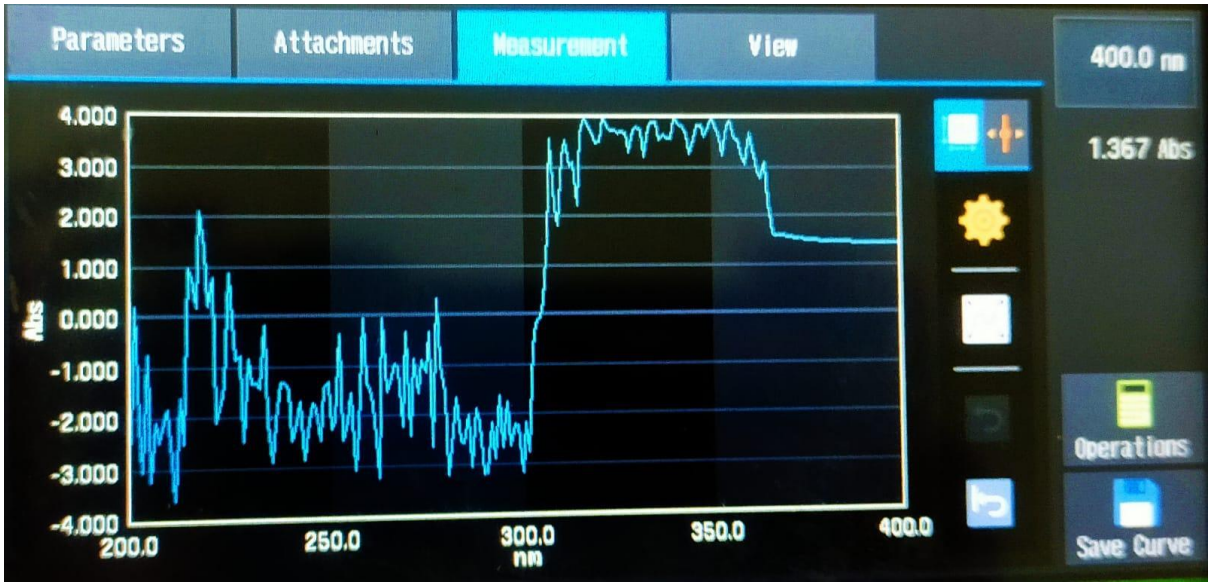
In the Fourth graph, the standard + test group(graph-32) is compared with other groups, where it shows a non-significant difference with all other groups, except the disease group, where the significant difference has been observed which ***denotes P<0.001

In the fifth graph, the disease group(graph-33) has compared with other groups, where we got significant differences with all groups. The significant difference with the normal group, **denotes P<0.01, with the Preventive treatment group significant difference has been observed, ***denotes P<0.001. With the standard + test group the difference is also significant, ***denotes P<0.001. When the disease group is compared with high dose group, a significant difference is also observed here, ***denotes P<0.001. The disease group shows significant difference from the normal group also, it is **,denotes P<0.01.

The sixth graph shows the compares between the High dose group (graph-34)with all other groups, where we got a non-significant difference for all groups, only with disease group showed a significant difference, ***denotes P<0.001.

In the seventh graph, we compared the normal group(graph-35) with all other groups, where we observed a non-significant difference with all groups, except with the disease group. A significant difference was observed, it is ***denotes P<0.001.

Graph36:Absorbance graph of Berberis vulgaris nano suspension



Graph 37:Absorbance graph of *Berberis vulgaris* extract 2mg/ml

4. Discussion:

4.1Phytochemical Analysis:

The phytochemical analysis of the extract of *Berberis vulgaris* revealed a rich presence of bioactive compounds such as berberine, triterpenoid, flavonoids, phenol, alkaloids & sesquiterpenes (table-5) these compounds are well-(table-6)documented for their anticancer, anti inflammatory, anti-mitosis, cell cycle arrest & antioxidant properties, which play a crucial role in treating lung cancer.

