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Research Article

Chemo protective activity of *Phyllanthus niruri* and *Opuntia ficus indica* plant extract

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Abstract



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Cancer is one of the leading causes of death. Several chemotherapeutic agents have been used to treat cancer, and yet a convincing cure is elusive in most cases. Therefore, there is continued need for development of new anticancer agents. Angiogenesis is one of the vital events for organ development and differentiation during embryogenesis as well as wound healing and reproductive functions in adults. Angiogenesis also contributes significantly to tumors growth and metastasis. Currently, search for effective anti-angiogenesis agents and therapy is an emerging field. Cytotoxic agents with anti-angiogenic activity are designated to control tumors endothelial cell growth and tumour cell survival. Therefore, combinatorial therapy in which anti-angiogenic agents with chemotherapeutic activity administered in a scheduled manner could result in a much more favorable therapeutic effect. India is one of the richest bio diversity centers with respect to medicinal plants. Many plants have been utilized in the traditional system of medicine for cancer treatment. The plants have been utilized as medicines from thousands of years ago. The isolation of active components from plants began with the morphine isolation from opium plant. In the present study, *Phyllanthus niruri* and *Opuntia ficus-indica* were screened for the phytochemical and anticancer evaluation.

Keywords: Anti-Cancer, Anti-Oxidant, *Phyllanthus niruri*, *Opuntia ficus-indica*, *in vivo* study.

INTRODUCTION

Cancer is one of the most dreaded diseases threatening the health conditions of mankind. It is among the killer diseases in Nigeria, and a name associated with a group of diseases in which the body cells divide, multiply and spread uncontrollably¹. They may also be regarded as a large family of diseases which show features suggestive of malignancy. They form a subsep of neoplasm. The disease causes about 13% of all world death². Worldwide, approximately 18% of cancer deaths are related to infections, such as hepatitis B, hepatitis C, and human papilloma virus. This proportion varies in different regions of the world from a high of 25% in Africa to less than 10% in the developed world. Deaths from cancer worldwide are projected to continue rising with an estimated 12 million deaths in 2030; case reports from 2007 indicated that about 7.6 million people died from cancer in the world³. In 2012, about 14.1 million new cases of cancer occurred globally. It caused about 8.2 million deaths or 14.6% of all human deaths⁵.

MATERIAL AND METHODS

Plant Extract Collection

Detail of Plant Extract

Plant Name	Family	Species	Drug
<i>Opuntia Ficus indica</i>	Cactaceae	<i>Opuntia Ficus indica</i>	Fruits
<i>Phyllanthus niruri</i>	Euphorbiaceae	<i>P. niruri</i>	Herb

Animal:

The animal experimental protocol was approved by the Department of Pharmacy, Mansarovar University Bhopal Institutional Animals Ethical Committee (IAEC), Deshpande Laboratories Pvt. Ltd. Bhopal (M.P.)

CPCSEA Registration no: **1582/PO/Re/11/CPCSEA**

In Vitro Study: Treatment groups for the individual study, *in vitro*: Activity of *Opuntia ficus indica* fruits extract and *Phyllanthus niruri* herb on NSCLC cells, A549 and H522 cells were treated with different concentrations of *Opuntia ficus indica* fruits extract and *Phyllanthus niruri* herb. Representative groups which were maintained for this study are as follows:

Group 1: Negative control (untreated) This group received no drug or alcohol. A549 and H522 cells were cultured in DMEM and RPMI-1640, respectively, supplemented with 10% heat inactivated FBS and 1% antibiotic antimycotic solution, maintained at 37°C with 5% CO₂ in a humidified incubator.

Group 2 & 3: *Opuntia ficus indica* fruits extract and *Phyllanthus niruri* herb –treated A549 and H522 cells were cultured in DMEM and RPMI-1640, respectively. Cells were treated with different concentrations of *Opuntia ficus indica* fruits extract and *Phyllanthus niruri* herb for 24h and 48h. For *Opuntia ficus indica* fruits extract and *Phyllanthus niruri* herb on NSCLC cell line, H460 H460 & HCT15 cells were treated with different concentrations of CGS. Representative groups maintained for this study are as follows:

Group 1: Negative control (untreated) This group received no

drug or alcohol. H460 & HCT15 cells were cultured in DMEM and RPMI-1640, respectively, supplemented with 10% heat inactivated FBS and 1% antibiotic antimycotic solution, maintained at 37°C with 5% CO₂ in a humidified incubator.

