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Research Article

PHYTOCHEMICAL INVESTIGATION AND ANTI-CANCER ACTIVITY OF AERIAL PARTS OF *VINCA DIFFORMIS*

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ABSTRACT

The study aimed to investigate the phytochemical and therapeutic effects of aerial parts of *Vinca differmis* for anticancer activity in normal and cyclophosphamide induced oxidative stress in mice brain. Aerial parts of *Vinca differmis* was extracted with ethanol:acetone mixture. Following extraction,extract was tested for the presences of phytochemical constituents and cyclophosphamide induced oxidative stress in mice brain. Presences of alkaloid, glycosides, steroids, phenolic compounds and volatile oils were identified in the extract. Among these total alkaloid content was 8% and 0.348mg/mg gallic acid was obtained from the extract. *Vinca differmis* extract was effective as antioxidant to reduce the cyclophosphamide toxicity. Thus the results suggests that the aerial parts of *Vinca differmis* extract contains some active principles which may possess significant anti-cancer activity.

INTRODUCTION

Cancer continues to be one of the major causes of mortality throughout the world. There will be an estimated 15 million new cases and 10 million new deaths in 2020, even if the current trends remain unchanged. 92 anticancer have been approved by the US Food and Drug Administration (FDA). Recently World Health Organization consultation considered only 17 drugs and 2 antiemetic as high priority and 12 more to have some advantage in particular clinical settings. Although the development of new anticancer drug has improved survival in a few diseases such as childhood leukemia and testicular cancer, there is an urgent need to continue to develop new and improved agents, as well as to optimize the use of conventional drugs[1].

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plant still makes an important contribution to health care[2]. Over the past decade, interest in drugs derived from plants, especially the phytotherapeutics, has increased expensively. It is estimated that about 25% of all modern medicines are directly or indirectly derived from plants. In some particular cases, such as antitumoral and antimicrobial drugs 60% medicines currently available on the market and most of these in the late stages of clinical trials are derived from natural products, mainly from plants. The World Health Organization estimates that upto 80% of the world's population relies on traditional medicinal system for some aspect of primary health care[3].

Anticancer activity can be exerted through a wide variety of mechanisms mostly targeting DNA, directly or indirectly by anti-cancer agents. Anti-cancer agents are drugs used to control the growth of cancerous cells and/or treat malignancies by eradicating cancer cells without harming the normal cells[4]. An important characteristics of an anticancer drug is to able to induce cancer cell apoptosis and such differentiates between an anticancer drug and a toxic compound[5]. Cyclophosphamide is alkylating and Platinating agents which classify as cytotoxic agents.

The genus Vinca (Apocyanaceae) is an evergreen shrubs or herbaceaous perennials, native to western Europe. *Vinca differmis* known commonly as intermediate periwinkle. There are at least 86 alkaloids extracted from plants in the Vinca genus.[12] The chemotherapy agent vincristine is extracted from Vincarosea (current name Catharanthus roseus), and is used to treat some leukemias, lymphomas, and childhood cancers, as well as several other types of cancer and some non-cancerous conditions. Vinblastine is a chemical analogue of vincristine and is also used to treat various forms of cancer. Dimeric alkaloids such as vincristine and vinblastine are produced by the coupling of smaller indole alkaloids such as vindoline and catharanthine. In addition, the nootropic agent Vincamine is derived from Vinca minor[11]. The present investigation was designed for Phytochemical and Cyclophosphamide induced anti-cancer activity of aerial parts of *Vinca differmis* extract.

MATERIALS AND METHODS

Collection of Plant material

The aerial part of *vinca differmis* was collected from garden of Cagliari, Italy and powdered using grinder mill. The powdered drug packed in a paper bags and stored in air tight container until use.

Authentification

The botanical identity was confirmed by Universitadeglistudi di Cagliari, Dipartimento di scienzebotaniche, Italy, the voucher specimen number is 925/B.

