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Comparative study on anti-cancer activities of Phytosome formulated from the root extract of *Clerodendron infortunatum Linn and Clerodendron paniculatum Linn* root

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ABSTRCT

Formulation and assessment of anti most cancer interest of *Clerodendron infortunatum* Linn *Clerodendron paniculatum* Linn, from the root extract by DAL cells. The foundation quantities of the *Clerodendron infortunatum* Linn and *Clerodendron paniculatum* extracted with ethanol by means of cold maceration process. The extracts had been vacuum dried and subjected to phytochemical screening for the detection of numerous phyto components. The formulated Phytosome from the extract exhibited activity towards cancer cells. The observation well-known shows that *Clerodendron infortunatum* Phytosome possess higher anti-cancer activity than the *Clerodendron paniculatum* extract.

KEY WORDS: *Clerodendron infortunatum* Linn root, *Clerodendron paniculatum*, anticancer pastime, DAL cells.

1. INTRODUCTION

Introduction of flora or their parts had been extensively used in medication seeing that historical instances and until nowadays use of Phytomedicines is vast. Maximum of the biologically active parts of vegetation are polar or water-soluble. but, water-soluble phytoconstituents like flavonoids, tannins, glycosides, aglycones etc. are poorly absorbed both due to their huge molecular length, which can't be absorbed via passive diffusion or due to their terrible lipid solubility, as a consequence critically proscribing their ability to move across lipid-wealthy biological membranes, ensuing of their bad bioavailability. Phytosome is a newly brought patented era developed to include the standardized plant extracts or water-soluble phytoconstituents into phospholipids to supply lipid compatible molecular complexes, which improves their absorption and bioavailability. *Clerodendron infortunatum* Linn, (family: Verbenaceae), was a species located in India, on this pronounced as people remedy for tumours, leprosy, fever, infection. The roots were mentioned to possess laxative, diuretic, analgesic, and anti-inflammatory, anti-cancer anti-bacterial activities. To our expertise there had been no medical reports on anti tumour activities of Phytosome formulated from *Clerodendron infortunatum* Linn, extract the key goal of the present look at is to expand the Phytosome of *Clerodendron infortunatum* Linn and *Clerodendron paniculatum* root extract to increase the solubility and bioavailability of drug. To prepared the Phytosome of *Clerodendron infortunatum* Linn, *Clerodendron paniculatum* by specific technique and compare its anti cancer activities with the aid of assessing tumour quantity, feasible and nonviable tumour cell, tumour weight, haematological parameters and biochemical estimations against DLA tumour cells.

2. MATERIALS AND METHODS

Plant Material and Extraction: The plant species *C. infortunatum, C. paniculatum* collected from Pathanamthitta district of Kerala and identified with the aid of Thomas Mathew, HOD of Botany, Marthoma College Tiruvalla, and Kerala. Voucher no. VSCI-13, VSCP-14 have been deposited in the Pharmacognosy department, Pushpagiri College of pharmacy, Tiruvalla. The root part of the plants were washed with water to dispose of soil and other count and dried in sunlight for 20 days, powdered, extracted 500gm with ethanol (EECI), EECp by using cold extraction to yield the respective extracts. The extracts have been reduced to molten mass by way of rotary vacuum evaporator and the yield was 18%w/w, 24% w/w initial phytochemical screening for detection of various phytochemical materials have been identified.

Preparation of Phytosome: Appropriately weighed amount of phosphatidylcholine and cholesterol have been dissolved in 10 ml of chloroform in spherical bottom flask and sonicated for 10 min using bath sonicator. Organic solvent removal is executed by means of Rotary evaporator (45-50°C). After complete elimination of solvent thin layer of phospholipids mixture formed. This became hydrated with ethanolic extract of Clerodendron infortunatum root, *Clerodendron paniculatum* root in rotary evaporator (37- 40°C for 1 hour). After hydration, mixture of lipid and plant extracts became sonicated for 20 minitues in presence of ice tub for heat dissipation. Then prepared phytosomes had been filled in amber coloured bottle and stored separately in freezer (2-8^{0}C) until used.