Group 2 & 3: *Opuntia ficus indica* fruits extract and *Phyllanthus niruri* –treated H460 & HCT15 cells were cultured in DMEM and RPMI-1640, respectively. Cells were treated with different concentrations of *Opuntia ficus indica* fruits extract and *Phyllanthus niruri* herb for 24h and 48h.

Determination of cell viability by MTT assay Principle

This is a colorimetric assay that measures the reduction of yellow coloured MTT by mitochondrial succinate dehydrogenase. The MTT enters into the cells and passes to the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. Cell viability was assessed using MTT followed by the method described by ⁷. NSCLC cells (1x10⁵ cells/ml) were cultured for 24h on 96-well micro plates. The cells were incubated for different time-points for different modes of studies with and without drugs.

Materials for MTT

Freshly prepared MTT (5mg/ml in PBS) was filtered and kept in dark, at 4°C. Acidic isopropanol (0.1N HCl in absolute isopropanol) 96 flat bottom well plate. Cell viability was assessed using MTT followed by the method described by ⁸.

DPPH and Hydroxyl (OH) radical scavenging assay Activity *Opuntia ficus indica* fruits extract and

Phyllanthus niruri in Lung Cancer cell lines Principle

DPPH and Hydroxyl (OH) catalyses the oxidation of lactate to pyruvate accompanied by the simultaneous reduction of NAD to NADH. DPPH AND HYDROXYL (OH) activity in any protein content is proportional to the increase in absorbance due to

the reduction of NAD. Working reagent 1.1ml of DPPH and hydroxyl (OH) (buffered substrate) was added to the vial labelled with 1DPPH and hydroxyl (OH) (co-enzyme), mixed well to dissolve and kept for 5min prior to use.

Observation of cell morphology by light microscopy (LM)

The changes in cell morphology were checked by phase contrast light microscope (Axiscope plus 2, Zeiss, Germany) following the method ⁹.

By scanning electron microscopy (SEM) Principle

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected which contain the information about the sample's surface topography and composition. The electron beam is generally scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. SEM can achieve resolution better than 1nm. Specimens can be observed in high vacuum, in low vacuum, and (in environmental SEM) in wet conditions. The most common mode of detection is by secondary electrons emitted by atoms excited by the electron beam. By scanning the sample and detecting the secondary electrons, an image displaying the tilt of the surface is created. To study the surface exposure and/or any deformity superficially of any unsectioned specimen, scanning electron microscopy is used.

RESULTS AND DISCUSSION:

In Vitro Antitumor Evaluation by MTT assay

Antitumor evaluation of extracts

Opuntia ficus indica and *Phyllanthus niruri* ethanol extracts were subjected to MTT assay. Different cell lines used were cultured in A459, H522, H460 cells. The results are depicted in the **TABLE 1**: It is very clear from the results that *Opuntia ficus indica* and *Phyllanthus niruri* are showing comparative activity comparative to control treated group.

TABLE 1: The IC₅₀ value of extracts by MTT assay

GROUP	IC ₅₀ VALUE CELL LINES		
	A549	H522	H460
<i>Negative control</i>	300	280	340
<i>Opuntia ficus indica</i>	32.88	38.8	42.77
<i>Phyllanthus niruri</i>	40	42.77	45.88

Antitumor evaluation of fractions: By first fractionation of the ethanolic extract of *Opuntia ficus indica* (OFI) and *Phyllanthus niruri* (PN) five different fractions namely OFI1 to OFI5 and PN1 to PN5. All the fractions were subjected to *in vitro* antitumor evaluation against the cancer cell lines A459, H522, H460. From the **TABLE 2**: Clear that PN3 and OFI4 showed better activity at 100µg/ml on different cell lines. **Table 3 and Fig.1, Fig.2**, show the result of *Opuntia ficus*

indica and *Phyllanthus niruri* fractions.