Preparation of extracts

The powdered drug was extracted with ethanol: acetone 1:1 by maceration. after completion of extraction solvent was recovered and the saturated solvent was dried over water bath at 40-50°C. The semisolid paste formed is transferred to petri plates and kept in hot air oven at 60°C for further drying and stored in air tight container and kept at 2-8°C for further use.

PHYTOCHEMICAL SCREENING[6]

The freshly prepared crude extracts of the aerial parts of *Vinca differmis* were qualitatively tested for the presences of alkaloids, Tannins and Phenolic compounds, Flavanoids, Steroids, Glycosides, Saponins, Proteins and Amino acids. Total alkaloidal and Phenolic content was also determined.

EXPERIMENTAL ANIMALS

Male Swiss albino mice 25-30gm of avg. wt. have been used. The animals maintained under standard environmental conditions had free access to standard diet and water ad libitum. Mice were housed in groups of six per cage. All the animals were maintained under standard conditions; that is room temperature 26±1°C, relative humidity 45-55% and 12:12 hrs light-dark cycle. The cages were maintained clean, and all experiments were conducted between 9 am and 4 pm.

Acute Toxicity Study[7]

Swiss Albino mice (25 - 30 gm weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD 425 and animals were observed for mortality and behavioral changes.

Ethical Approval

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC).All the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

PHARMACOLOGICAL SCREENING

Anti-Cancer activity of *Vinca differmis* against cyclophosphamide induced oxidative stress in mice brain.

IN VIVO STUDY-BIOASSAY[8]

- Mice weighing (25-30gm) was acclimatizes for 2 weeks.
- Mice was divided into three groups.
- Group 1 (control) was feed basal diet (corn flour 67%, ground nut cake 14%, fish meal 5%, ground nut oil 10% and vitamin mineral).
- Group 2 was feed basal diet (corn flour 67%, ground nut cake14%, fish meal 5%, ground nut oil 10% and vitamin mineral).
- Group 3 was feed with basal diet with extract.
- The rats in groups 2-3 was treated with cyclophosphamide by i.p (75mg/kg body weight).
- The malonoaldehyde content of the brain was determined and serum glutamate oxaloacetate transferase (sgopt), glutamatepyruvatetransferase (sgpt) was determined by diagnostic kits.

PREPARATION OF TISSUE HOMOGENATE

- Rats was decapitated and its brain was removed.
- The tissue was homogenized in cold saline (1/10w/v) and homogenizes in Teflon glass at 1200rpm.
- Centrifugation for 10min at 3000rpm.
- *S*.I fraction (tissue homogenate)

LIPID PEROXIDATION ASSAY[9]

- 0.2ml tissue homogenate add 0.2ml sodium doudecylsulphate (8%) add 1.5% acetic acid add 1.5ml 8% thiobarbituric acid (8%).
- Makeup volume to 4ml with distilled water.
- Heat for 1hrs at 95°C on heating mantle.
- Cool and add1ml water and add 5ml butanol:pyridine (15:1)
- Shake using vortex shaker for 5min and centrifuge at 4000rpm foe 10 min.
- Absorbance at 532nm on spectrophotometer

.GLUTATHIONE (GSH) DETERMINATION[10]

Take 0.05ml of supernatant and added 0.2ml of DTNB Stock and 0.4ml of 1M Tris (P^H 8). Then add 3.350ml distilled water and put for incubation for 5min and after took absorbance at 412nm with blank.

SGOT DETERMINATION

Reagent Composition

Reagent No.	Reagent	Composition
1.	Buffer	Tris buffer(Ph 7.8), MDH,LD L-Aspartate
2.	Substrate	α –ketoglutarate, NADH

Procedure

Add reagent 2 to reagent 1 in 1:4 ratio. Pipette into tube marked serum/plasma 100µl and working AST reagent 1000µl, mix well and aspirate immediately for

measurement. Program the analyser with purified water and read the absorbance after 60sec.repeat reading after every 30sec,upto 120sec. at 340nm wavelength. Determine the mean absorbance change per min.

AST activity (IU/L) = ΔA/minute*Kinetic factor

Where ΔA/minute=change in absorbance per minute.

