ejpmr, 2016,3(4), 253-262

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

EVALUATION OF ANTIOXIDANT, CYTOTOXIC AND ANTICANCER EFFECTS OF *CLEOME VISCOSA LINN*

Jayaprakash A. P.*, Krishnakumar K. R., Dr. K.K. Srinivasan, Dr. Jyoti Harindran, Shan P. Mohammed

Department of Pharmaceutical Sciences, Mahatma Gandhi University, RIMSR, Rubber board P.O, Kottayam-686009, India.

***Correspondence for Author: Jayaprakash A. P.**

Department of Pharmaceutical Sciences, Mahatma Gandhi University, RIMSR, Rubber board P.O, Kottayam-686009, India.

Article Received on 29/01/2016 **Article Revised on 20/02/2016** **Article Accepted on 11/03/2016**

ABSTRACT

From time immemorial, natural products, including plants, animals and minerals have been the basis of treatment of human diseases. Plants, in particular, have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies. Plant-based medicines initially dispensed in the form of crude medicines such as tinctures, teas, poultices, powders, and other herbal formulations. In this present context, one traditional medicinal plant namely *Cleome viscosa* was selected and its antioxidant effect by DPPH, ABTS and Nitric oxide scavenging assay methods, cytotoxic and *in vitro* anticancer effects in different fractions of solvent were evaluated phytoconstituents.The results of studies show that the plant has good antioxidant activity low cytotoxic effect and good anticancer properties.

KEYWORDS: *Cleome viscosa***,** cytotoxic, DPPH, ABTS.

INTRODUCTION

Traditional medicine is an evolutionary process as communities and individuals continue to discover new techniques to heal the aliment that can renovate into medical practices. We should credit our forefathers with the wisdom, which identified the species with medicinal properties. The information regarding specific plants to be used and the methods of their application in different pathological conditions were passed down by word of mouth. However, with the advent of the development of written communication, these were eventually recorded in texts. Ayurveda remains one of the most ancient and yet living traditions practiced widely in India, Sri Lanka and other countries and has a sound philosophical and experiential basis[1]

The word cancer was derived from the Greek word karkinos (crab or crayfish).This name comes from the appearance of the cut surface of a solid malignant tumor, with "the veins stretched on all sides as the animal the crab has its feet, whence it derives its name.Celsus (ca. 25 BC – 50 AD) translated karkinos into the Latin cancer, also meaning crab. Cancer includes a large number of distinct diseases that are characterized by abnormal growth of cells out of control. The phenotypic abnormality that is virtually pathogenomonic of all cancer cells is dysregulation of cell cycle controls, including a lack of appropriate responses to the stimuli that normally inhibit the cell cycle and a lack of a cellular death program to the appropriate signals and stresses.

One of the most significant examples of plant derived compound used in cancer treatment is the vinca alkaloid family isolated from the periwinkle Catharanthus roseus**,** which is found in the rain forests of Madagascar. The introduction of the vinca alkaloid "vincristine" was responsible for an increase in cure rates for Hodgkin's disease and some forms leukemia.[2] Another example of a highly active anticancer agent derived from a natural product is etoposide, derived from the mandrake plant Podophyllumpeltatumand the wild chervil Podophyllum emodi.[3] It has produced high cure rates in testicular cancer when used in combination with bleomycin (also derived from a natural product) and cisplatin[4] and has significant activity against small-cell lung carcinoma[5]

In this present context, we selected a traditional medicinal plant namely *Cleome viscosa*. *Cleome viscosa* belongs to the family Capparaceae, the plant is an annul, sticky herb with a strong penetrating odour and clothed with gladular and simple hairs; leaves 3-5 foliate, gradually becoming shorter upwards; flowers yellow in lax raceme; fruits capsules, compressed, hairy throughout; seeds brownish black when ripe. This plant was reported to be highly useful in treating different ailments in traditional medicine.*Cleome viscosa* had been reported for various biological properties like antioxidant, cytotoxic, antimicrobial, hyper-lipidemic etc. Interestingly other plants from the same familiy have been proved to be potential for different biological activities including antitumor properties.[6] From literature review, it is understood that *Cleome viscosa*

was found to have a variety of phytoconstituents which are biologically active. So, in the present study its antioxidant effect by DPPH, ABTS and Nitric oxide scavenging assay methods, cytotoxic and *in vitro* anticancer effects in different fractions of solvent were evaluated.

MATERIALS AND METHODS

Plant material

All the plant materials were collected from different places in and around of Kottayam, Kerala in the month of September 2010. *Cleome viscosa* (Capparaceae) whole plant was collected (5 kg), root part was washed with plain water.The whole plant material was authenticated by Dr. K.V. George, Professor of Botany, CMS College, Kottayam,Kerala.The voucher specimens of C. viscosa (CVW/JP/UCP/CMS/508) is deposited in CMS college, Kottayam and UCP Cheruvandoor. The collected plant materials were shade dried, powdered by using ball mill and used for further extraction process[7].