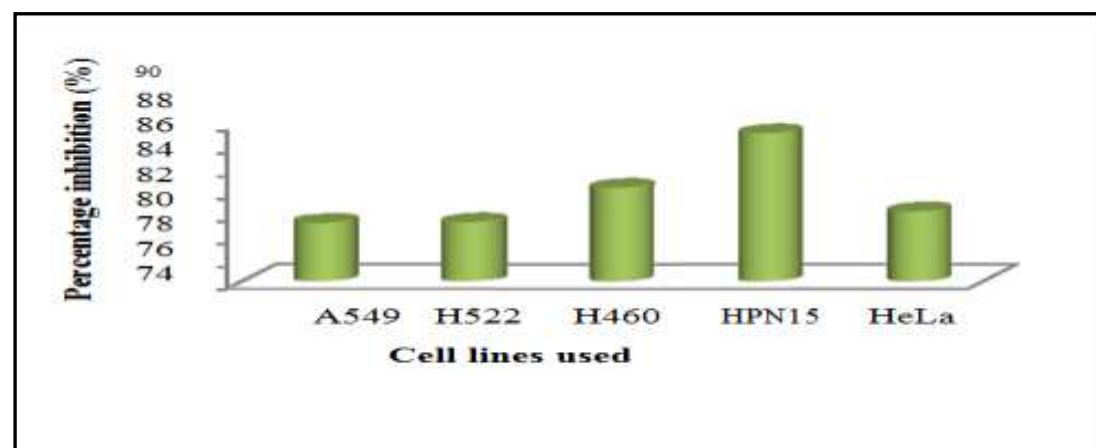
Antitumor evaluation of isolated components: The compounds isolated from *Opuntia ficus indica* and *Phyllanthus niruri* respectively. They showed the better activity was exhibited by OFI4 of *Opuntia ficus indica* and PN3 of *Phyllanthus niruri*. It is shown in the **TABLE 2**: and graphically shown in the **Fig.1**.

TABLE 2: The percentage inhibition by MTT assay of the fractions (*Opuntia ficus indica* & *Phyllanthus niruri*) at 100µg/ml

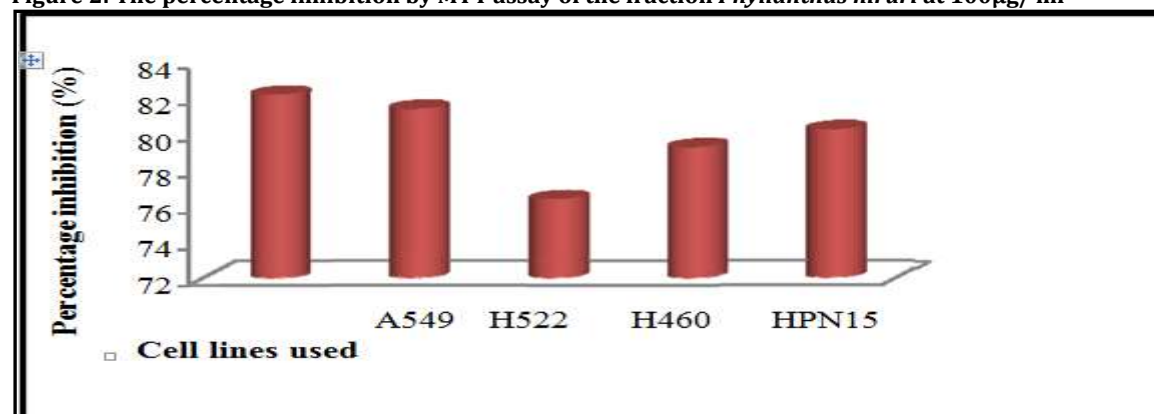
Cell lines used	Percentage inhibition (%)									
	OFI1	OFI2	OFI3	OFI4	OFI5	PN1	PN2	PN3	PN4	PN5
A549	26.66	37.55	26.56	50.33	35.70	25.25	45.95	52.56	22.33	35.26
H522	36.56	35.67	25.33	45.26	20.33	24.36	22.23	67.56	25.12	30.12
H460	26.42	42.33	30.65	39.53	25.76	38.37	25.78	55.59	20.22	22.33

TABLE 3: The percentage inhibition by MTT assay of the fractions from *Opuntia ficus indica* and *Phyllanthus niruri* at 200 and 100µg/ml

Different cell lines	Percentage inhibition(%)	
	OFI4	PN3
A549	55.78	46.34
H522	60.33	52.45
H460	50.34	36.35

Figure 1: The percentage inhibition by MTT assay of the fraction *Opuntia ficus indica* at 200µg/ ml

A549 H522 H460 HPN15 HeLa
Cell lines used

Figure 2: The percentage inhibition by MTT assay of the fraction *Phyllanthus niruri* at 100µg/ ml

