Int J Pharma Res Health Sci. 2016; 4 (5): 1399-1402
DOI:10.21276/ijprhs.2016.05.11
Leena P N *et al.*
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Development and Assessment of Anti Cancer Activity of Phytosome Using Isolated Compound from *Clerodendron Paniculatum* Linn Root Extract

Sundaraganapathy [1],Leena P N [*]

*Research scholar, Karpagam University, Coimbatore , Tamil Nadu, India.
[1]Dean, Faculty of Pharmacy, Karpagam University, Coimbatore,Tamil Nadu, India.

ARTICLE INFO

Received: 15 Oct 2016
Accepted: 29 Oct 2016

ABSTRACT

Components and assessment of anti most cancer activity of Clerodendron paniculatum Linn formulated Phytosome from the extract by means of DAL cells. The foundation portions of the Clerodendron paniculatum Linn root extracted with ethanol by using bloodless maceration method .The extracts were vacuum dried and subjected to phytochemical screening for the detection of diverse phyto ingredients .The formulated Phytosome from the extract exhibited amazing pastime against cancer cells .The look at well-known shows that Clerodendron paniculatum Phytosome own higher anti most cancers activity than the extract.

Key words: Clerodendron paniculatum Linn root, anticancer activity.DAL cells.

1. INTRODUCTION

Guidance of flowers or their components have been broadly used in medication since ancient times and till nowadays use of Phytomedicines is sizable. Most of the biologically lively components of flora are polar or water-soluble. However,water-soluble phytoconstituents like flavonoids, tannins, glycosides aglycones and many others are poorly absorbed either because of their large molecular length, which cannot be absorbed by way of passive diffusion or due to their poor lipid solubility, consequently critically proscribing their capacity to transport throughout lipid-wealthy biological membranes, ensuing of their poor bioavailability[1]. Phytosome is a newly added patented

Corresponding author *
Leena P N,
Karpagam University, Coimbatore , Tamil Nadu, India
Email: leena09narayanan@gmail.com

IIIIIIII© International Journal of Pharma Research and Health Sciences. All rights reserved

(%) Yield = (Practical yield) × 100 (Theoretical yield)

technology developed to contain the standardized plant extracts or water-soluble phytoconstituents into phospholipids to produce lipid well suited molecular complexes, which improves their absorption and bioavailability[2].Clerodendron paniculatum Linn, (family :Verbenaceae)[3] become a species observed in India, in this pronounced as people treatment for tumours , leprosy, fever, contamination ,inflammation. The roots have been suggested to possess laxative, diuretic, analgesic, and anti inflammatory, anti cancer antibacterial activities[4]. To our know-how there were no medical reviews on anti tumour activities of Phytosome formulated from Clerodendron paniculatum Linn, extract. The key objective of the prevailing take a look at is to expand the Phytosome of Clerodendron paniculatum Linn to boom the solubility and bioavailability of drug. To organized the Phytosome of Clerodendron paniculatum Linn through unique approach and examine its anti most cancers activity by using assessing tumour volume, viable and nonviable tumour cell depend, tumour weight, haematological parameters and biochemical estimations in opposition to DLA tumour cells [5].

2. MATERIALS AND METHODS

Plant material and Extraction

The plant C. paniculatum turned into gathered from Pathanamthitta district of Kerala and diagnosed by Thomas Mathew, HOD of Botany, Marthoma College Tiruvalla, Kerala. Voucher no. VSCp-14, have been deposited in the Pharmacognosy branch, Pushpagiri College of pharmacy, Tiruvalla. The portion of the plant had been washed with water to do away with soil and other material and dried in shade for 20 days, powdered, extracted 500gm with ethanol (EECp) through cold extraction to yield the respective extract. The extracts were decreased to molten mass by using rotary vacuum evaporator and the yield turned into 21%tw/w preliminary phytochemical screening turned into carried out as in step with fashionable method and numerous phytochemical materials were recognized [6,7]