Preparation of extract

The powdered shade dried material LCL (400 g each) was soxhleted in 8 batches using 70% ethanol (2.5 L). The sox let extraction was performed for 40 cycles. Extraction of the shade dried whole plant material of CVW was performed in the same manner. The solvent was evaporated under reduced pressure to get the dry extract. Yield of the extract was calculated by using the formula given below and result tabulated in table.

$$\% \text{ of Yield} = \frac{\text{Weight of dried extract}}{\text{Weight of fresh material}} \text{ X } 100$$

The extract were subjected to various phytochemical screening test to confirm the active constituents

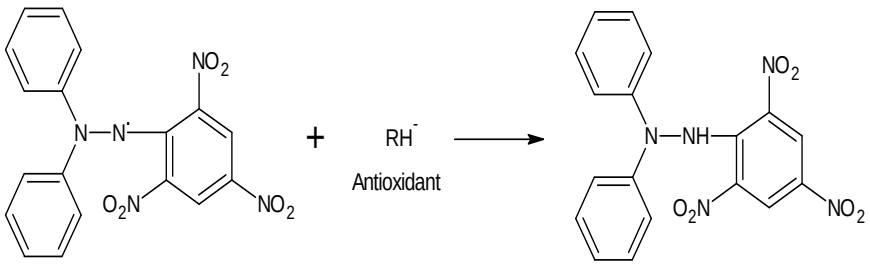
Selection of animals

Healthy swiss albino mice of both sexes weighing between 20-25 gm was collected from Animal house, Aptus Biosciences Pvt Ltd, Mahabubnagar, Andra Pradesh(APTUS/IAEC/01/081/2013). All the animals were fed with standard diet and water ad libitum. Before the commencement of the experiment (12h), the animals were deprived of food but not water.

Antioxidant studies

DPPH scavenging assay

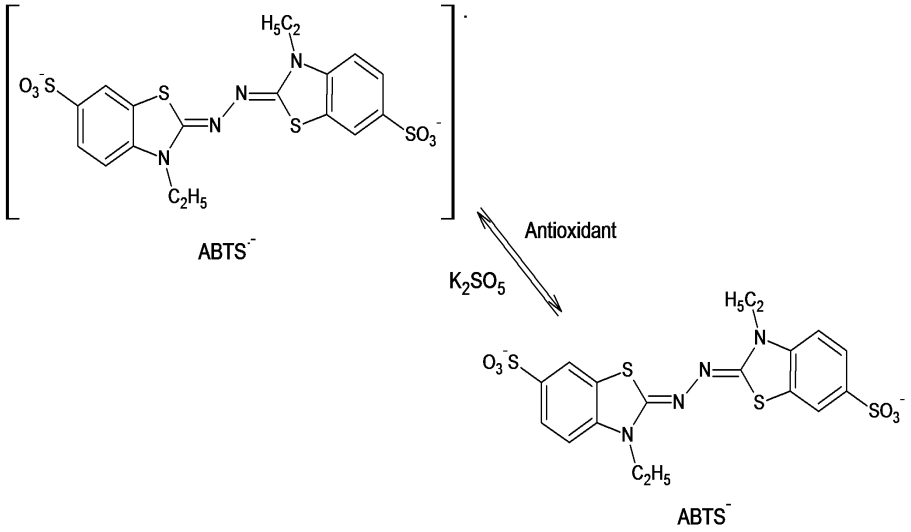
2, 2-diphenyl-l-picrylhydrazyl (DPPH) is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the methanolic solution of DPPH shows a strong absorption band at 517 nm. DPPH radicals react with various electron-donating molecules (reducing agents or antioxidants) when electrons become paired off leading to the bleaching of the solution. This occurs as a result of the formation of the colourless 2,2-diphenyl-l-picryl hydrazine. Reduction of the DPPH radicals can be estimated quantitatively by measuring the decrease in absorbance at 517 nm[8]



2, 2-diphenyl-l-picrylhydrazyl (purple) 2, 2-diphenyl-l-picrylhydrazine (colourless)

ABTS radical anion scavenging assay

ABTS is chemically 2, 2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid). The ABTS assay is based on the inhibition of the absorbance of the radical anion ABTS", which has a characteristic long wavelength absorption spectrum. The ability of the test compounds to scavenge the ABTS radical anion is measured at 734 nm.



Nitric oxide Radical scavenging activity

This assay relies on a diazotization reaction that was originally described by Griess in 1879. Modifications have been made to the original reaction through the years. This procedure is based on the chemical reaction shown below, which uses sulfanilamide and naphthylethylenediamine dihydrochloride (NED) under acidic conditions. Sulfanilamide and NED compete for nitrite in the Griess reaction. This reagent detects NO_2^- in a variety of biological and experimental liquid samples such as plasma, serum, urine and tissue culture medium. The limit of detection is 2.5 µM (125pmol) nitrite (in distilled, deionized water); however, the sensitivity will vary depending upon the sample used. Individual researchers must determine the limits for their individual experiments and referred to the absorbance at standard solutions of potassium nitrite, treated in the same way with Griess reagent.