Assessment of phytosome:

Determination of % yield: (%) Yield = (sensible yield) × one hundred (Theoretical yield)

Visualisation: The morphology of phytosomes became observed by digital microscopy, transmission electron microscope.

Digital microscopy: Phytosome components shaken in distilled water and viewed beneath digital microscope at 400X goal lens.

TEM analysis: The complex changed into shaken in distilled water and considered the usage of Transmission Electron Microscope.

Determination of Entrapment Performance: Phytosome complex of extract changed into diluted 1-fold with 10 ml of methanol and then centrifuged at 18,000 rpm for half of hour at -4°C the usage of cooling centrifuge device. The supernatant become remoted and the amount of unfastened extract turned into decided by way of UV/Vis spectroscopy at 269 nm. To decide the entire quantity of extract, 0.1 ml of the extract phospholipid suspension becomes diluted in methanol, adjusting the extent to 10 ml. The Entrapment efficiency turned into calculated consistent with the following system:

$$\text{Entrapment efficiency (\%)} = \frac{\text{(overall amount of drug)} - \text{(quantity of loose drug)}}{\text{(Total amount of drug)}} \times \text{one hundred}$$

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

Induction of cancer the use of DAL cells: Daltons Lymphoma ascites (DAL) supplied through Amala cancer research, Trissur, Kerala, India. The cells maintained in vivo in Swiss albino mice via intra peritoneal transplantation. At the same time as transforming the tumour cells to the grouped animal the DAL cells had been aspirated from peritoneal cavity of the mice the usage of saline. The cell counts have been carried out and similarly dilutions have been made so that overall mobile must be 1×10^6; this dilution become given intra peritoneal. Allow the tumour develop within the mice for minimal seven days before beginning treatments.

Animals: Male Swiss albino mice (20-25 gm) Animal moral Committee no PCP/2013/IAEC/602/02 have been created from animal experimental laboratory, and used in the course of the study. They had been housed in micro nylon packing containers in a control surroundings (temp. 25±2°C) and 12 hours darkish /light cycle with popular laboratory eating regimen and water ad libitum. The look at became conducted after acquiring institutional animal ethical committee clearance. As in line with the usual exercise, the mice have been segregated based totally on their gender and quarantined for 15 days earlier than the commencement of the test. They have been eaten up healthful eating regimen and maintained in hygienic environment in our animal residence.

Protocol for *Clerodendron infortunatum* root: Swiss Albino mice had been divided into five group of six each. All the animals in four groups have been injected with DAL cells (1×10^6 cells consistent with mouse) intra peritoneal, and the closing one institution is ordinary control group.

G1 served as the normal manage.
G2 served as the tumour manage. Group1 and Group 2gets ordinary diet and Water.
G3 Served with injection 5-FU (20mg/kg) Intra peritoneal.
G4 Served as a low dose treatment turned into administered EECi (200mg/kg, n=6)
G5 Served as a high dose treatment and was administered EECi (400mg/kg, n=6)
G5 Served as a excessive dose treatment control and become administered with formulated Phytosome using extract (20mg/kg)

Protocol *Clerodendron paniculatum* root: Swiss Albino mice had been divided into 5 institution of six every. All the animals in four businesses had been injected with DAL cells (1×10^6 cells in line with mouse) intra peritoneal, and the ultimate one organization is normal control group.

G1 served as the regular control.
G2 served because the tumour manage. Group1 and a pair of receives normal food plan and Water.
G3 Served as the fine manipulate; changed into treated with injection 5-FU (20mg/kg) Intra peritoneal.
G4 Served as a low dose remedy manipulate and became administered EECp (200mg/kg, n=6)
G5 Served as a high dose remedy control and became administered EECp (400mg/kg, n=6)
G5 Served as a high dose treatment control and become administered with formulated Phytosome the use of extract (20mg/kg)

Treatment: On this look at, drug treatment become given after the 24 hrs of inoculation, as soon as daily for 14 days. On day 14, after 24 hrs. The ultimate dose, all mice became sacrificed; blood became withdrawn from each mouse by means of orbital plexus method and the following parameters have been checked.

Haematological parameters: White blood cells (WBC), red blood cells (RBC), Haemoglobin content material (Hb), Platelet rely, Packed cellular extent (PCV).