7.1Phytochemical Analysis:

The phytochemical analysis of the extract of *Berberis vulgaris* revealed a rich presence of bioactive compounds such as berberine, triterpenoid, flavonoids, phenol, alkaloids & sesquiterpenes - these compounds are well-documented for their anticancer, anti inflammatory, anti-mitosis, cell cycle arrest & antioxidant properties, which play a crucial role in treating lung cancer.

7.2Amount of Berberine from this plant extract:

For extraction, we used the Soxhlation process & Ultra Sonication extraction process. For Soxhlation we used methanol & for Ultra Sonication we used water as solvent. The percentage of yield of Berberine is, 6% w/w by the Soxhlation method & 9% w/w by the Ultra Sonication method. We detected the amount of plant extract,9.36mg/ml(table-7) by ultrasonication method .

4.3Analysis method for plant Phytoconstituents:

For TLC, we got a good & standard result, because the Rf value for berberine is 0.7, which is in the range of standard Rf value, Along with the yellow spot was also identified on the TLC paper, which is the mark of the presence of the high amount of berberine.

For UV spectroscopy analysis, we got standard value for the limit of detection, Limit of quantification(table-8), Absorption maxima(graph-1) & also the high amount of concentration(graph-2) was also observed in this analysis.

The amount of berberine was detected 9.36 mg/ml , which is almost the maximum amount according to the standard range.

For HPLC, we got a standard retention time for berberine of 5.3 minutes(graph-3), which is also within the range of the standard value, and this retention time can be observed when the highest amount of berberine is present in the sample.

So, we can easily decide that for all analysis processes, we got the maximum amount of presence & yield of berberine, with the standard result.

4.4. The cell line inducing by the Intranasal route:

We induced the cell line by the intranasal route, for that we used cell suspension, which contained just 1.14x10^6 no cells. After inducing cell suspension, we observed the injured lung & also the formation of a moderate tumor after 7 days & on the 13th day the proper formation of the tumor was observed.

This was a remarkable success of this project, because the formation of lung cancer is rarely observed within this short period. And the mortality rate was also less than expected rate, we observed the mortality for

1st rat on 7th day after the cell suspension induction, 2nd rat on 13th day, 3rd on the 18th day . After that we started our treatment so before starting treatment, we observed the mortality in only 3 rats. After starting the treatment we observed mortality for another 3rd & 4th rats on the 20th day, 5th on 22nd day, and 6th on the 24th day. On the 13th day, the proper formation of the tumor was observed. According to this result the mortality rate was less & the tumor was also starting to form within a short period. Undoubtedly, it is a unique & the easiest way to induce lung cancer in the rat without any painful process.

4.5. Lung cancer Symptom observation:

For observing the lung cancer symptom, we observed first the decrease of the body weight(graph-4,5,6,7) 5 days after inducing the cell line suspension to the rat & it continued, after 4-5 days of starting the treatment.

The food intake was also decreased(graph-8,9,10,11), after inducing the cell suspension, which was occurring as the major symptom of lung cancer. After 12hrs of cell line suspension induction the inflammation in the rat's penis & scrotum area we observed, it is a rear symptom, but in our research work, we observed, this within 12hrs & for male rats this is a symptom of lung cancer. After 3-4 days of cell line suspension induction we observed skin redness & itchy skin in rats, it is a common symptom of lung cancer.

4.6. Haemoglobin assay:

We took absorbance by UV-visible spectroscopy, after dissolving the sample with Drabkin reagent & we got the standard absorbance for all samplestable-9), which we determined from graph for test high dose(graph-12),test low dose(graph-13),standard group(graph-14),standard +test group(graph-15),preventive group(graph-16),normal group (graph-17),disease group(graph-18). Then we calculated the haemoglobin concentration for different groups (table-10)from the graph of uv absorbance of different groups(graph-19)& haemoglobin concentration of different group (graph-20) to determine the haemoglobin level in normal, disease, and treated rats(table-11).

According to the concentration of haemoglobin also performed statical analysis to compare the obtained result & to determine the activity of the drug.