***In vitro* cytotoxicity studies**

Chemicals

3-(4,5–dimethylthiazol–2–yl)–5–diphenyltetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. DimethylSulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India

Cell lines and Culture medium

Vero, MCF-7, Pc3 and HeLa cell lines was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 g/mL) and amphotericin B (5 g/mL) in an humidified atmosphere of 5% CO_2 at 37^0C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions

For cytotoxicity studies, each weighed test drug was separately dissolved in distilled DMSO and the volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/mL concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies

Determination of cell viability by MTT Assay

Principle: The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/mL using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 mL of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hr, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37^o C for 3 days in 5% CO_2atmosphere, and microscopic examination was carried out and observations were noted every 24 hr interval. After 72 hr, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and

incubated for 3 h at 37° C in 5% CO_2 atmosphere. The supernatant was removed and 100 l of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line[9]

$$\% \text{ growth inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \right) \text{X } 100$$

***In vivo* anticancer studies**

Mice and tumor system

Female Swiss albino mice (20±2 g, 6–8 weeks old) were used for acute toxicity and anticancer study. Mice were housed in open top cages and maintained on food and water ad labium. Room temperature was maintained at 22±2 ◦C with light and dark cycle of 14/10 h. Dalton's lymphoma ascites (DLA) is maintained in ascetic form by serial transplantation in Swiss albino mice or *in vitro* cell culture system by serial passage. Irrespective of whether the cells are obtained from *in vitro* culture or from ascetic fluid they exhibited typical phenotypic features.

Preparation of suspensions and solutions

The test drugs and standard 5-Fluorouracil (5-FU, procured from Ranbaxy Laboratories, New Delhi) were suspended in distilled water using sodium carboxy methyl cellulose (0.3%) and administered orally to the animals with the help of an intragastric catheter.

Evaluation of antitumor activity of test drugs in Dalton's lymphoma-bearing mice model

The animals, total of 84 were divided into seven groups (Twelve mice in each group) Group 1-Group 7. Under sterile condition, about 0.5 mL of DLA cell suspension (2×10^6cells/mL) was inoculated intraperitoneally to each mouse of Group 2 to Group 7 (total of 72) at day zero. After two days tumor inoculation the animals were treated as fallows,

Group 1: Normal control and received sodium CMC suspension (0.3%).
Group 2: tumor control and received sodium CMC suspension (0.3%).
Group 3: Positive control was treated with 5-fluorouracil (20 mg/kg, p.o).
Group 4: Treated with CVW (250 mg/kg, p.o) in sodium CMC suspension
Group 5: Treated with CVW (500 mg/kg, p.o) in sodium CMC suspension

The treatment was continued for the next 10 days. On day 11th day, i.e. after the last dose and 24 h fasting, six mice from each group were sacrificed for the study of antitumor and hematological parameters. The rest of the animals were kept to check the average life span and change in the body weight. Blood was collected from the animals by retro-orbital puncture under slight anesthetic (diethyl ether) condition and the hematological parameters such as RBC, WBC, differential count and hemoglobin were studied. The significance of the *in vivo* data was analyzed by one-way analysis of variance (ANOVA) followed by Turkey-Kramer multiple comparison tests. $P<0.05$ was considered as statistically significant.

Parameters analyzed

Average life Span

Average life span of the animals of all the groups was determined and noted.

Percentage increase in life span (% ILS)

The effect of CVW and LCL on tumor growth was monitored by recording the mortality daily for a period of 6 weeks and percentage increase in life span (% ILS) was calculated, by using following formula,

$$\% \text{ ILS} = \left(\frac{\text{Mean survival of treated group}}{\text{Mean survival of control group}} - 1 \right) \text{X } 100$$

Body weight analysis

Body weights of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (day 0) and sequentially on every day during the treatment period. Percent increase in body weight was calculated on day 11 of the experiment using following formula.

$$\% \text{ Increase in body Weight} = \left(\frac{\text{Body wt. of animal on 11 day} - \text{Body wt of animal on 0 day}}{\text{Body wt. of animal on 0 day}} \right) \text{x } 100$$

Packed cell volume

Peritoneal fluid from the each animal was collected on 11th day and 5 mL of fluid was transferred to sterile centrifuge tubes and centrifuged at 2000rpm for 10 minutes. Cell sedimentation levels were measured and expressed in mL.

Viable tumor cell count

Peritoneal fluid from the each animal was collected on 11th day and cell viability was measured by using tryptan blue dye exclusion method.

Hematological Parameters

The blood collected was immediately used for the estimation Hemoglobin (Hb) content, red blood cell count (RBC) and white blood cell count (WBC) using haemocytometer[10]

RESULTS AND DISCUSSION

Phytochemical screening

The extract shows positive test for carbohydrates, Glycosides, Flavonoids, Phenolics and Tannins, Phytosterols and Triterpenoids.

Antioxidant activity

Table 1.Shows antioxidant activity of different fractions of *Cleome viscosa* by using different methods.values are mean±SD.