Preparation of Phytosome

Accurately weighed quantity of phosphatidylcholine and cholesterol were dissolved in 10 ml of chloroform in spherical flask and sonicated for 10 min the usage of tub sonicator. Natural solvent removal is finished by Rotary evaporator (forty five-50°C). After whole elimination of solvent thin layer of phospholipids combination was formed. This film became hydrated with ethanolic extract of Clerodendron paniculatum root in rotary evaporator (37-40°C for 1 hour). After hydration, mixture of lipid and plant extract turned into sonicated for 20 minutes in presence of ice bathtub for heat dissipation. Then organized phytosomes were stuffed in amber colored bottle and stored in freezer (2-80C) till used[8]

Evaluation of phytosome.

1. Determination of % yield:

2. Visualisation-

The morphology of phytosomes was observed by digital microscopy, transmission electron microscope.

i. Digital microscopy-

Phytosome formulation shaken in distilled water and viewed under digital microscope at 400X objective lens.

ii. TEM analysis-

The complex was shaken in distilled water and viewed using Transmission Electron Microscope

Determination of entrapment efficiency

Phytosome complex of extract was diluted 1-fold with 10 ml of methanol and then centrifuged at 18,000 rpm for 1/2 h at -4oC using cooling centrifuge machine. The supernatant was isolated and the amount of free extract was determined by UV/Vis spectroscopy at 269 nm. To determine the total amount of extract, 0.1 ml of the extract phospholipid suspension was diluted in methanol, adjusting the volume to 10 ml.

The Entrapment efficiency was calculated according to the following formula: [15-16]

Entrapment efficiency (%) = (Total amount of drug) – (amount of free drug) / (Total amount of drug) × 100

Evaluation of *In Vivo* anti cancer activity of formulated phytosome against DLA tumour cells:

Induction of cancer using DAL cells

Dalton's Lymphoma ascites (DAL) cell was supplied by Amala cancer research center, Trissur, Kerala, India. The cells maintained ***in vivo*** in Swiss albino mice by intra peritoneal transplantation. While transforming the tumor cells to the grouped animal the DAL cells were aspirated from peritoneal cavity of the mice using saline. The cell counts were done and further dilutions were made so that total cell should be 1 x 10^6; this dilution was given intra peritonealy. Let the tumor grow in the mice for minimum seven days before starting treatments.

Animals

Male Swiss albino mice (20-25 gm) Animal Ethical Committee no PCP/2013/IAEC/602/02 were produced from animal experimental laboratory, and used throughout the study. They were housed in micro nylon boxes in a control environment (temp 25±2°C) and 12 hours dark /light cycle with standard laboratory diet and water *ad libitum.* The study was conducted after obtaining institutional animal ethical committee clearance. As per the standard practice, the mice were segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygienic environment in our animal house.

Treatment Protocol

Swiss Albino mice were divided into five group of six each. All the animals in four groups were injected with DAL cells (1×10^6 cells per mouse) intra peritoneal, and the remaining one group is normal control group.

IIIIIIII© International Journal of Pharma Research and Health Sciences. All rights reserved

Treatment

In this study, drug treatment was given after the 24 hrs of inoculation, once daily for 14 days. On day 14, after 24 hrs. The last dose, all mice from each group were sacrificed; blood was withdrawn from each mouse by retro orbital plexus method and the following parameters were checked.

Cancer cell count

The fluid (0.1ml) from the peritoneal cavity of each mouse was withdrawn by sterile syringe and diluted with 0.8 ml of ice cold Normal saline or sterile Phosphate Buffer Solution and 0.1 ml of tryphan blue (0.1 mg/ml) and total numbers of the living cells were counted using heamocytometer.

Group	Treatment group
1	DLA Control
11	DLA + 5FU (20mg/kg)
111	DLA + 200mg of EECi
1V	DLA +40 mg of isolated compound
V	DLA +40mg of Phytosome developed

Hematological parameters:

WBC count

Total WBC count was found to be increased in cancer control, when compared with normal and treated tumor-bearing mice. The total WBC count was found to decrease significantly in animals treated with extract when compared with cancer control.

