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ORIGINAL RESEARCH ARTICLE

Cytotoxic Screening of Plant extracts and Angiogenesis: A Landmark approach to cancer

A.R. Chavhan [1], Ayaz Ahmed[1], M I Irfan[1], Naiyer Shahzad[1*], Amit Deshpande[2]

[1] *School of Pharmacy and Medical Sciences, Singhania University, Rajasthan, India.*
[2] *Deshpande Lab., and Pvt., Ltd., Bhopal, MP. India.*

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ABSTRACT

Andrographis paniculata and Aegle marmelos are the important medicinal plants. In the Indian Systems of Medicine, they are used against blood cancer, as an analgesic, antithrombotic, thrombolytic, hypoglycemic and antipyretic, antidote for snake venom, diarrhea and constipation. The major objective of this study is to evaluate cytotoxicity of this medicinal plants with help of MTT and Trypan Blue Assay. Angiogenesis model plays an important role to introduce to vascularisation process that plays an important role to focus on organogenesis and to study antiangiogenesis effect of different drugs. The study of angiogenesis is making a profound impact on the biological and medical world. The hope of being able to build new, functional, and durable blood vessels in ischemic tissues, or conversely, to prevent their further growth in malignant and inflamed tissues is becoming more realistic every day. Conc. are prepared of each plant extracts which are 100 µg/ml, 10 µg/ml , 0.1 µg/ml, 0.01 µg/ml and 5-10×10^{3} cells/ml are taken into each well which are exposed to different conc. of plant extracts for 96 hr and then treated with MTT and Typan Blue Dye with in 1 hr- 24 hr. For MTT absorbance taken at 570 nm. From IC50 values of MTT assay and percentage of cell viability from trypan Blue Assay of kalmegh for MCF7,A549, DU145 is less as compare to bael fruit for same cell lines, from this it may concluded that Bael Fruit shows potent cytotoxicity than Kalmegh for respective cell lines Angiogenesis was only implicated in number of diseases, such as, cancer, arthritis, and psoriasis. Research in angiogenesis offers a potential to cure a variety of diseases such as Alzheimer's and AIDS. Modulation of angiogenesis may have an impact on diseases in the twenty-first century similar to that which the discovery of antibiotics had in the twentieth century. Further studies are necessary for chemical characterization of the active principles and more extensive biological evaluations for above cell lines which leads to novel drug innovation.

Key Words: Androgaphis paniculata, Aegle marmelos, MTT Assay, Trypan Blue Assay.

INTRODUCTION

The challenging and expensive activity of pharmaceutical industry is drug development and discovery. The success rate and economics and rime involved in drug discovery is staggering for each pharmaceutical industry, research institute. The aim to discover new drug are [1] Invention of completely new drug with newer clinical advantages over older one, economically viable, Most efficacious and safer drug, Pharmacodynamic /mechanism should explore. The discovery of new drugs and their development into commercial products takes place across the broad scope of the pharmaceutical industry and research institutes. The basic underpinning for this effort is the cumulative body of scientific and biomedical information generated worldwide in research institutes, academic centers, universities and industry[1]. It is currently used and applied in developmental stages of anticancer drug discovery are able to select the most potent compounds [2]. Each and every new drug cosmetics, additives, food and so on go through extensive, cytotoxicity testing before they are released to market for the use by people. This study involves large number of animal testing or experiments, although in Europe, these experiments will be subjects to new legislation. There is much pressure, both human and economic, to perform at least part of cytotoxicity