Samples	IC_{50} values g/mL by methods		
	DPPH	ABTS	Nitric oxide
CVW PETETHER soluble	820.00±20.00	24.67±0.58	260.00±17.32
CVW ETHYLACETATE soluble	236.67±15.28	4.40±0.10	80.67±0.58
CVW ETHANOL soluble	210.00±17.32	14.67±0.58	436.67±20.82
Standard	Rutin	Rutin	Rutin
	10.11	4.0	60.14

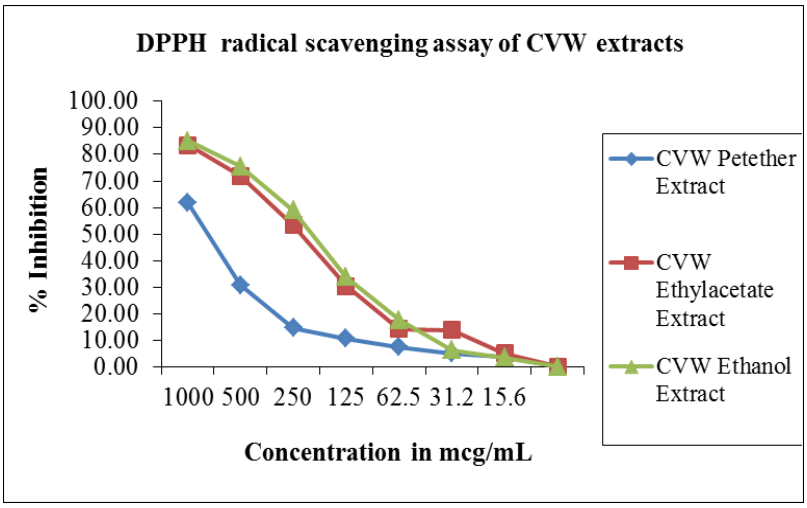


Figure1

Values are mean±SD in each fraction and standard.

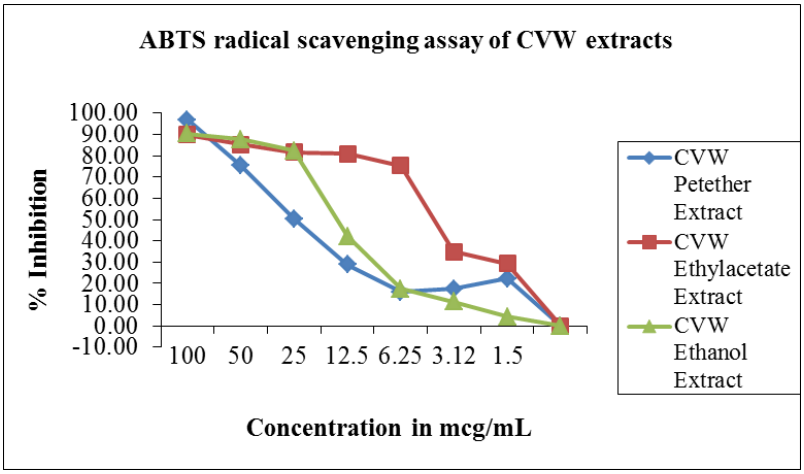


Figure2

Values are mean±SD in each fraction and standard.

NITRIC OXIDE SCAVENGING ASSAY

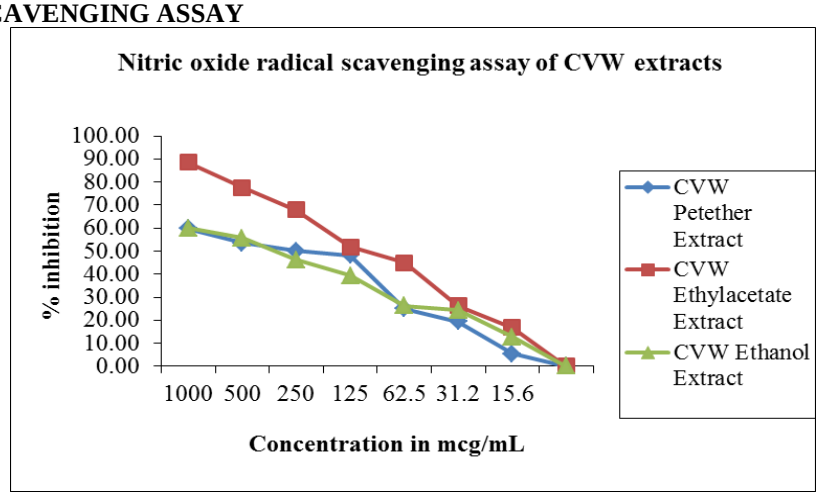


Figure3

Values are mean±SD in each fraction and standard.

Table 2.Shows cytotoxic properties of test drugs against various types of cell lines.

Samples	CTC_{50} (µg/mL)			
	Vero cell line	Pc3 cell line	HeLa cell line	MCF-7cell line
CVW PETETHER soluble	<1000.00	483.33±11.55	>1000±0.00	293.33±75.06
CVW ETHYLACETATE soluble	126.67 ±15.28	226.67±11.55	326.67±5.7	130.00±8.66
CVW ETHANOL soluble	>1000	>1000±0.00	>1000±0.00	>1000±0.00

values are mean±SD.