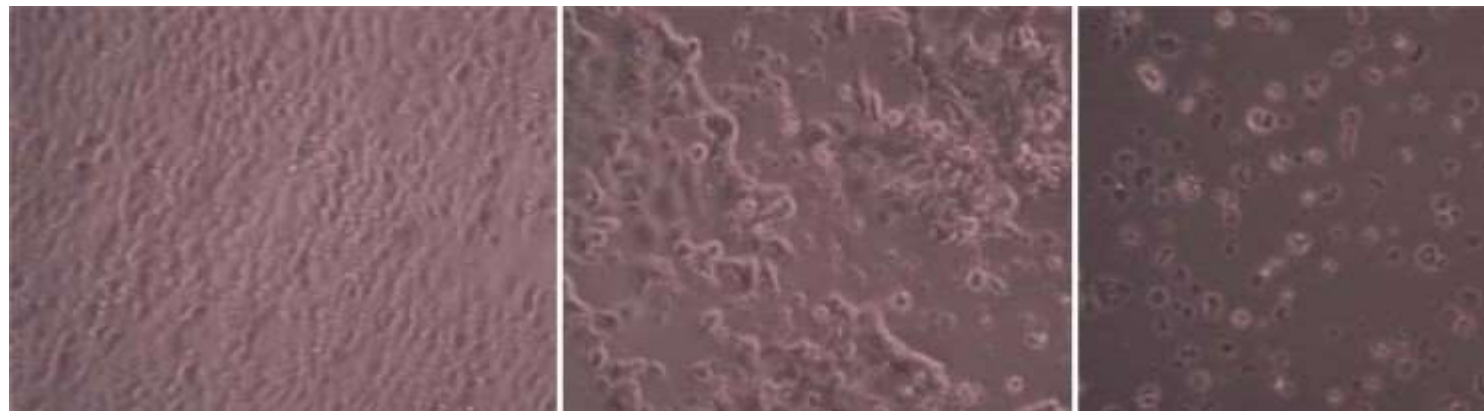
A549 H522 H460 HPN15
Cell lines used

The following figure represents the pathophysiology of cell lines.

Figure 3: (a) indicates A549 control **Figure 3(b):** indicates A549 cell lines treated with OFI4 and **Figure 3(c):** indicates A549 cells treated with PN3.

Figure 4: (a) Indicates H522 control, **Figure 4: (b)** Indicates H522 cell lines treated with OFI4 and **Figure 4: (c)** Indicates H522 cells treated with PN3. **Figure 5:** (a) Indicates H460 control **Figure 5: (b)** Indicates H460 cell lines treated with OFI4 **Figure 5: (c)** Indicates H460 treated with PN3.

Figure 3: A549 control and the cells treated with the isolated compounds (MTT assay)



(a) A549 Control

(b) A549 OFI4

(c) A549 PN3

Figure 4: H522 control and the cells treated with the isolated compounds (MTT assay)



H522 Control

(b) H522 OFI4

(c) H522 PN3

Figure 5: The H460 control and the cells treated with the isolated compounds (MTT assay)



(a) H460 Control

(b) H460 OFI4

(c) H460 PN3

In vitro antitumor evaluation by Lactate Dehydrogenase Assay of isolated compounds: The isolated components from *Phyllanthus niruri* PN1, PN2, PN3, PN4, and from *Opuntia ficus indica*, OFI1, OFI2, OFI3 and OFI4 were subjected to Lactate Dehydrogenase Assay. The cell lines used were A549, H522, and H460 at 100µg/ml. The results are showed in the **TABLE 4**: It is represented graphically in the **figure6.18**. In Lactate Dehydrogenase Assay, nuclear condensation is taking place and the dead cells become shrunk and the percentage inhibition can be calculated by counting the number of nor

OFI1 cells and shrunk cells. The following figure represents the pathophysiology of cell lines. **Fig.6: (a)** Indicates **A549** control, **Fig.6: (b)** indicates **A549** cells treated with **OFI4** Figure **Fig.5: (c)** indicates **A549** cells treated with **PN3**. **Fig.6: (a)** indicates **H522** control, **Fig.6: (b)** indicates **H522** cells treated with **OFI4** and **Fig.6: (c)** indicates **H522** cells treated with **PN3**. **Fig.7: (a)** indicates **H460** control, **Fig.7: (b)** indicates **H460** cells treated with **OFI4** and **Fig.7: (c)** indicates **H460** cells treated with **PN3**. The results were comparable with the results obtained by MTT assay.

