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Cell line studies for Anticancer and Wound healing activity and Antioxidant potential evaluation of Naturamore Tulsi & Licorice Lung detox herbal green tea

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STUDY CONDUCTED AT:

National Facility for biopharmaceuticals

A project sponsored by department of science and technology, Govt. of India

विज्ञान एवं प्रौद्योगिकी मंत्रालय
MINISTRY OF SCIENCE AND TECHNOLOGY
सत्यमेव जयते

G.N. Khalsa college, Mumbai

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Study 1:

Tulsi & Licorice Lung Detox tea was dissolved in solvent to get a sample for trial. 10mg of the sample was dissolved in 1mL DMSO to give a stock concentration of 10mg/mL. The working concentration of 1mg/mL was used for the studies.

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Test Cell Lines:
A. A549 (Lung Carcinoma) – Human epithelial lung carcinoma cells
B. L132 (Lung cell line)- Healthy cells
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Protocol:

1. A549 cells were revived and 0.05 million cells were seeded in 96 well plate.
2. The cells were incubated in CO2 incubator at 37° C, 5% CO2 overnight.
3. After observing the fully confluent cells under microscope the cells were treated with the given samples at 6 different concentrations.
4. The cells were incubated overnight in the presence of sample in CO2 incubator at 37° C, 5% CO2.
5. After observing the cells under microscope 10µL of 5mg/mL MTT reagent was added in the wells and incubated for 4 hours.
6. The media was discarded and the formazan crystals were dissolved by adding 100µL of DMSO.
7. The absorbance was measured at 570 nm, the values are as follow.
8. The study was performed in triplicates.

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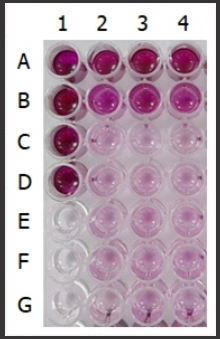
Results:

1.For cell line A-549



Legend:

Lane 1 A-D: Untreated cells

Lane 1 E-G: Media control

Lane 2-4: Sample at different concentrations (2.5, 5, 10, 20, 40, 60, 80µL) in triplicates.

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Results:

µL of sample	Average OD	% viability	% cytotoxicity
2.5	1.632	59.9394	40.0606
5	0.892	32.761	67.239
10	0.213	7.822973	92.17703
20	0.275	10.10008	89.89992
40	0.267	9.806262	90.19374
60	0.286	10.50409	89.49591
80	0.219	8.043339	91.95666

The given sample was tested on A549 Cell line for toxicity.

The IC50 of the given sample was found to be 3.2 µL i.e. 32µg/mL.

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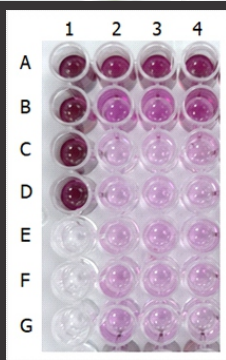
Results:

1.For cell line L132



Legend:

Lane 1 A-D: Untreated cells

Lane 1 E-G: Media control

Lane 2-4: Sample at different concentrations (2.5, 5, 10, 20, 40, 60, 80µL) in triplicates.

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Results:

µL of sample	Average OD	% viability	% cytotoxicity
2.5	1.977	70.607	29.393
5	0.817	29.179	70.821
10	0.279	9.952	90.048
20	0.254	9.083	90.917
40	0.266	9.500	90.500
60	0.233	8.321	91.679
80	0.229	8.190	91.810

The given sample was tested on L132 Cell line for toxicity.

The IC50 of the given sample was found to be 4.03µL i.e 40.4µg/mL.

Mode of Action:

A549 (Human Lung Carcinoma cell lines) cells were treated with the given samples at IC10 and IC25 of the drug to study DNA degradation.