Serum enzyme and lipid profile: general ldl cholesterol (TC), Triglycerides (TG), Aspartate amino Transferase (AST), Alanine amino Transferase (ALT), Alkaline Phosphatase (ALP).

Derived parameter: Body weight, Life span (%), cancer cell matter.

Cancer cellular count: The fluid (0.1ml) from the peritoneal hollow space of every mouse changed into withdrawn by means of sterile syringe and diluted with 0.eight ml of ice cold regular saline or sterile Phosphate Buffer solution and zero.1 ml of tryphan blue (zero.1 mg/ml) and general numbers of the dwelling cells were counted the use of haemocytometer.

Haematological parameters:

WBC: Total WBC be counted changed into located to be elevated in most cancers manipulate, when compared with everyday and handled tumour-bearing mice. The total WBC rely was observed to decrease substantially in animals handled with extract while as compared with cancer manipulate.

RBC and Hb: RBC and Hb content decreases with tumour bearing mice whilst compared with ordinary control mice.

Platelets: In Hodgkin lymphoma, boom in platelet remember was often said in laboratory findings. Subsequently, we investigated this parameter within the observe

Packed cell extent: Anyhow of anaemia the packed mobile extent decreases.

Derived parameters

Body weight: All of the mice had been weighed, from the start to the 15th day of the have a look at. Common growth in frame weight on the 15th day became determined.

Percent increase in life span (ILS): All biochemical investigations were done by using COBAS MIRA PLUS-S vehicle analyzer from Roche Switzerland. Haematological test are finished in COBAS MICROS OT 18 from Roche. Newly introduced hello-Tech gadgets MAX MAT used for an automobile analyzer for all biochemistry investigations in blood sample.

Impact of extract and the formulated Phytosome on Survival Time: Animals had been divided into five companies of six animals each. except the regular manage group, the closing agencies had been inoculated with DAL cells (1x106cells/mouse) intraperitonealy on day 'zero' and remedy with extract started 24 hrs after inoculation, at a dose of 200mg and 400mg/kg/day. p.o. The regular and tumour manage group become handled with same volume of 0.9% sodium chloride answer. All of the treatments were given for fourteen days. The boom in existence span (ILS) of every organization, which include 6 mice turned into cited.

The antitumor efficacy of extract and the formulated Phytosome changed into as 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 the usage of the subsequent calculation:

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

In which T = variety of days the handled animal survived, C = wide variety of days manipulate animals survived.

Statistical analysis: All the experimental facts are expressed as the mean SEM. The records was statistically analyzed by using the use of one manner evaluation of Variance (ANOVA) observed with the aid of Dennett's test

Preparation of phytosomes: Solvent evaporation approach became used, percentage yield become determined to be 87.85%, entrapment performance 95.6 ± 0.7, 93.6 ± 05.

Assessment of Phytosome:

Visualisation: Digital microscopic, TEM view of Phytosomes.

Table.1. The effect of EECi, and formulated phytosome on heamatological parameters

Parameters	G_1	G_2	G_3	G_4
Tumour Volume	-	3.01 ± 0.18	1.81 ± 0.17b,*	1.17 ± 0.15 b,*
Tumour weight	-	2.92 ± 0.21	1.33 ± 0.14 b,*	0.98 ± 0.07 b,*
Viable cell	-	8.55 ± 0.28	3.67 ± 0.18 b,*	1.40 ± 0.16 b,*
Non Viable cell	-	0.35 ± 0.07	1.29 ± 0.15 b*	2.57 ± 0.18 b,*
MST (days)	-	21.6 ± 0.47	30.7 ± 0.90	37 ± 0.39
%ILS	-	00	42.87	74.41
RBC	6.08 ± 0.06	3.14 ± 0.38a*	4.13 ±0.24 b,*	5.19 ± 0.18 b,*
WBC	5.18 ± 0.06	12.14 ± 0.76 a*	8.39 ± 0.49 b,*	6.39 ± 1.12 b,*
Haemoglobin	13.18 ± 0.06	7.21 ± 0.41 a*	9.18 ± 0.96 b,*	10.93 ± 0.60 b,*