TABLE 4: The percentage inhibition by Lactate Dehydrogenase Assay of the isolated compounds at 100µg/ml

CELL LINES USED	OFI1	OFI2	OFI3	OFI4	PN1	PN2	PN3	PN4
A549	75.88	44.25	80.62	44.34	56.67	88.53	80.34	55.48
H522	79.45	47.35	79.52	40.22	44.36	85.66	83.78	52.43
H460	76.78	43.22	75.33	49.23	54.33	80.99	80.44	60.56

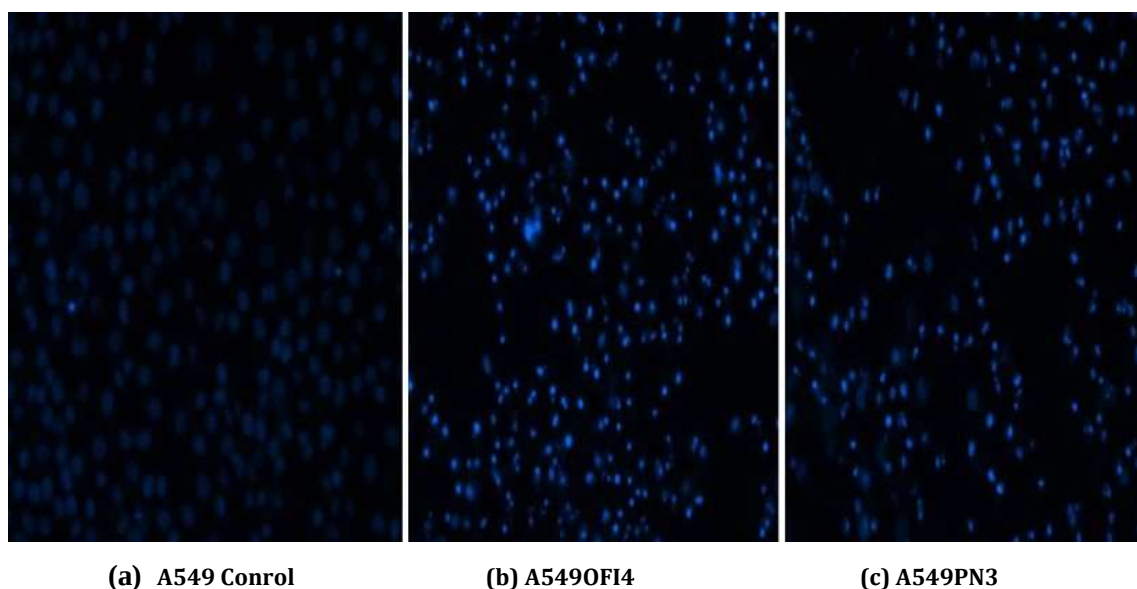


Figure 6: A549 control and the cells treated with the isolated compounds (Lactate Dehydrogenase Assay)

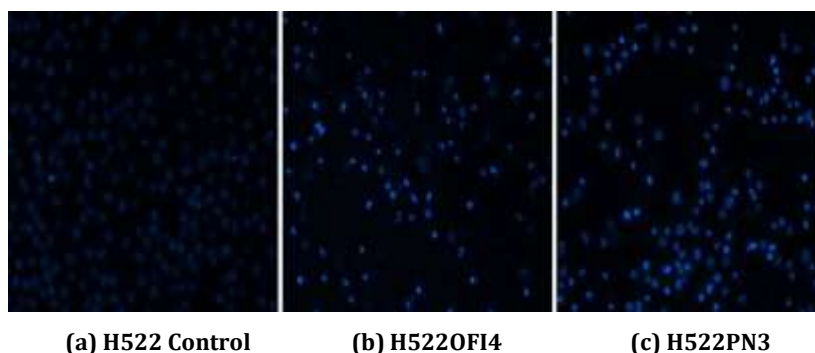


Figure 7: H522 control and the cells treated with the isolated compounds (Lactate Dehydrogenase Assay)