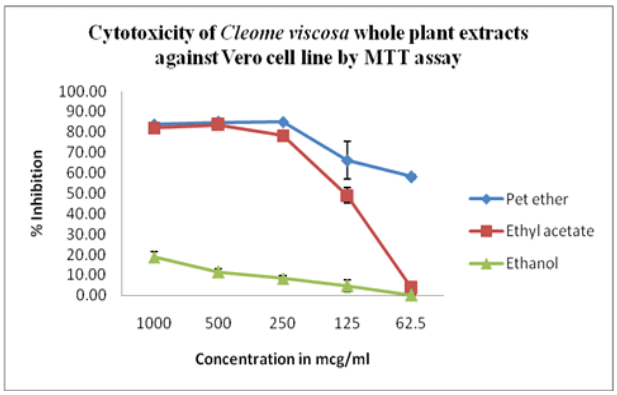


Figure4

Values are mean±SD in each fractions.

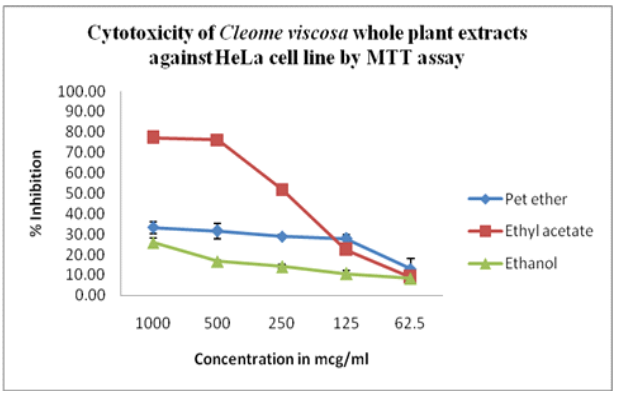


Figure6

Values are mean±SD in each fractions.

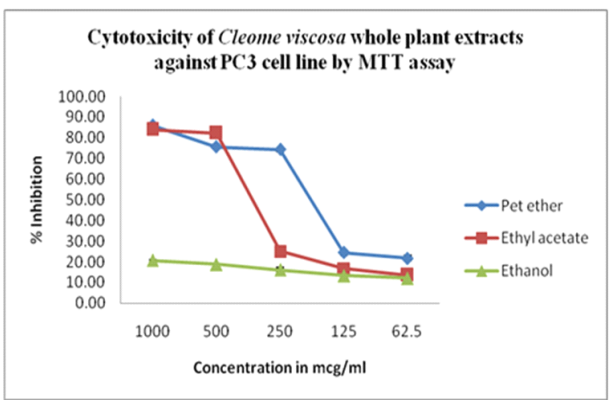


Figure5

Values are mean±SD in each fractions.

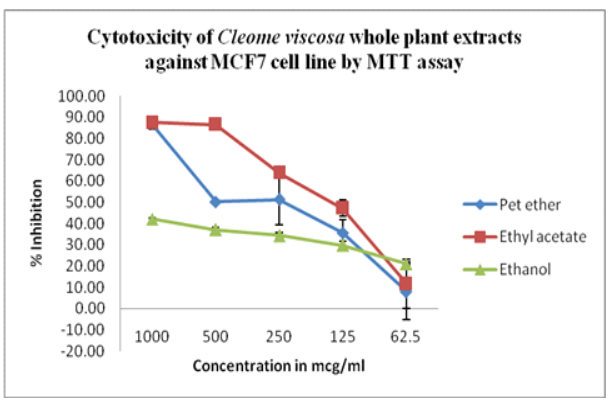


Figure7

Values are mean±SD in each fraction and standard.