***Corresponding Author:** Mr. Naiyer Shahzad, **Email:** shahzad.naiyer@gmail.com, **Contact No:** 09784504486

testing. The nature of the response must also be considered carefully. A toxic response *in-vitro* may be measured by changes in cell survival or metabolism while the major problem *in-vivo* may be a tissue response (eg. an inflammatory reaction, fibrosis, kidney transport) or a systemic response (eg. pyrexia, vascular dilation). For *in-vitro* testing to be more effective, models of these responses must be constructed, perhaps utilizing organotypic cultures resembled from several different cell types and maintained in the appropriate hormonal milieu. It should not be assumed that complex tissue and even systemic reactions cannot be stimulated *in-vitro.* Assays for inflammatory responses, teratogenic disorders and neurological dysfunction may be feasible *in-vitro* given a proper understanding of cell-cell interaction. In addition to cancer research and virology, other areas come to depend heavily on tissue culture techniques. Introduction of cell diffusion techniques and genetic manipulation established somatic cell genetics as a major component in the genetic analysis of animals, including humans[3]. Tissue culture has contributed greatly, via the monoclonal antibodies technique[4] , to the study of immunology, already dependent on cell culture for assay techniques and the production of haematopoietic cell lines. This field has supplied much basic information on the control of gene transcription, and a vast new technology [5]. Tissue culture technology has also been adopted into many routine applications in medicine and industry. Chromosomal analysis of cells, toxic effects of pharmaceutical compounds, potential environmental pollutant can be measured in colony forming and other in-vitro assay [5-7].

MATERIAL AND METHODS

Reagents-

Alcohol 70%, 100% Alcohol, MEM media (Minimal Essential Media) [8], Trypsin[9], MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole)[10], Trypan Blue[11], Distilled Water, Dimethyl sulphoxide (DMSO) [12], etc.

Requirements-

Laminar air flow, Autoclave, N_2 liquid container, CO_2 incubator, Inverted microscope, Filtration assembly, Hemocytometer, Centrifuge machine, Micropipette.

Preparation of Plant Extract-

Weighed 20 gm of drug and transferred it into container containing menstruum and covered it with help of cotton or other closure which is suitable one. Kept it for adequate duration of time. Shaking of drug during maceration is essential in order to replace the saturated layers around the drug with fresh menstruum. After straining, the marc was pressed in filter press, hydraulic press or hand press. Then went for concentration of drug.

Isolation of Human Cancer Cells-

Human cancer cells are isolated from the patients and characterized at cellular and molecular levels. Isolated cells are cultivated in specialized mediums and specialized incubators to provide them physiological conditions required for the growth [13, 14].

Cell line-

The subculturing of the primary culture gives rice to cell lines. The term continuous cell line implies the indefinite growth of the cell in the subsequent subculturing. On the other hand, finite cell lines represent the death of cell after several subcultures. The studied cell lines are DU145, A549, and MCF7 [15, 16].

Assay Performed [16-20]

MTT Assay [16-18]

Method-Laminar air flow was prepared. Dilutions of concentration 100 µg/ml, 10 µg/ml, 1 µg/ml, 0.1 µg/ml, 0.01 µg/ml from stock solution (test drug +DMSO) having concentration 10mg/ml are done. Then normal count on haemocytometer before seeding the cells in plate was done. 10µl from each conc. in 4wells i.e. 20 wells for one drug was added. Plate contained 5,000-10,000 cells/ml into each well of 96-well culture plate. The cells were incubated for 96 hr in CO_2 incubator. After it cells are incubated with basal medium containing 0.5 mg/ml MTT in CO_2 incubator at 37ºC for appropriate duration of time.

Table-1 Concentrations of Plant Extracts

Sr. No.	Dilutions	Media	Drug Amount
1	100 µl	1000µl	10 µg/ml from stock in 990 µl
2	10 µl	90 µl	10 µg/ml from 1st in 90 µl
3	1 µl	90 µl	10 µg/ml from 1st in 90 µl
4	0.1 µl	90 µl	10µg/ml from 1st in 90 µl
5	0.01µl	90 µl	10µg/ml from 1st in 90 µl

The medium is aspirated, and the formazan product is solubilized with dimethyl sulfoxide (DMSO). Absorbance at 570 nm is measured for each well using a microplate reader on colorimeter. Analyse data of test with standard drug and plot graph.