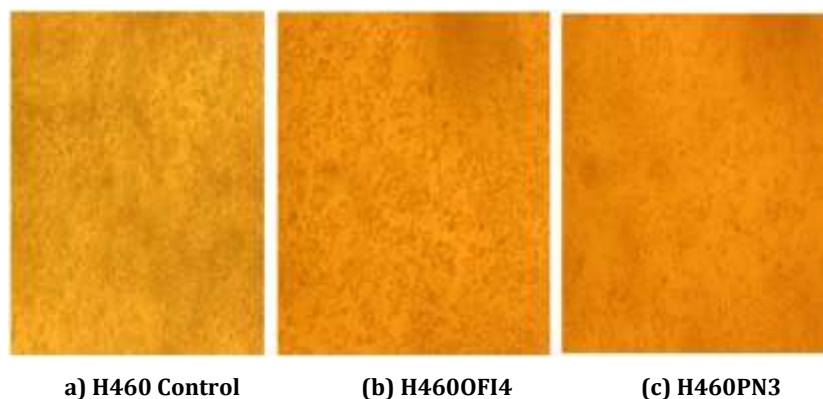


Figure 8: The HPN15 control and the cells treated with the isolated compounds (MTT assay)

In Vivo Study

In vivo antitumor evaluation of the fractions OFI4 and PN3

The *in vivo* evaluation was done using PN3 fraction obtained from *Phyllanthus niruri* and OFI4 fraction obtained from *Opuntia ficus indica*. Two different models, solid tumour study and ascites tumour analysis were done. Dalton's lymphoma ascites (DLA) cell lines were used for the study. Both the fractions showed marked activity against cancer since there is marked reduction in tumour volume in case of solid tumour study. It is represented in the **TABLE 6**. Also there is increase in life span of mice in case of ascites tumour study. It is shown in the **TABLE 5**.

Ascites tumour model

Effect on tumour growth response

Body weight: Tumour development was noticed from day 6 and there was a gradual rise in body weight due to accumulation of ascites fluid in the peritoneum of EAC mice, especially in the positive control group (45.54 ± 6.66), whereas a significant reduction of gain in body weight was found in the standard (cisplatin) treated groups (7.12 ± 0.9). On comparing the extract treated groups, all groups exhibited significant differences in a dose-dependent OF Inner and OFI 200 mg/kg showed a maximum decrease in body-weight (19.38 ± 1.9). Both extract groups showed significant differences of gain in body weight when compared with the positive control group indicating the effectiveness of the test extracts. Cisplatin

(Standard) treated group caused 91% decrease in body weight, while group IV and V OFI (200mg/kg) and PN (100mg/kg) registered only 60.95 and 54.24% respectively **TABLE 6**. A regular sudden rise in ascites tumour volume was seen in EAC bearing hosts. The direct nutritional source of tumour cells is the ascites fluid and perhaps the sudden rise in ascites fluid with tumour growth could be a way to meet the nutritional needs of tumour cells.¹⁰

Effect on mean survival time (MST) and percentage increase in life span (% ILS)

Mean survival time and percentage increase in life span are two important parameters used for screening of any anticancer agent. A compound can be considered as cytotoxic if it exhibits more than 25% increase in percentage life span. A significant drop in MST was observed in EAC induced mice (14 days) when compared with standard mice (33 days). In the control group, on day 15 the first death was reported and all mice were dead by day 18. However, cisplatin caused a significant increase in the MST (33 days) and % ILS (115) when compared to the vehicle group. Both petroleum and ethanol extracts significantly improved the MST and all groups exhibited a dose-dependent increase in the life span (Figure 5.1f). Among the six treatments, the petroleum ether extract of OFI200 mg/kg was found to be the most effective percentage inhibition of 81.8% (**TABLE 5**).

TABLE 5: Effect of different concentrations of petroleum ether and ethanol extracts of *Opuntia ficus indica* and *Phyllanthus niruri* gain in body weight, MST and % ILS.

GROUPS	% Gain in	% Decrease in	MST (days)	% ILS
	body weight	bodyweight (g)		
Control	2.21 ^{x,ns}	-	-	-
PositiveControl	45.54 $\pm$ 6.66 ^a	-	15.5 $\pm$ 0.53 ^a	-
Standard	7.12 $\pm$ 0.9 ^x	93	33 $\pm$ 0.56 ^x	115.25
OFI 200mg	19.38 $\pm$ 1.9 ^{x,a}	65.85	30 $\pm$ 1.39 ^{x,c}	84.87
PN 100mg	20.12 $\pm$ 2.2 ^{x,a}	57.28	24 $\pm$ 0.48 ^{x,a}	55.54

n=6, values are expressed as mean $\pm$ SEM. Statistical analyses were done by one-way ANOVA followed by Tukey's multiple comparison tests. ^xp < 0.001, ^yp < 0.01 and ^zp < 0.05 as compared to control, ^ap < 0.001, ^bp < 0.01 and ^cp < 0.05 as compared to standard, ns – non significant.