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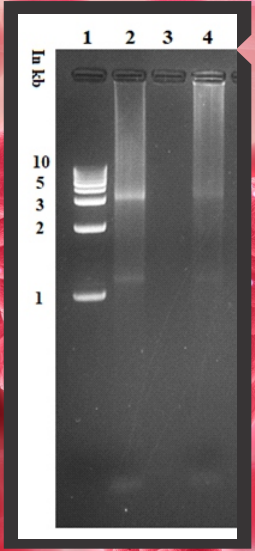
Results:



Legend:

Legend
Lane 1- DNA Ladder (1kb)
Lane 2- Untreated (Control)
band 4 – IC50

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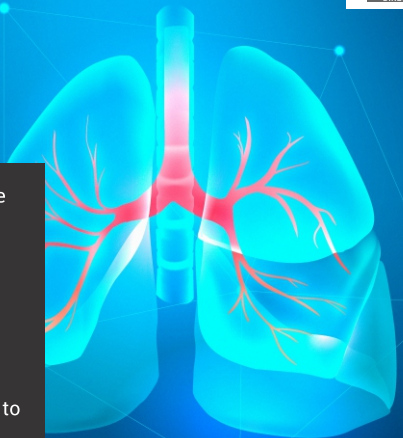
Conclusion:

In order to delineate the mechanism of cell death mediated by Tulsi & Licorice herbal green tea it was tested on A549 Cell line for DNA degradation assay, which is characteristic of apoptosis. The results show DNA degradation at IC50. This data suggests that Tulsi & Licorice herbal green tea is a potent inducer of apoptosis, a mechanism by which it kills Lung cancer cells.

The Tulsi & Licorice herbal green tea showed difference in the IC50 values for lung epithelial cells and lung carcinoma cell line. The herbal tea is more toxic to cancer cells as compared to normal cells.

Cell line studies suggest that Tulsi & Licorice herbal Green Tea has potential to kill Lung Cancer cells However, further detailed clinical trials needs to be done for evaluating its anticancer activity.



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Study 2:

Wound healing activity
Test Cell line: L132 cell line

Protocol:

1. L132 cells were revived and 0.05 million cells were seeded in 6 well plate.
2. The cells were incubated in CO2 incubator at 37° C, 5% CO2 overnight.
3. After observing the fully confluent cells under microscope the scratch was made in the cells.
4. The media was discarded from the cells and the wells were washed with PBS.
5. The cells were replenished with fresh media and the scratch was observed under the microscope and the image was documented.
6. The given sample was added to the respective wells.
7. The plate was incubated in CO2 incubator at 37° C, 5% CO2 for 3 days and microscopic images were captured at 24, 48 and 72hrs and documented.
8. The wound healing properties of the sample was compared with the control well where the scratch was replenished only with the media without addition of any sample.

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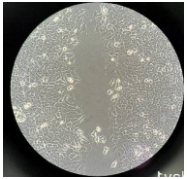
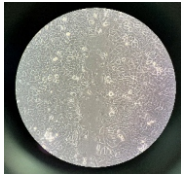
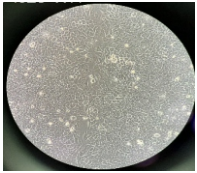
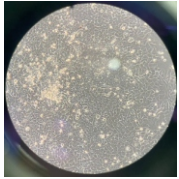
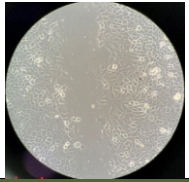
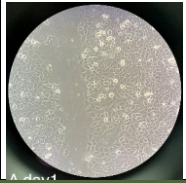
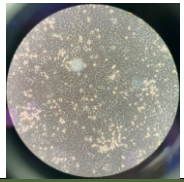
Results:

Sr. No.	Sample name	Time of incubation			
		0hr	24hrs	48hrs	72hrs
1	Control				
2	Tulsi & Licorice herbal green tea				

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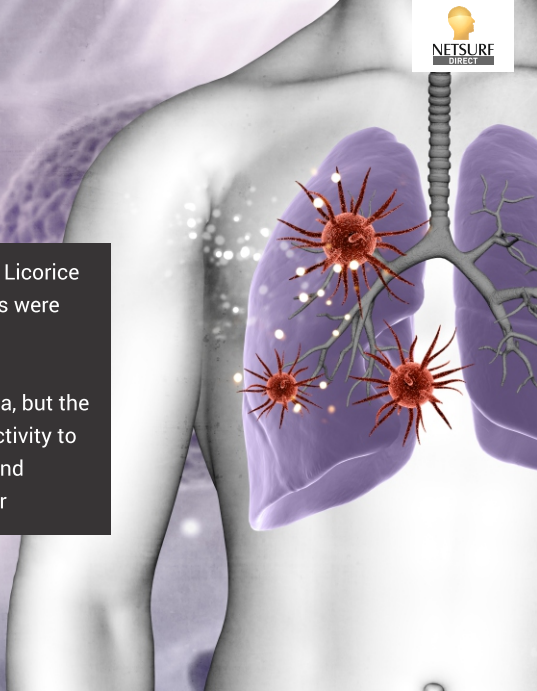
Conclusion:

Wound healing study was carried out on L132 cell line for Tulsi & Licorice herbal green tea. The wound was observed for 3 days and images were captured at 0hr, 24hr, 48hr and 72hrs.