Trypan Blue Assay [19-21] Cell suspension being tested on petri dish mix with 0.4% trypan blue. Mixture was allow to incubate 3 min at room temperature. Cells are counted within 3 to 5 min of mixing with trypan blue, as longer incubation

periods will lead to cell death and reduced viability counts. Mixing is performed in a well of a microtiter plate or a small plastic tube using 10 to 20 µl each of cell suspension and trypan blue. A drop of the trypan blue/cell mixture to a haemocytometer was added. Then hemocytometer was placed on the stage of a binocular microscope and focus on the cells. Counted the unstained (viable) and stained (nonviable) cells separately in the hemocytometer. Percentage of viable cells by using following formula were calculated-

Viable cells (%) = total number of viable cells per ml/total number of cells per ml *100

RESULTS

Table-2

Cell line	**MCF7**		**A549**		**DU145**	
Sample code	A	B	A	B	A	B
IC50 (µg/ml)	10.5	15	60	30	5	9
Trypan Blue	56	48	56	48	54	48

A= Kalmegh, B= Bael Fruit ***Kalmegh***

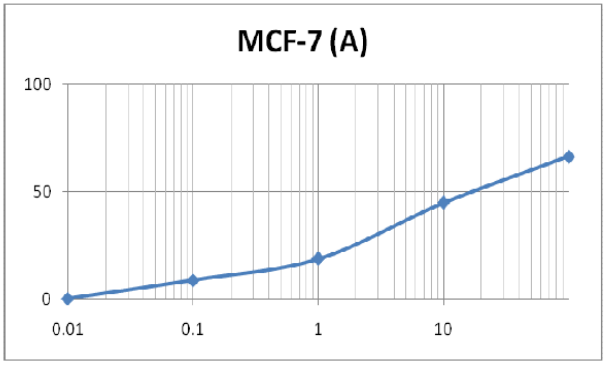

Fig- 1 MCF7 Cells were treated with Methanolic extract of *Andrographis paniculata* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT within 1 hr- 24 hr. Response Of MCF7 Cell to *A. paniculata* For % Inhibition on Y-axis and Concentration on X-axis.

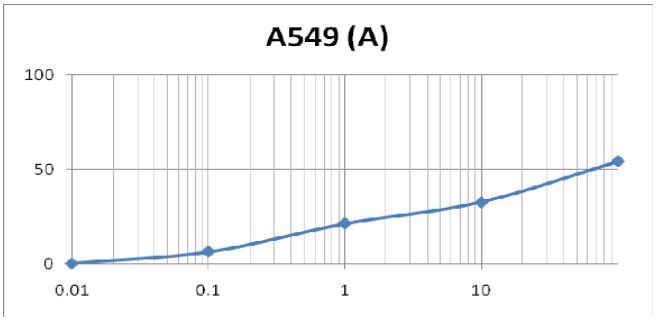

Fig- 2 A549 Cells were treated with Methanolic extract of *Andrographis paniculata* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT within 1 hr- 24 hr. Response of A549 to *A. paniculata* For % Inhibition on Y-axis and Concentration on X-axis.

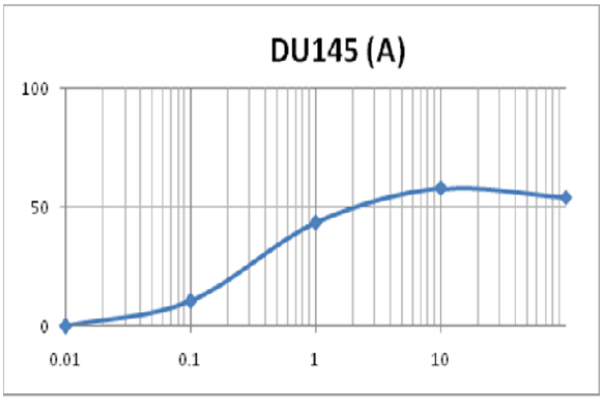

Fig- 3 DU145 Cells were treated with Methanolic extract of *Andrographis paniculata* dissolved in DMSO at 0.01,0.1,1,10,100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT within 1 hr- 24 hr. Response of DU145 to *A. paniculata* For % Inhibition on Y-axis and Concentration on X-axis. Bael Fruit