Tumour volume and packed cell volume:

At the doses of 200 mg/kg OFI4 treated groups significantly (p < 0.001 and p < 0.001) reduced tumour volume and packed cell volume in a dose dependent manner compared with that

of the EAC control. All groups showed significant differences in tumour volume and packed cell volume, when compared with the positive control group except low dose of the ethanol extract of OFI4 (**TABLE 6**)

TABLE 6: Effect of petroleum ether and ethanol extracts of *Opuntia ficus indica* and *Phyllanthus niruri* on tumour growth response of EAC inoculated Swiss albino mice.

Group	Tumour	Tumour	Packed cell
	weight (g)	volume (mL)	volume (mL)
Control	-	-	0.33±0.02 ^x
Positive Control	9.96±0.1180 ^a	8.875±0.2045 ^a	5.867±0.275 ^a
Standard	2.645±0.0234 ^x	0.9878±0.0174 ^x	0.4856±0.1787 ^x
OFI 200mg	3.675±0.1080 ^{x,a}	1.783±0.0667 ^{x, c}	0.9687±0.0589 ^x
PN 100mg	4.745±0.1479 ^{x, a}	3.784±0.1877 ^{x, a}	1.937±0.8767 ^{x, a}

n=6, values are expressed as mean ± SEM. Statistical analyses were done by one-way ANOVA followed by Tukey's multiple comparison tests. ^x p<0.001, ^y p<0.01 and ^z p<0.05 as compared to control, ^a p<0.001^b p<0.01 and ^c p<0.05 as compared to standard, ns- non significant.

The reliability criterion for judging the value of any anticancer drug is the prolongation of life span of the ani OFI (Clarkson D. & Burchneal JH. 1965) and reduction of WBC (Obeling *et.al.*, 1954). The OFI4 and PN3 have decreased the ascites fluid volume. In all OFI4 treated groups viable cell count was greatly reduced in a dose dependent OFI. In contrast, in the treated groups compared with EAC control hosts the percentage of trypan blue positive dead tumour cells (non-viable) also increased. It OFIly be concluded that OFI4 caused a decrease in the fluid volume by arresting the tumour growth and thereby increased the life span of EAC bearing mice.

Solid tumour model

Effect of ethanol extracts of *Opuntia ficus indica* and *Phyllanthus niruri* on solid tumour development in DLA inoculated mice.

Tumour volumes of mice treated with different doses of extracts were found significantly lower than that of control group. The average tumour volume of the vehicle control group on 30th day was 3.21±0.02 mm³. Animals treated with extracts of *Opuntia ficus indica* 200 mg/kg and *Phyllanthus*

niruri 100 mg/kg for a period of 30 consecutive days showed the reduction in tumour volume to 1.88±0.03 mm³, 1.26±0.04mm³ respectively. The standard drug (cisplatin 3.5 mg/kg) was found to be efficient (1.26±0.04 mm³) in preventing the development of DLA induced tumour as shown in Figure 5.7. Treatment with *Opuntia ficus indica* and *Phyllanthus niruri* also revealed a decrease in the tumour volume as compare to Positive control respectively. These observations revealed that the *Opuntia ficus indica* and *Phyllanthus niruri* had the ability to inhibit the tumour progression.

Percentage inhibition of tumour weight and tumour volume

To confirm these findings, the tumour weight after excision was also recorded. A significant reduction in average tumour weight was found in the treated Animals compared to the control Animals (TABLE 7). There was a reduction in tumour weight of *Opuntia ficus indica* 200 mg/kg and *Phyllanthus niruri* 100 mg/kg treated Animals (77.54% and 75.42%) and was comparable with that of standard cisplatin (95%).

TABLE 7: Effect of *Opuntia ficus indica* and *Phyllanthus niruri* on percentage inhibition of tumour weight and tumour volume of DLA induced Swiss albino mice.