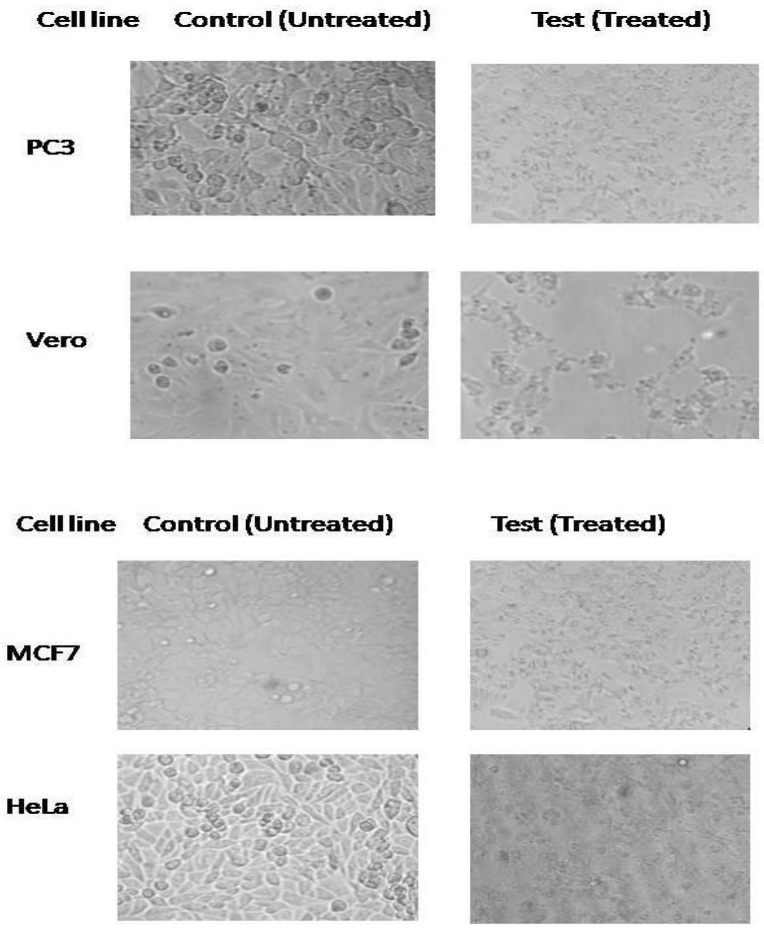


Figure 8 shows Histo pathology of various cell lines

***In vivo* studies**

Effect of test extracts on antitumor parameters of DLA bearing mice

Table3.Values are expressed as mean ±SD for six animals in each group $^{a}P<0.05$, $^{b}P<0.01$and$^{c}P<0.001$ between normal and tumor group values. $^{d}P<0.05$,$^{e}P<0.01$ and $^{f}P<0.001$ between tumor control and treated groups, one-way analysis of variance followed by Turkey-Kramer multiple comparison tests.

Parameters	Normal	DLA control (1×10^6 cells/ml/ mice)	DLA + 5-FU	DLA + CVW (250 mg/kg)	DLA + CVW (500 mg/kg)
Average life span, Days	---	18.0 ± 0.22	34.50 ± 0.85^{f}	26.78 ± 0.45^{f}	32.00 ± 1.05^{f}
Increase in life span, %	---	---	91.66	48.77	77.77
Increase in body wt, %	---	39.56 ± 0.78	14.65 ± 1.00^{f}	30.47 ± 2.10^{f}	23.00 ± 0.70^{f}
Packed cell volume, ml	---	0.95 ± 0.05	0.51 ± 0.01^{a}	0.79 ± 0.01^{a}	0.66 ± 0.01^{f}
Viable tumour cell count ($\times 10^7$ cells/ml)	---	546.00 ±11.45	306.00 ± 8.21^{f}	495.00 ± 3.78^{d}	379.33 ± 7.65^{f}
Total WBC (10^3/mm^3)	9.81 ± 0.75	19.65 ± 0.43^{c}	9.95 ± 1.34^{f}	15.83 ± 0.72^{a}	12.13 ± 0.60^{f}
RBC (1×10^6/mm^3)	11.38 ±0.65	5.98 ± 0.71^{c}	9.78 ± 0.56^{f}	8.76 ± 0.36^{f}	10.33 ± 0.10^{f}
Hgb (g/dl)	14.92 ±0.38	7.96 ± 0.58^{b}	13.69 ± 0.85^{d}	9.15 ± 1.00	13.50 ± 1.35

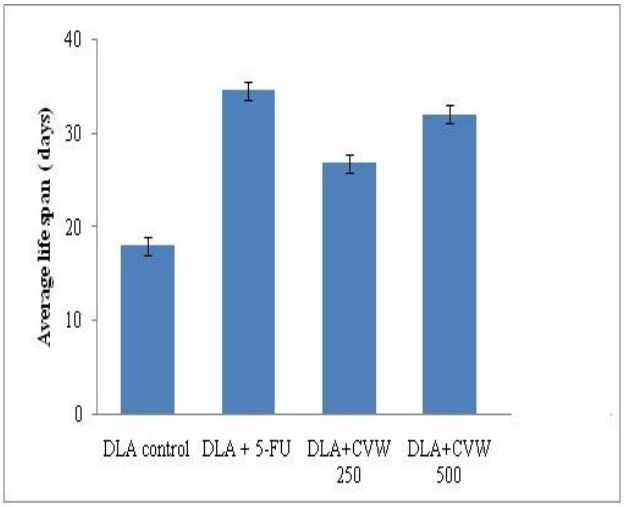


Figure 9.Effect of test extracts on survival of tumor bearing mice

Values are mean±SD in each fraction and standard.One-way analysis of variance (ANOVA) followed by Turkey-Kramer multiple comparison tests.

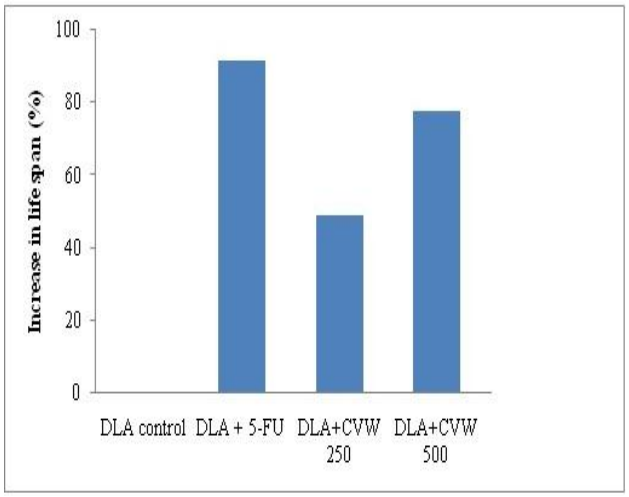


Figure10: **Effect of test extracts on increase in life span of tumor bearing mice.**