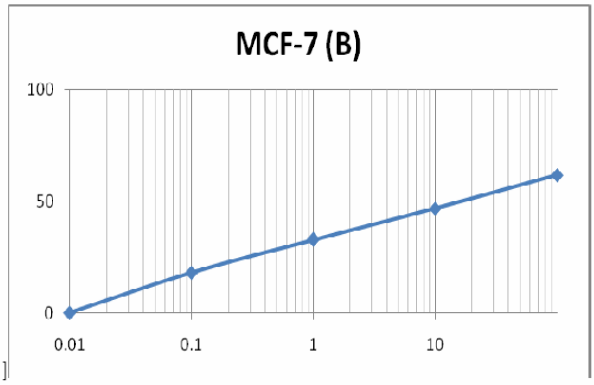

Fig- 4 MCF7 Cells were treated with Ethanolic extract of *Aegle marmelos* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT within 1 hr – 24 hr. Response of MCF7 to *A. marmelos.* For % Inhibition on Y-axis and Concentration on X-axis.

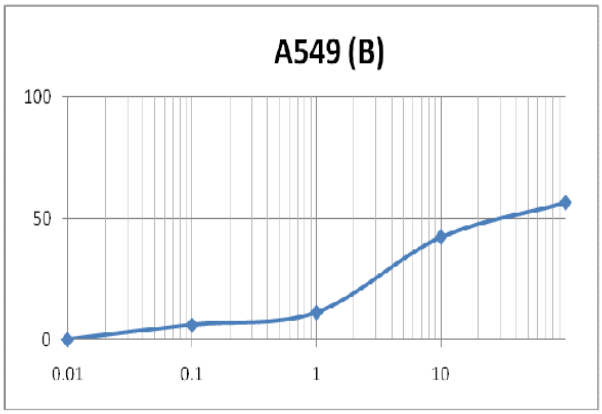

Fig- 5 A549 Cells were treated with Ethanolic extract of *Aegle marmelos* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT 1 hr- 24 hr. Response of A549 to *A. marmelos.* For % Inhibition on Y-axis and Concentration on X-axis.

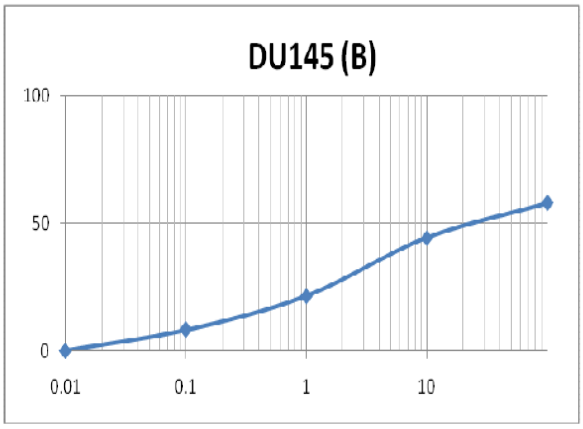

Fig- 6 MCF7 Cells were treated with Ethanolic extract of *Aegle marmelos* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT 1 hr- 24 hr. Response of DU145 Cell to *A. marmelos* For % Inhibition on Y-axis and Concentration on X-axis.