The wound has a capacity to heal on its own in presence of media, but the Tulsi & Licorice herbal green tea showed marginal proliferative activity to heal the wound as compared to the control. That Suggest Tulsi and Licorice herbal green tea may help heal the damaged lungs faster



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Study 3:

Antioxidant Activity activity

AIM: To carry out an ANTI-OXIDANT ASSAY of Hydro-ethanolic Extract of Tulsi and Licorice Tea using DPPH [α, α-diphenyl-β-picrylhydrazyl] REAGENT.

1. DPPH ASSAY:

a. To each of the 5 test-tubes, the ascorbic acid standard was added in increasing volumes of 100µL, 200µL, 300µL, 400µL and 500µL and the volumetric addition of methanol was carried out to make the volume up to 1000µL. To this mixture, 1000µL of 0.008%w/v DPPH was added and the test-tubes were incubated in the dark for 30 minutes.

b. The exact procedure was repeated for a triplicate set of the ascorbic acid standard, under the same conditions.

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Study 3:

Antioxidant Activity activity

c. Similarly, for the Tulsi & Licorice Tea extract, to each of the 5 test-tubes, the ascorbic acid standard was added in increasing volumes of 10µL, 20µL, 30µL, 40µL and 50µL and the volumetric addition of methanol was carried out to make the volume up to 1000µL. To this mixture, 1000µL of 0.008%w/v DPPH was added and the test-tubes were incubated in the dark for 30 minutes. The samples were analyzed in triplicate sets.

d. After the 30-minute incubation, the samples were subjected to spectrophotometric determination for free radical scavenging activity at 517nm using the Multiscan sky cuvette Touch Drop Spectrophotometer.

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Study 3:

Antioxidant Activity activity

e. A set of 3 test-tubes was filled with 1000µL of and 1000 µL of the DPPH reagent and incubated in dark for 30 minutes similar to the standards and the samples were spectrophotometrically analyzed on the Multiscan sky cuvette Touch Drop Spectrophotometer at 517nm. This set of triplicates was used as the control.

f. Formula used for calculating the % Free Radical Scavenging Activity of the Ascorbic Acid standard and the Tea sample was as follows:

$$\%INH+\left(\frac{(\text{Abs of Control}-\text{Abs of Sample})}{(\text{Abs of Control})}\right)*100$$

g. The IC50 Values for Ascorbic Acid & Tulsi and Licorice were calculated using the following formula:

$$y=mx+c$$

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Results:

The following tables and Graphs indicate the comparative anti-oxidant properties of the Tulsi & Licorice Tea Extracts:

Table : Absorbance of the DPPH Control

VOL. OF SOLVENT	VOL. OF DPPH REAGENT	ABS-1	ABS-2	ABS-3	ABS-AVG
1000µL	1000µL	0.927	0.928	0.927	0.927

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Results:

Figure : Image of the DPPH Control

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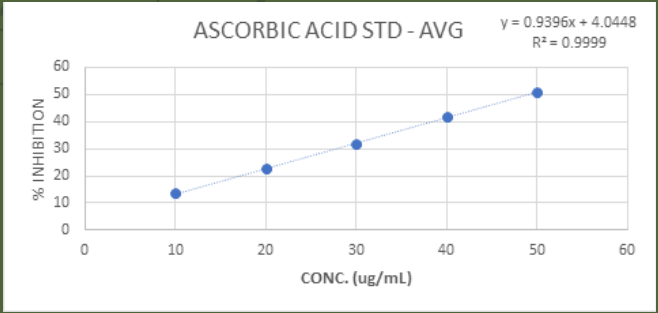
Results:

Figure : Graph for the free radical scavenging activity of Ascorbic Acid Standard



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Results:

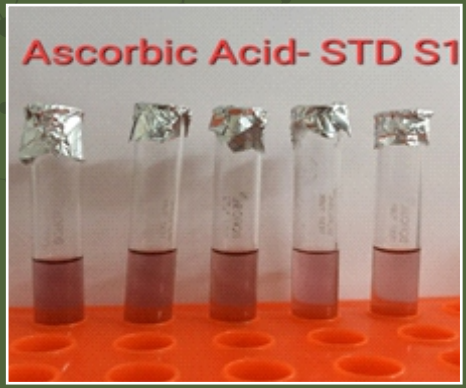

Figure 3-A: Ascorbic Acid DPPH Assay

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Results:

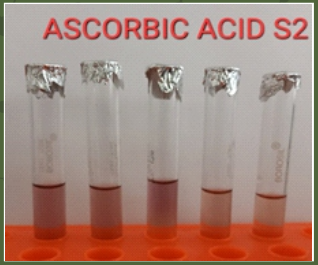

Figure 3-B: Ascorbic Acid DPPH Assay

Figure -C: Ascorbic Acid DPPH Assay

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Results:

Table 2: DPPH free radical scavenging activity of the Ascorbic Acid Standard

VOLUME	CONC. (ug/mL)	S1-ABS	S2-ABS	S3-ABS	AVG	AVG-%INH
100	10	0.732	0.726	0.744	0.734	13.51985
200	20	0.656	0.629	0.658	0.648	22.83305
300	30	0.561	0.555	0.573	0.563	31.96645
400	40	0.476	0.461	0.477	0.471	41.85498
500	50	0.391	0.382	0.387	0.387	50.98839

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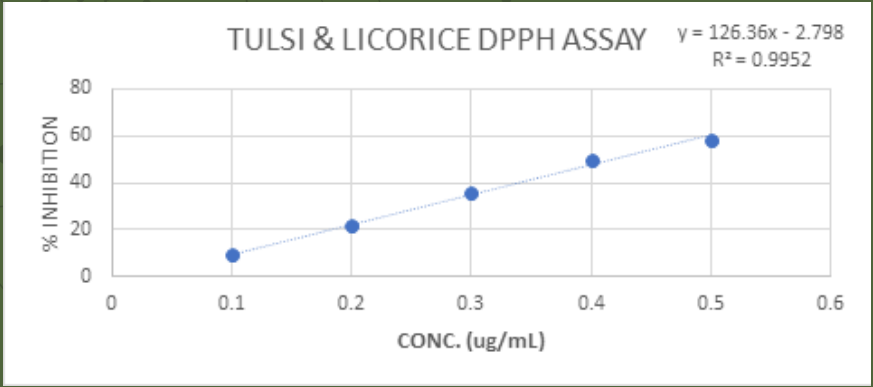
Results:

Figure 4: Graph for the free radical scavenging activity of Tulsi & Licorice Tea Extract



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Results:

Figure 5-A: Tulsi & Licorice DPPH Assay

Figure 5-B: Tulsi & Licorice DPPH Assay

Figure 5-C Tulsi & Licorice DPPH Assay

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Results:

Table 3: DPPH free radical scavenging activity of Tulsi & Licorice Tea Extract

VOLUME	CONC. (ug/mL)	S1-ABS	S2-ABS	S3-ABS	AVG	AVG-%INH
10	0.1	0.78	0.769	0.773	0.774	9.204854
20	0.2	0.657	0.652	0.653	0.654	22.14984
30	0.3	0.528	0.522	0.529	0.526	35.92186
40	0.4	0.405	0.394	0.399	0.399	49.62197
50	0.5	0.312	0.317	0.318	0.316	58.6475

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Results:

From the above observation table, IC50 was calculated using formulae: y = mx + c
Where “y” is 50, “m” and “c” are the constants as taken from the graphs above. The following table summarizes the values and the calculation of the IC50 Values.

SAMPLE	“y”	“m”	“c”	IC 50
ASCORBIC ACID	50	0.9396	4.0448	48.889µg/mL
TULSI & LICORICE	50	126.36	-2.798	0.418µg/mL

CONCLUSION: The IC50 for ascorbic acid standard and Tulsi & Licorice tea extract was found to be 48.889µg/mL and 0.418µg/mL respectively. The Tulsi & Licorice tea extract possessed remarkable anti-oxidant properties by the virtue of its ability to scavenge free radicals.