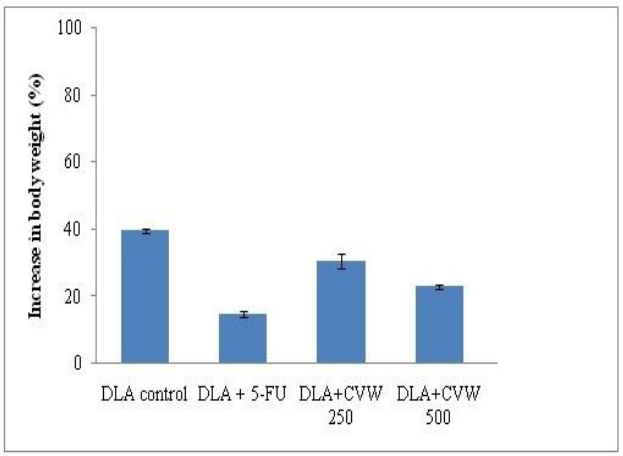


Figure11. Effect of test extracts on weight of tumor bearing mice

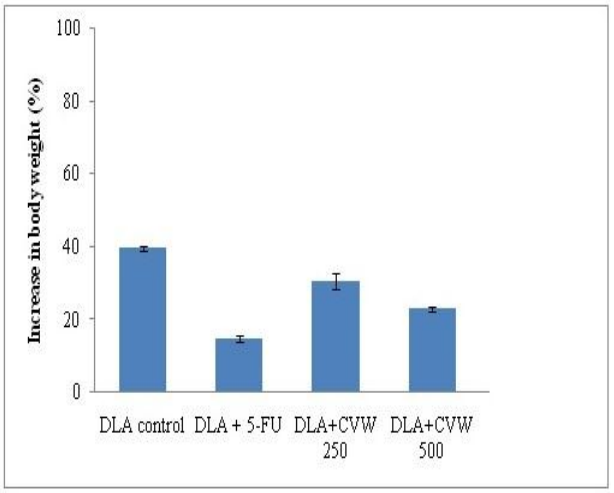
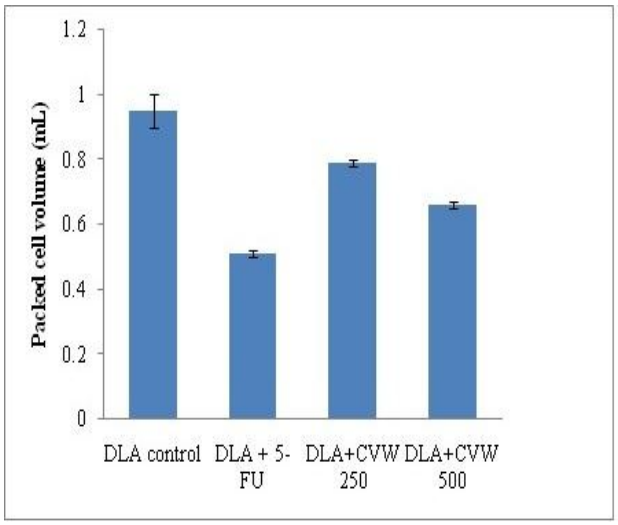


Figure12. Effect of test extracts on weight of tumor bearing mice



Fifure13.Effect of test extracts on packed cell volume of tumor cells in tumor bearing mice

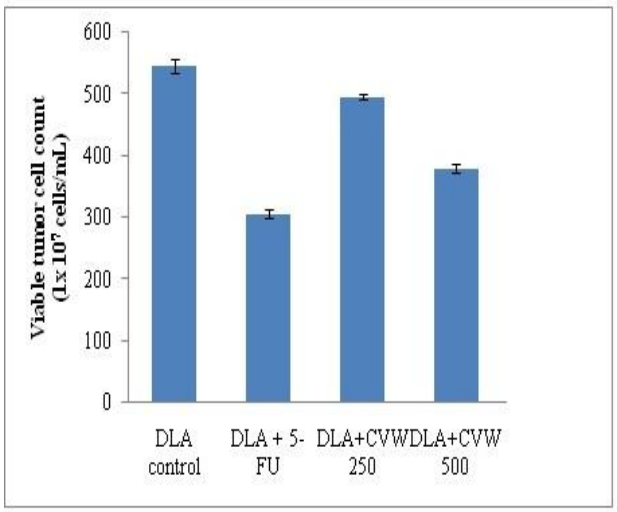


Figure14.Effect of test extracts on viablity of tumor cells from tumor bearing mice