Our target was the inhibition of angiogenesis, so we needed to decrease the level of haemoglobin in rats. According to this target, we observed that when the normal group is compared with other groups(graph-21), we

got significant differences **** denotes (P<0.0001). For the disease group(graph-22) with other groups also showed significant differences, **** denotes (P<0.0001).

The standard drug-treated group(graph-23) showed a non-significant difference with the standard+ test group & high dose group. The significant difference between the low dose & preventive treated group.

The preventive group showed (group-24)the significant difference with all groups.

The test (high dose) group(graph-26) showed a significant difference to the preventive group & the highly significant difference (****) with all other groups, even with the Standard drug & Standard + test drug also.

The Standard + test treated group(graph-27) , we observed, a non-significant difference among the standard, high & preventive group .The significant difference with the low dose group.

The test (low dose)(graph-25), we observed significant difference with the standard & the standard + test group & highly significant with the preventive group.

According to this result, we can decide that super significant activity of the test drug was observed in the Preventive treated group. Then we also observed a significant result for the test (high dose) group, which was remarkable activity in comparison with the standard drug.

4.7 Histopathological Analysis:

In this analysis, we observed all normal parts of the lung in the normal group. In the disease group, we observed the symptoms, which were exactly related to large cell carcinoma, such as Thickened alveolar septum, Hyperplasia, Inflammatory cell filtrate, fibrotic area, cancerous area & the main feature of LCC which is an increased no of cell & larger size of the cell was also observed.

The Anti-lung cancer activity of the drug was observed properly in the Standard+ test group, because in this group we observed all cells with perfect size, and the cell numbers were also as healthy as normal lung, along with perfect and normal size of all alveoli.

In the standard group, we observed that the cell no & size were perfect & most of the alveoli were normal in size & shape, though some irregular size & shape of the alveoli were also observed. In the Test(high dose) group, Normal size & shape of alveoli and the normal no & size of cells were observed & as in the Standard group, no irregular size of alveoli was observed here. However, the number of the cell was more decreased in the Standard group. In the Test (low dose) group alveoli were observed with irregular size & shape, but the cell in no & size were normal, so we can decide here that in this group also the main criteria of LCC treated successfully. In the Preventive group, the Alveolar size & shape were normal & the cells were also observed with normal size & shape. Only some necrosis area was observed in this group.

So, according to this observation, we can decide that the anti-lung cancer activity of our test drug in this analysis was exactly effective and & it treated the abnormal cell number and shape successfully, even at a low dose. Though some irregular alveoli were observed, most probably it happened for a short period of treatment because, after getting lung cancer, the period of survival of the rat was too short.

4 .8 RT–PCR Analysis for the Determination of the TNF–∞ expression:

In our research work, our target was to decrease the TNF–∞ expression, and the TNF–∞ expression was determined by the RT–PCR. In the RT–PCR graph, the CT

value is mentioned, and the CT value is related to mRNA expression, and mRNA expression is related to TNF−∞ expression.

The CT value on the RT-PCR graph (graph-28) represents the point at which an amplification curve intersects a threshold line. It is a relative measure of the concentration of the target mRNA in the PCR process. The Ct values are presented on the amplification plot's X axis.

The Threshold cycle value is inversely related to the initial quantity of the target gene. If the starting mRNA quantity is high, the amplification signal emerges early (lower Ct value). conversely, when mRNA is scarce, more cycle are indeed for detection (higher Ct value).So, an increasing Ct value corresponds to decreasing mRNA levels in the sample. According to the result of the RT-PCR curve(table0, we observed the highest CT value for the standard + test group and then for the preventive treated group, the standard group, the test (high dose) group, the test (low dose) group, the normal group, and the disease group, respectively.

So, The mRNA expression was the highest in the disease group & then in the Normal, Test(low), Test(high), Standard, Preventive treated & Standard + test group respectively. According to this observation, we can decide that the TNF -∞ expression was lower in the Standard + test group & the preventive treated group. According to the statistical analysis, the standard group & preventive group did not show a significant difference with the test(high dose) , test (low dose) & standard + test group. Because the CT value differences were minor & we didn't observe any significant difference among these groups. Here, the effective activity of our test drug has been observed which was very similar to the activity of the standard drug.