Kinetic factor(K)= 1768

SGPT Determination

Reagent composition

Reagent No.	Reagent	Composition
1.	Buffer	Tris buffer (ph 7.8), LD L-Alanine
2.	Substrate	α – ketoglutarate NADH

Add reagent 2 to reagent 1 in 1:4 ratio. Mix serum/plasma 100µl to working AST reagent 1000µl and aspirate immediately for measurement. Programme the analyser as per assay parameters, Blank the analyser with purified water. Read the absorbance after 60sec. repeat reading after every 30sec,upto 120sec. at 340nm wavelength. Determine the mean absorbance change per min. (ΔA/minute)

AST activity (IU/L) = ΔA/minute*Kinetic factor

Where ΔA/minute=change in absorbance per minute.

Kinetic factor (K)= 1768.

Statistical Analysis

Statistical analysis was carried out using primer of Bio-statistical software. All results were expressed as mean ±standard error mean (SEM). Data were analyzed using one-way ANOVA followed by Bonferroni t-Test. In the entire tests the criterion for statistical significance was P‹ 0.05.

RESULTS

PHYTOCHEMICAL ANALYSIS

The results of the chemical tests performed in the screening, revealed the presences of flavonoids, alkaloids, tannins, glycosides, saponins, terpenoid in the extract of aerial parts of *Vinca difformis*.

1. Colour – Grayish Black
2. Consistency – Solid Powder
3. Odour – Characteristic
4. Percentage yield – 5.43%

Table 1:Phytochemical analysis of aerial parts of *Vinca difformis*

Sl.No.	Test	Inference
Class : Carbohydrate		
1	Molish test	+
2	Iodine test	+
3	Benedict test	+
4	Inversion test	+
Class : Alkaloid		
1	Dragendroff test	+
2	Mayer test	+
3	Hager test	+
4	Wagner test	+
Class : Gycosides		
1	Keller-Killani test	+
2	Foam test	+
3	Haemolytic activity	+
4	Odour set	+
5	Alkaline test	+
6	Filter paper test	+
Class : Flavanoids		
1	Shinoda test	+
Class: Tannin and Phenolic Compounds		
1	Fecl3 5%	+
2	Lead acetate solution	+
3	Gelatin solution	+
4	Acetic acid solution	+
5	Potassium dichromate	+
6	Dil. Iodine solution	+
7	Dil. Nitric oxide	+
8	Mirror test	+
9	Dil.Potassium Permaganate	+
Class : Steroid		
1	Salkowaski Reaction	+
2	Libermann-burchad reaction	+
Class : Volatile Oils		
1	Odour test	+

2	Filter paper test	+
3	Solubility test	+

Total Alkaloidal Determination

Total Extract Weight	250 mg
Total Alkaloid Dry Weight	20mg
Percentage of Total Alkaloid Content	8 %

Total Phenolic Content Determination

Absorbance of Sample	0.921
Sample Amount	1ml
Concentration of Sample	100mg/ml
Standard Curve Equation	Y = 0.026X + 0.019 Here X = Gallic acid conc.(µg/ml) Y = Absorbance
Total Phenolic Content	0.348 mg/mg Gallic acid equivalent.

Acute oral toxicity

Acute oral toxicity studies revealed the non-toxic nature of the *Vinca differmis*. The extract of *Vinca differmis* did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose after fourteen days of study. This indicates that the extracts were found to be safe up to the dose levels studied. Since, all the animals survived at a dose of 2000 mg/kg body weight, the LD_{50} of the extract will be >2000 mg/kg body weight. No major behavioural changes were observed during the period of study. Therefore 1/10th and 1/5thof the maximum tolerated safe dose was selected for further pharmacological activity.

Anti-Cancer activity

Cyclophosphamide induced Oxidative stress

The extract treated showed a significant protection as compared to cyclophosphamide groups.