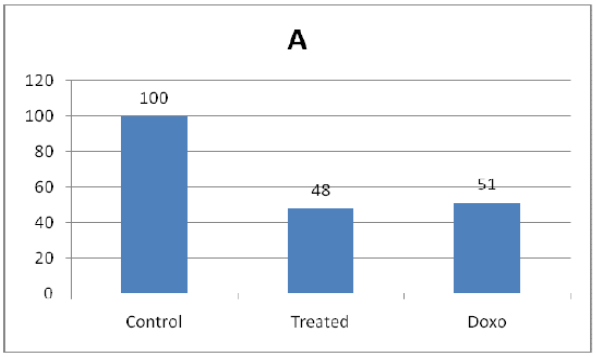

Fig- 7 MCF7 Cells were treated with Methanolic extract of *Andrographis paniculata* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 Conc. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to Trypan Blue Dye. Response of MCF7 Cell To *A. paniculata* For % viable cells on Y-axis and Control, Treated and Doxorubicin on X-axis.

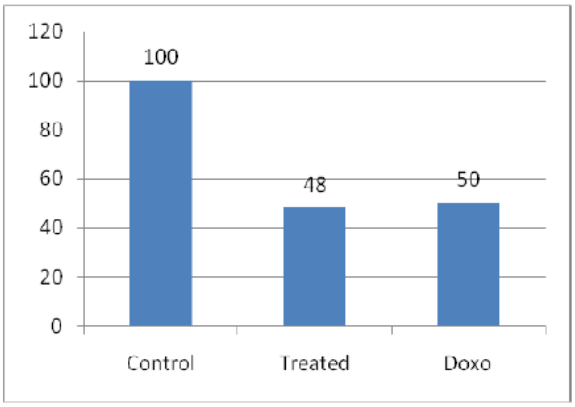

Fig- 8 A549 Cells were treated with Ethanolic extract of *A. Paniculata* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 Conc. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to Trypan Blue Dye. Response of A549 Cell to *A. paniculata*. For % Viable Cells on Y- axis and Control, Treated and Doxorubicin on X-axis.

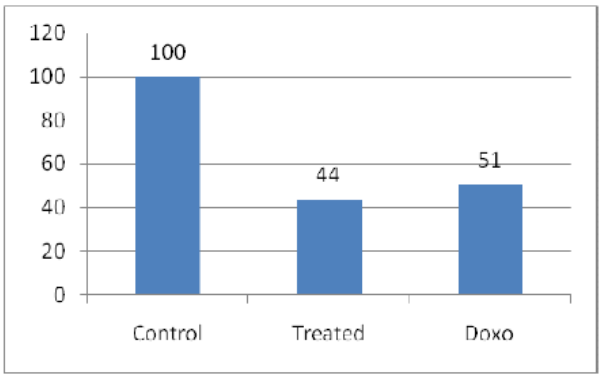

Fig- 9 DU145 Cells were treated with Methanolic extract of *A.paniculata* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 Conc. For 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to Trypan Blue Dye. Response of DU145 Cell to *A.paniculata* For % of Viable cells on Y-axis and Control, Treated and Doxorubicin on X-axis.

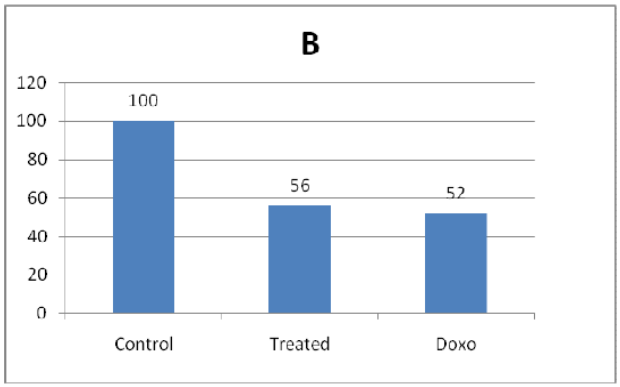

Fig 7. MCF7 Cells were treated with Ethanolic extract of *Aegle marmelos* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 Conc. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to Trypan Blue Dye. Response of MCF7 Cell to *A. marmelos*. For % Viable Cells on Y- axis and Control, Treated and Doxorubicin on X-axis.