4.9.Evaluation of Nano Suspension:

4.9.1Morphological Characterization of Nanoparticle

Generally, nano suspensions have particle sizes in the range of **10 nano meter to 1000 nano meter**. According to this range, our nano particles size is perfect which is 500nm & the stability & sedimentation rate are also accurate. We have also observed the absorption by UV spectroscopy .The absorption value was also in standard range(fig-32) .

4.9.2.The UV-visible spectroscopy Graph for nano suspension:

The UV-vis spectra of pure berberine & berberis nanoparticle solution in distilled water at the same concentration 2.0mg/ml are mentioned here. According the peaks & absorption, it's clearly visible that, the graph of berberine nano particle suspension showed higher peaks(graph-36) than pure berberine absorption(graph-37), 2.057 for berberine nano suspension & 1.367 for pure berberine.

This could happen because the saturation concentration of pure berberine in 2 mg/mL is less than that of the berberine nanosuspension because in water the nanosuspension showed complete solubility.

The, standard range of wavelength in UV-visible spectroscopy of berberine chloride nanosuspension is normally between 300-400nm, our nano suspension showed the peak also between this range. [53,49]

5. Conclusion:

For Lung cancer ,Novel drugs are essential to overcome resistance mechanisms and enhance treatment outcomes. At this point our research work is a remarkable project work, where we focused on specially Large Cell Carcinoma, in this type the aggressive malignancy, poor prognosis, and lowest survival rate . So we tried to explore the anti-lung cancer activity of *BV* by the analysis of a detailed well planned in -vivo process by inducing lung cancer in male .Wister rats by NCI-H460 cell line. We obtained successful positive results undoubtedly in

in-vivo haemoglobin assay and histopathological assay along with a developed & significant Real-Time PCR assay. We tried to diminish the angiogenic activity to improve the tumor microenvironment. According to this, our test drug showed effective anti-lung cancer activity in all tests,& we also observed almost similar activity of our test drug (high dose) like Standard drug in some analyses. We formulated the nano suspension of *BV* ,the suspension is with exact particle size & by UV-visible absorption we determined the proper concentration of Berberine in this suspension, which also provided us the standard value. Our nanosuspension formulation contains the herbal phytoconstituent, which is almost side effects free & nano suspension, improves the antigen activity with lower side effects & also decreases the morbidity of advanced cancer therapies, which need lower dosages to treat cancerous conditions. For patients with LCC, nowadays the most common process for the treatment is mainly surgery. So our successful research work is like a new era for a huge no LCC patients as well as patients with cigarette smoking-inducing cancer. Our test drug has treated the NCI-H460 cell line-induced lung cancer, which is strongly related to smoking-induced cancer. At this present moment, the most conventional drugs are available with huge side effects, our drug will be the most suitable option for the treatment of large cell carcinoma & cancer related to cigarettes which is one of the major causes of lung cancer.

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Abbreviation

LC- Lung Cancer

RT PCR- Real-Time Polymerase Chain Reaction

LLC-Large Cell Carcinoma

ANOVA-Analysis of Variance

BSL-Brine Shrimp Lethality

DNA-Deoxyribo Nucleic Acid

MTX-Methotrexate

TAE BUFFER- Tris Acetate Ethylenediaminetetraacetic acid

TNF- Tumor Necrosis Factor

ACK BUFFER-Ammonium-Chloride-Potassium

CT- Threshold cycle

MTT- 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

SEM- Scanning Electron Microcope

OD-Optical density

UV spectroscopy- Ultraviolet Spectroscopy

HPLC- High-performance liquid chromatography

PVA-Polyvinyl Alcohol

RPM- Revolutions Per Minute

RNA- Ribonucleic acid

Dntps- Deoxynucleotide triphosphates

Author Contribution

All authors have studied the final manuscript for communication. The authors were responsible for study conceptualization complete literature search, protocol development, data gathering, statistical analysis, and development of the manuscript. The authors have read and approved the final version of the manuscript.

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Ethics approval and consent to participate.Not applicable.

Competing interests

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