RBC and Hb

RBC and Hb content decreases with tumor bearing mice when compared with Normal control mice.

Percentage increase in life span (ILS)

% ILS was calculated by the following formula

Life span of treated group

$$\%ILS = \frac{Life\ span\ of\ treated\ group}{Life\ span\ of\ control\ group} - 1 \times 100$$

- All biochemical investigations were done by using COBAS MIRA PLUS-S Auto analyzer from Roche Switzerland.
- Hematological test are carried out in COBAS MICROS OT 18 from Roche.
- Newly added Hi-Tech instruments MAX MAT used for an auto analyzer for all biochemistry investigations in blood sample.

Effect of extract and the formulated phytosome on Survival Time

Animals were divided into five groups of six animals each. Except the normal control group, the remaining groups were inoculated with DAL cells ($1x10^6$cells/mouse) intraperitonealy on day '0' and treatment with extract started 24 hrs after inoculation, at a dose of 200mg and 400mg/kg/day. *p.o.* The normal and tumor control group was treated with same volume of 0.9% sodium chloride solution. All the treatments were given for fourteen days. The increase in life span (ILS) of each group, consisting of 6 mice was noted.

The antitumor efficacy of extract and the formulated Phytosome was compared with that of 5-fluorouracil (Dabur pharmaceutical ltd. India; 5-FU, 20 mg/kg/day, *i.p*, for 14 days). The ILS of the treated groups was compared with that of the control group using the following calculation:

Increase in lifespan = [(T - C) / C] x100

Where T = number of days the treated animal survived.

C = number of days control animals survived.

Statistical analysis

All the experimental data are expressed as the mean SEM. The data was statistically analyzed by using one way Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test .

3. RESULTS AND DISCUSSION

Preparation of phytosomes

Solvent evaporation method was used , percentage yield was found to be 89.81%,entrapment efficiency 96.4 ± 0.7

Evaluation of phytosome:

Visualisation

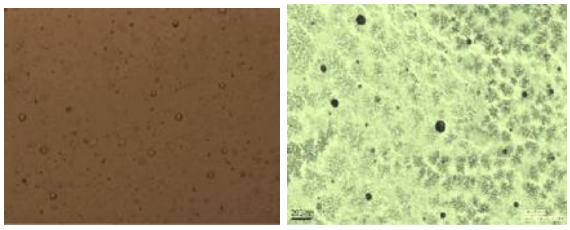

Fig 1: Digital micropic, TEM view of phytosome

Table 1: Anticancer activity of of *Clerodendron paniculatum Linn* ethanolic extract, Isolated compound,,Formulated phytosome using isolated compound on tumor volume, viable tumor cells count and non viable tumor cells count.

S. No	Treatment group	Tumor volume (ml)	Viable tumor cells count (10^6 cells/mouse)	Non viable tumor cells (10^6 cells/mouse)
1	DLA Control	3.81 ± 0.18	8.55 ± 0.28	0.35 ± 0.07
2	DLA + 5FU (20mg/kg)	1.81 ± 0.17*	0.87 ± 0.18 *	3.09 ± 0.15 *
3	DLA + 200mg of EECi	1.30 ± 0.05 *	1.10 ± 0.16 *	2.37 ± 0.16*
4	DLA +40 mg of isolated compound	0.39±0.01	0.42± 0.08*	5.10 ± 0.22 **
5	DLA +40mg of Phytosome developed	0.61±0.13	0.68± 0.05*	5.91 ± 0.11 **

Statistical significance (p) calculated by one wayANOVA followed by Dunnett's test, *p<0.01 calculated by comparing treated groups with DLA Control

Table 2: Anticancer activity of *Clerodendron paniculatum Linn ethanolic* extract, Isolated compound,Formulated phytosome using isolated compound on Mean survival time and Percentage increase in life span

S. No	Treatment group	Mean survival time (in days)	Percentage increase in life span
1	DLA Control	21.60 ± 0.47	-
2	DLA + 5FU (20mg/kg)	31.91 ± 0.90	95.34