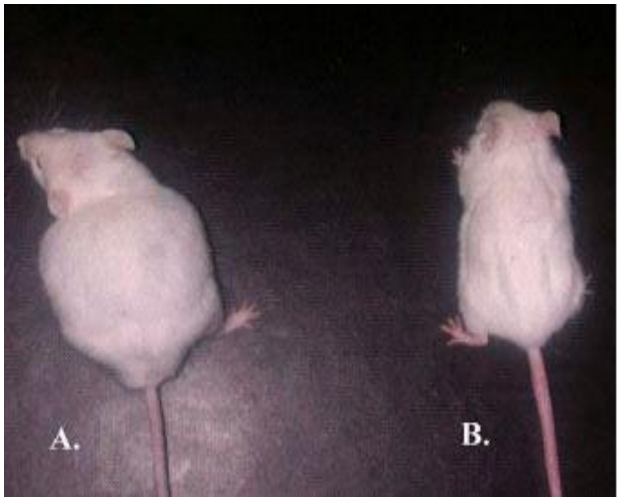


Figure 15.Photograph of Tumor bearing (A) and Normal (B) mice

Phytochemical constituents present in the plants were identified by preliminary phytochemical screening; in *Cleome viscosa* carbohydrates, glycosides, flavonoids, phytosterols and triterpenoids were present. The compounds quercetin, rutin and gallic acid were isolated from plant extract of *Cleome viscose*.

The *in vitro* study conducted with plant extracts the following results were obtained; in antioxidant assay by DPPH and ABTS method the petroleum ether soluble fraction of the plant has given higher IC_{50} values compared to other extracts and on the other hand in nitric oxide scavenging assay of the plant ethanol soluble fraction has shown higher IC50 value. Higher IC_{50}value signifies less antioxidant activity.

Cytotoxicity study on vero cell line shows that ethanol soluble extract of *Cleome viscosa* has less cytotoxic activity compared to other extracts. Cytotoxicity study on pc3 cell line shows that ethanolic extract of *Cleome viscosa* has less cytotoxic activity compared to other extracts. Cytotoxicity study on HeLa cell line shows that ethanolic extract of *Cleome viscosa* has less cytotoxic activity compared to other extracts. Cytotoxicity study on MCF-7 cell line shows that ethanolic extract of *Cleome viscosa* has less cytotoxic activity compared to other extracts and also *Cleome viscose* whole plant ethyl acetate fraction showed potent toxicity with CTC50 of 130.00±8.66 µg/ml. *In vivo* studies were performed and it indicated that the test extract, *Cleome viscosa* at 500 mg/kg dose found to have very good anticancer properties.

CONCLUSION

Cancer can develop in virtually any of the body's tissues, both hereditary and environmental factors contributing to its development. There are over 200 different types of cancer, each with its own methods of diagnosis and treatment. It was estimated that there were approximately 8.2 million deaths from cancer in the world every year of which 4.7 million (57%) is in males and 3.5 million (43%) in females, giving a male: female ratio of 10:8. Many natural products are available as chemoprotective agents against commonly occurring cancers worldwide. Here we had selected a traditional medicinal plants namely *Cleome viscose Linn*.

In this work different phytochemical constituents present in the plants were identified by preliminary phytochemical screening and carbohydrates, glycosides, flavonoids, phytosterols and triterpenoids were present and it can be concluded that the plant extract shows anti oxidant,cytotoxic and anticancer properties. The reported activities may be due to presence of the above chemical constituents, but further studies are necessary to confirm the mechanism of action of the above activities.

ACKNOWLEDGEMENTS

We thank all the staffs of Department of pharmaceutical sciences, Mahatma Gandhi University, RIMSR, Manipal College of pharmacy and Radient Reasearch laboratory banglore for timely assistance for the completion of this work

REFERENCES

1. Chopra A and Diophode VV, Ayurvedic medicine. Core concept, therapeutic principles, and current relevance, Med Clin North Am, Jan, 2002; 86(1): 75-89.
2. Devita, V.T., Jr., Canellos, G.P.: Treatment of the lymphomas. Sem. Hemat (1972).9, 193–209.
3. Stahelin H. Activity of a new glycosidic lignan derivative (VP 16-213) related to podophyllotoxin in experimental tumors. Eur J Cancer, 1973; 9: 215-21.
4. Williams SD, Birch R, Einhorn LH, Irwin L, Greco FA, Loehrer PJ. Treatment of disseminated germ-cell tumors with cisplatin, bleomycin, and either vinblastine or etoposide. N Engl J Med, Jun 4, 1987; 316(23): 1435-40.
5. Chabner.The Language of Medicine Fourth Edition 1991Chase, CR. Pratt R. Flourescence of powdered vegetable drugs with particular reference to development of a system of identification.Journal of American Pharmaceutical Association Sciences, 1949; 28: 324-331.
6. Nadkarni, K.M., a nd Na dkarni, A.K., "Indian Materia Medica". Popular Prakashan, Bombay, 1976; 1: 498.
7. Harborne JB, 'Phytochemical methods: A guide to modern technique of plant analysis', Champman and Hall, London, 1998.
8. Kato K, Terao S, Shimamoto N, Hirata M. Studies on scavengers of active oxygen species. 1. Synthesis and biological activity of 2-Oalkylascorbic acids. J. Med. Chem, 1988; 31: 793-798.
9. Francis D and Rita L. Rapid colorometric assay for cell growth and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. Journal of Immunological Methods, 1986; 89: 271-277.

10. Gupta M, Mazumeder UK, Haldar PK, Kandar CC. Anticancer activity of Indigofera aspalathoides andWedelia calendulaceae in Swiss albino mice. Iran J Pharm Res., 2007; 6: 141–5.