Group	**Biochemical parameters**			
	LPO (nM/mg protein)	**GSH (nmg/mg)**	**GPT (IU/ml)**	**GOT (IU/ml)**
Control	3806±81.33	2315±116.6	1.989±0.4232	6.63±1.111
Cyclophosphamide	4970.16±61.62**	561.525±1977**	14.144±0.6251**	42.211±1.88**
Drug+ Cyclophosphamide	4198.75±63.27**	2822.4±26.84**	9.724±1.488**	15.028±3.405*

Values are expressed as Mean ± SEM, for 4 animals, **P<0.05, significantly when compared with control group.

DISCUSSION

The results of the present study demonstrated that the extract of *Vinca differmis* possessed alkaloid, carbohydrate, glycoside, steroids, phenolic compounds and volatile oils but amino acid, protein and anthraquinone type glycoside is absent. The chemical constituent flavanoids are responsible for anticancer activity. The total alkaloid content was 8% in extract ethanol/acetone(1:1) solvent system. Among these V.D.E. contained 0.348mg/mg Gallic acid equivalent phenolic compound.

Phenolics are diverse secondary metabolites abundant in plant tissues. Polyphenols possess ideal structural chemistry for radial scavenging activity 81. The LPO showed that there is a significance differences between the control and cyclophosphamide treated groups. Cyclophosphamide causes GSH depletion in brain and the extract protect against cyclophosphamide toxicity and increases the GSH level. In GPT the protection against liver toxicity were assesses on different parameters. The differences showed that drug protects the liver from cyclophosphamide induced toxicity but the drug has no capacity to reduce the level. Level of GOT in cyclophosphamide group was significantly high as compared to that of vehicle treated. On treatment with extract it was observed the extract decreased the elevated level of GOT as compared to cyclophosphamide only treated group. Thus the extract is potent in protecting the hepatic cells by the damaged caused by cyclophosphamide.

CONCLUSION

In the present study *Vinca differmis* extract showed significant protection from cyclophosphamide induced toxicity in mice brain by reducing LPO level in brain and reducing the GPT and GOT level in serum and increasing the GSH level in mice brain. Raising the level of all three parameters showed the toxicity phenomena in response to oxidative stress and depletion of GSH in brain indicates oxidative stress. So ,on the basis of result it is concluded that *Vinca differmis* extract possessed antioxidant activity and it might be beneficial for protecting brain from oxidative damage caused by cyclophosphamide. Due to its antioxidant potential the anticancer activity was tested because a good antioxidant may be anticancer agent.

REFERENCES

1. Fig WD, Mcleod HL. Handbook of anticancer pharmacokinetics and pharmacodynamics. Published by human press, Totowa, New Jersey. 2002; 1-2.
2. Introduction to cancer: available at.WWW.cancer.gov/cancertopics/what-is-cancer

3. Calixto JB, Efficacy, Safety, Quality control, Marketing and Regulatory Guidelines for herbal medicines (Phytotherapeutic Agents). Braz J Med Biol Res, 2000;33:179-189.
4. Chabner BA, Thompson EC. Prevention and Treatment of cancer. United States of America: Merck Research Laboratories. 2003;2nd edition:1043.
5. Frankfurt OS, Krishnan A. Apoptosis-based drug screening and detection of selective toxicity to cancer cells. Anticancer Drugs.2003;14:555-561.
6. Khaldelwal KR. Practical pharmacognosy. Nirali Prakashan,Pune.2005:149-153.
7. Ghosh MN. Toxicity studies. In fundamental of Experimental Pharmacology. Scientific Book Agency,Calcutta.1984;153-158.
8. Oboh G, Ogunrukku OO. Cyclophosphamide-induced oxidative stress in brain: Protective effect of hot short pepper. Experimental and Toxicologic Pathology. Accepted 31 March 2009.
9. OhkawaH, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by Thiobarbituric Acid Reaction. Analytical Biochemistry.1979;95:351-358.
10. Ellman LL. Tissue Sulphahydryl group. Arch Biochem Biophys.1959;82:70-77.
11. Introduction to vincadifformis: available at. https://en.wikipedia.org/wiki/Vinca_difformis.

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