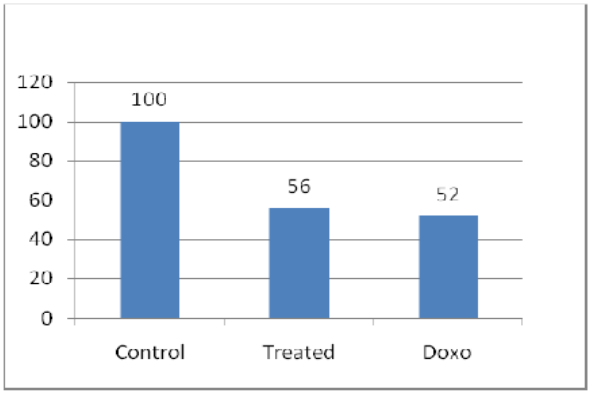

Fig.7.1 A549 Cells were treated with Ethanolic extract of *A.marmelos* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 Conc. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to Trypan Blue Dye. Response of A549 Cell to *A. marmelos*. For % Viable Cells on Y- axis and Control, Treated and Doxorubicin on X-axis.

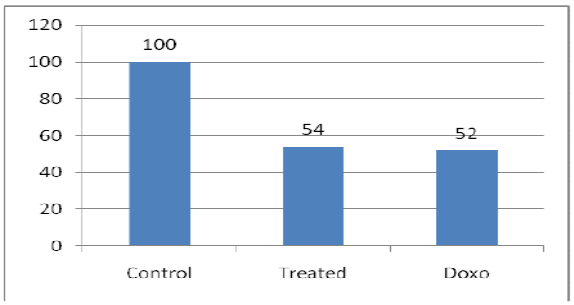

Fig 7.2 DU145 Cells were treated with Ethanolic extract of *A.marmelos* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 Conc. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to Trypan Blue Dye. Response of DU145 Cell to *A. marmelos*. For % Viable Cells on Y- axis and Control, Treated and Doxorubicin on X-axis.

DISCUSSION

In this study Cytotoxic potential of two Indian medicinal plant extracts was investigated. The *invitro* cytotoxic potentiality was investigated as the ability of these two extracts to inhibit tumour cell line growth. With this investigation we had also focused on angiogenesis. The studied cell lines are MCF7, A549 and DU145.

After exposure of cells to plant extracts the cell line were treated with MTT Dye which results into the live cells covert the MTT to purpled colour formazan crystals, which are soluble in Dimethyl sulphoxide (DMSO). After solubilisation of crystals then absorption is taken on spectrophotometer at 570 nm. With respect to readings the graphs were plotted for % inhibition on Y-axis and Conc. of drug on X-axis. The readings were directly converted into percentage. From above graph IC50 value of *A. paniculata* for MCF7, A549, DU145 is less as compare to *A. marmelos* for MCF7, A549, DU145.

In case of Trypan Blue Assay, normal counting of cells were taken before exposing to different drug conc. and after exposing cells to drug Conc. which then directly converted into % of viable cells in which doxorubicin is standard and medium as control was taken, from this it seems that *A. marmelos* showing more inhibition of cell gowth than *A. paniculata* which deals to the cytotoixcity of plant extracts to A549, DU145 and MCF7 cell lines.

Angiogenesis model plays an important role to introduce to vascularisation process that plays an important role to focus on organogenesis and to study antiangiogenesis effect of different drugs. Above graphs and figures shows the how angiogenesis occur and inhibition of vascularisation.

Conclusion:

With respect to above results and discussion it may concluded that the *Aegle marmelos* (Bael Fruit) having more cytotoxicity than *Andrographis paniculata* (Kalmegh) for MCF7, DU145 and A549 cell lines.

Further studies are necessary for chemical characterization of the active principles and more extensive biological evaluations for above cell lines which leads to novel drug innovation.

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