Parameters	G5	G6
Tumour Volume	0.31± 0.05 b,*	0.31± 0.05 b,*
Tumour weight	0.21± 0.05 b*	0.41± 0.05 b,*
Viable cell	0.43± 0.08 b*	0.48± 0.05 b,*
Non Viable cell	0.31± 0.13 b*	
MST (days)	42.21 ± 0.05 b*	40.01± 0.05 b*
%ILS	98.10	82.13
RBC	1.31± 0.06 b*	1.11± 0.04 b*
WBC	4.31± 0.02 b*	4.81± 1.02 b*
Haemoglobin	11.31± 0.05 b	12.89± 0.02 b*

Group 1- Normal control (5ml/kg),Group 11- DLA Control (2x10^6cell/ ml), Group 111- DLA+EECi (200mg/kg), Group 1V- DLA+EECi, (400mg/kg), Group V- DLA+Formulated Phytosome (20mg) Group V1- DLA+5-FU (20mg/kg) Values are represented as mean ±SEM, where n=6, a P<0.01 as compare to normal control

Table.2. The effect of EECp, and formulated Phytosome on heamatological parameters

Parameters	G_1	G_2	G_3	G_4
Tumor Volume	-	3.01 ± 0.18	1.81 ± 0.17b,*	1.17 ± 0.15 b,*
Tumor weight	-	2.92 ± 0.21	1.33 ± 0.14 b,*	0.98 ± 0.07 b,*
Viable cell	-	8.55 ± 0.28	3.67 ± 0.18 b,*	1.40 ± 0.16 b,*
Non Viable cell	-	0.35 ± 0.07	1.29 ± 0.15 b*	2.57 ± 0.18 b,*
MST (days)	-	21.6 ± 0.47	30.7 ± 0.90	37 ± 0.39
%ILS	-	00	42.87	74.41
RBC	6.08 ± 0.06	3.14 ± 0.38a*	4.13 ±0.24 b,*	5.19 ± 0.18 b,*
WBC	5.18 ± 0.06	12.14 ± 0.76 a*	8.39 ± 0.49 b,*	6.39 ± 1.12 b,*
Hemoglobin	13.18 ± 0.06	7.21 ± 0.41 a*	9.18 ± 0.96 b,*	10.93 ± 0.60 b,*

Parameters	G5	G6
Tumor Volume	0.30± 0.05 b,*	0.35± 0.05 b,*
Tumor weight	0.19± 0.05 b*	0.41± 0.05 b,*
Viable cell	0.43± 0.08 b*	0.48± 0.05 b,*
Non Viable cell	0.31± 0.13 b*	
MST (days)	42.21 ± 0.05 b*	40.01± 0.05 b*
%ILS	90.10	83.13
RBC	1.31± 0.06 b*	1.11± 0.04 b*
WBC	15.31± 0.02 b*	4.81± 1.02 b*
Hemoglobin	13.31± 0.05 b	12.89± 0.02 b*

Group 1- Normal control (5ml/kg), Group 11- DLA Control (2x106cell/ ml), Group 111 -DLA+EECp (200mg/kg), Group 1V- DLA+EECp, (400mg/kg), Group V- DLA+Formulated phytosome (20mg) Group V1- DLA+5-FU (20mg/kg) Values are represented as mean ±SEM, where n=6, a P<0.01 as compare to normal control b P<0.01 as compare to DLA control.

Impact on Tumour Growth: The effect of EECi, and formulated phytosome on tumour growth responses had been discovered and proven in table -1. Within the DAL tumour, the common life span of animals become determined to be 48% while, 200, and 400 mg/kg of EECi and formulated Phytosome confirmed growth in life span to 42.87, 74.41 and 98.10% respectively. The impact of EECp and formulated phytosomes on tumour increase responses have been determined and shown in table.1. In the DAL tumour manage institution, the average existence span of animals became discovered to be 48% whereas, 200, and 400 mg/kg of EECp, and formulated Phytosome confirmed growth in life span to 42.87, and 90.10%.

3. CONCLUSION

From above studies we're concluded that phytosomes has better bodily characteristics than that of extract. In-vivo research found out that phytosomes of EECi showed extra anticancer that phytosomes of EECp, and of 5-fluorouracil.

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