IIIIIIII© International Journal of Pharma Research and Health Sciences. All rights reserved

3	DLA + 200mg of EECi	38.03 ± 0.39	74.54
4	DLA +40 mg of isolated compound	44.12 ± 0.05 *	98.10
5	DLA +40mg of Phytosome developed	41.01± 0.05 *	92.3 3

Statistical significance (p) calculated by one way ANOVA followed by Dunnett's test, *p<0.01 calculated by comparing treated groups with DLA Control

Table 3: Haematological parameters of *Clerodendron paniculatum Linn ethanolic* extract, Isolated compound,Formulated phytosome using isolated compound treated mice bearing DAL cell line

Treatment group	Hb(g%)	RBC	WBC	Differential count (%)		
				Lymphocytes	Neutrophils	Monocytes
DLA Control	3.14 ± 0.38*	4.13 ±0.24*	5.19 ± 0.18*	76.80±0.47	29.51±0.27	1.9±0
DLA + 5FU (20mg/kg)	12.14 ± 0.76	8.39 ± 0.49	6.09 ± 1.12	61.24 ± 0.42	23.2±0.42	1.2±0
DLA + 200mg of EECi	7.21 ± 0.41 *	9.11 ±0 .96*	10.00 ± 0.60*	40.42 ± 0.36	19.52±0.35	1.4±1.1
DLA +40 mg of isolated compound	1.31± 0.06 *	1.01± 0.04*	1.30± 0.06 *	64.16 ± 0.42	27.88±0.41	1.2±0
DLA +40mg of Phytosome developed	3.23± 0.02 *	3.72± 1.02 *	4.38± 0.02 *	60.21 ± 0.42	28.2±0.42	2.1±0

Statistical significance (p) calculated by one way ANOVA followed by Dunnett's test, *p<0.01 calculated by comparing treated groups with DLA Control

Effect on Tumor Growth

The effect of EECp and formulated phytosome using isolated compound Tumor volume (ml) Viable tumor cells count (106 cells/mouse Non viable tumor cells (106 cells/mouse, Mean survival time (in days), Percentage increase in life span, Haematological parameters were observed and shown in Table -.Developed Phytosome using isolated compound possess better anti cancer activity

4. CONCLUSION

From above studies we are concluded that phytosomes has better physical characteristics than that of extract. In-vivo studies revealed that phytosomes showed more anticanceractivity than that of 5-fluorouracil

5. ACKNOWLEDGEMENT

The authors acknowledge Karpagam University, Coimbatore and management, for the support.

6. REFERENCES

1. Manach C, Scalbert A and Morand C: Polyphenols food sources and bioavailability. Am J Clin Nutr 2004; 79:727-747.
2. Bombardelli E, Curri SB, Loggia Della R, Del NP, Tubaro A and Gariboldi P: Complexes between phospholipids and vegetal derivatives of biological interest. Fitoterapia 1989; 60:1-9.
3. Nandkarni KM, Nandkarni AK and Chopra RN: Indian MateriaMedica, Popular Prakasan, Bombay, 3rd Ed, 1954.
4. Vaidyaratnam P.S Varier: Indian Medicinal plants Volume2, AryaVaidyasala 124-125.
5. Priya K, Krishankumari S, Vijaykumar M Cyathula prostrata: A potent source of anti cancer agent against Dalton's ascites in Swiss albino mice. Asian Pacific J Trop Med 2013; 6: 776-779.
6. Patel J, Patel R, Khambholja K, Patel N, "An overview of phytosomes as an advanced herbal drug delivery system", Asian Journal of Pharmaceutical Sciences, 2009, 4 (6); 363-371.
7. Patel S., In-vitro cytotoxicity activity of Solanum nigrum extract against hela cell line and vero cell line, International Journal of Pharmacy and Pharmaceutical Sciences, 2009, 1(1);38-46.

Conflict of Interest: None

Source of Funding: Nil

IIIIIIII© International Journal of Pharma Research and Health Sciences. All rights reserved