Group	Average tumour weight	% Reduction of tumour volume after 30 th day	Percentage inhibition
	±SEM (g)		
Positive control	0.3020±0.01020	3.21±0.02	00.00
Standard	0.02020±0.00090	1.09±0.06	95.074
OFI 200mg	0.2250±0.01300	1.88±0.03	77.544
PN 100mg	0.2100±0.01010	1.26±0.04	75.426

N=6, values expressed as mean ± SEM

Effect on mean survival time (MST) and percentage increase in life span (% ILS)

Mean survival time and percentage increase in life span are two important parameters used for screening of any anticancer agent. A compound can be considered as cytotoxic if it exhibits more than 25% increase in percentage life span. A significant drop in MST was observed in EAC induced mice (15.6 days) when compared with standard mice (20 days). In the control group, on day 15 the first death was reported and all mice were dead by day 19. However, cisplatin caused a significant increase in the MST (22 days) and % ILS (58) when compared to the vehicle group. Both petroleum and ethanol extracts significantly improved the MST and all groups exhibited a dose-dependent increase in the life span. Among the six treatments, the petroleum ether extract of OFI 200

mg/kg was found to be the most Effective showing a percentage inhibition of 48.8% and survival time is (21 days)

TABLE 8: Effect on mean survival time (MST) and percentage increase in life span (% ILS) on solid tumour study of *Opuntia ficus indica* and *Phyllanthus niruri*

GROUP OF ANIMALS	MEAN SURVIVAL DAYS	INCREASE IN LIFE SPAN (%)
Normal	15.67±3.63	-
Positive Control	20.40±4.41	22.34
Standard	22.17±3.62	58.87
OFI 200mg	21.33±4.38	48.89
PN 100mg	20.36±1.50	45.20

CONCLUSION

The *in vitro* anticancer activity of the ethanolic extracts of the four plants was carried out using three different cell lines, A549, H522, and H460 cells. Based on the results two plants, *Phyllanthus Niruri* and, *Opuntia ficus-indica* were selected for further study. IC₅₀ value of the most active extract, the ethanol extract of *Phyllanthus Niruri* and, *Opuntia ficus-indica* was found to be 32.88, 38.80, 42.77 µg/mL A549, H522, H460, and, *Opuntia ficus-indica* was found to be 40.00, 42.77, 45.88 µg/mL also increased the mean life span and exhibited 71% of tumour inhibition. The active component isolated (*Phyllanthus Niruri* and, *Opuntia ficus-indica*) from the same could bring down the IC₅₀ values to 10 and 15 µg/mL.

Under the phytochemical investigation, the plant extracts were subjected to fractionation by column chromatography. five different fractions PN1 to 5 from *Phyllanthus Niruri* and OF11 – OF15 from *Opuntia ficus-indica* were obtained. Among the *Phyllanthus Niruri* fractions, PN4 and among the *Opuntia ficus-indica* OF13 fractions, were found to be the more active fractions based on the *in vitro* anticancer evaluation and were selected for further column chromatographic fractionation. Again they were subjected to *in vitro* anticancer evaluation and it was found that the PN4 and OF13 were comparatively the more active fractions. From the gas chromatography of these fractions it was clear that they contain four components each and by preparative thin layer chromatography all the eight components were separated. They were again subjected to the *in vitro* anticancer evaluation by MTT assay. PN4 and OF13 were found to be more active. These selected components were subjected to further characterization to identify the structure of the compounds.

The *in vivo* study was also carried out using the PN4 and OF13 fractions by solid tumour and ascites tumour study using the DLA cell lines. The plant fractions were also exhibited better anticancer activity. Effect of different doses of *Phyllanthus Niruri* and, *Opuntia ficus-indica* on solid tumour development, percentage inhibition of tumour growth and histopathological studies were also assessed. *Phyllanthus Niruri* (100mg/kg) and, *Opuntia ficus-indica* (200 mg/kg) exhibited 77% and 55% inhibition in tumour growth, whereas in the standard drug (cisplatin) caused an inhibition of 95%. Histopathological studies revealed the presence of fibrous cells. Absence of necrosis, inflammatory changes and blood vessels in the high dose treated group proved its efficacy as an anticancer agent. Hence we selected petroleum ether extract of *Phyllanthus Niruri* and *Opuntia ficus-indica* for the isolation of the active compounds.

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