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Research Article

Cytotoxic and Antioxidant Activity of Aqueous Extract of *Neriumindicum* Linn.by *in-vitro* Method

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ABSTRACT

Cancer is a characterized by abnormal cell growth with the potential to invade or other parts of the body. The medical plant extracts and compounds derived from plants have played an important part in the development of novel anticancer drugs. From the literature it was found that the plants belonging to *Apocynaceae* family is well documented for their antioxidant and anticancer potential. Hence in the present study a commonly available plant belonging to the family *Apocynaceae* known as Arali botanically equated *Nerium indicum* Linn., was selected and screened for its antioxidant and anticancer activity of against EAC cell lines employing *in-vitro* studies.Preliminary phytochemical analysis of the drug powder revealed the presence of flavones, Alkaloids, Phenols and Tannin. *in-vitro* antioxidant studies were carried out for the Aqueous extract of the test drug using various free radical model such as a DPPH, Reducing power assay, Inhibition production of Nitric oxide assay, $ABTS^{+}$ and superoxide radical scavengingassay. The result revealed that the extracts have significant antioxidant potentials. *in-vitro* cytotoxic assay such as Trypan blue dye exclusion and MTT assays were carried out against EAC cell lines. The result revealed significant cytotoxic effect of the extract against EAC cells. From the study it was observed that the aqueous extract of *Nerium indicum* Linn.,showed significant free radical scavenging and potent anticancer activity against EAC cell lines.

Key words: Ehrlich Ascite Carcinoma, Nerium indicum Linn, DPPH, Trypan blue, MTT.

INTRODUCTION

Cancer cells are raised due to the uncontrolled cell proliferation.The majority of cancers (90-95%) are due to environmental factors. The remaining 5-10% is due to inherited genetics (Anand *et al*., 2008). Environmental factors that includes are tobacco consumption (25-30%), diet and obesity (30-35%), infections (15-20%), radiation (both ionizing and non-ionizing, upto 10%), stress, lack of physical activity and environmental pollutions (Tolar *et al*., 2003). The major treatment of methodology includes surgery, radiation and chemotherapy therapy. Chemotherapy is considered as a major treatmentmodality for the control of advanced stages of malignancies. All the treatment models have their own side effects. (Madhuri and Pandey, 2009). Despite the development of new drugs, cancer endures torepresent the largest cause of mortality in the worldand claims over 6 million lives every year. (Lawal RA *et al.*, 2012). Medicinal plants have been known to be good sources of effective anticancer drugs. (Cragg and Newman 2005). Over the years, researchers have focused their research on development of anticancer drugs from medicinal plants. The literature survey revealed the *Apocynaceae* plants were well documented for their anticancer activity, Hence in the present *Nerium indicum* Linn., a common plant of *Apocynaceae* was selected for its antioxidant and anticancer potential employing various *in-vitro* methods.

MATERIALS AND METHODS

Plant source selected for the present study was *Nerium indicum* Linn. Leaves of the selected plant was collected from in, around Trichy, identified with the help of Flora of Presidency of Madras (Gamble 1997), and authenticated with the specimen deposited at RAPINAT Herbarium, Department of Botany, St. Joseph's college, Trichy.

Tumor cells

Ehrlich Ascites Carcinoma (EAC) cells were obtained from Amala Cancer Research Centre, Trissur, Kerela, India and they were maintained by weekly intra-peritoneal inoculation of $1X10^6$cells/mouse (Gothosakar *et al.*, 1971).

Preliminary phytochemical screening

Preliminary phytochemical screenings of aqueous extract and drug powder were carried out as per the standard textual procedure.(Brindha *et al*., 1981)

in-Vitro Antioxidant Assay

The Aqueous extract of the *Nerium indicum* Linn., (AENI) was screened for the antioxidant activity employing various *In-vitro* models such as DPPH Radical Scavenging Assay (Gyamfi*et al*.,

2002), Reducing Power Assay (Manmohansighal *et al*., 2011), $ABTS^+$ Radicals Scavenging Activity (Robert Re *et al*., 1999), Nitric Oxide Scavenging Activity (Sreejayan *et al*., 1997) and Superoxide Radical Scavenging Activity (Halliwell, 1985).

Anticancer Screening

in-Vitro Cytotoxicity

The cytotoxic effect of the aqueous extract of the test drug wasevaluated against EAC cell lines using trypan blue exclusion method (Sheeja *et al*., 1997)and MTT assay procedure (Sridharan *et al*., 2012).

RESULTS AND DISCUSSION

Cancer is a group of diseases characterized by the deregulated proliferation of abnormal cells that invade and disrupt surrounding tissues. These tumors are characterized by malignant potential and show growth with an annual incidence of 4-6% death. Due to the increasing clinical awareness and more wide-spread use of diagnostic tools the incidence of cancer has increased annually by 3 to 10% over the last three decades (Bernhard svejdal *et al*., 2010). Over the past few years, cancer has remained a major cause of death and the number of individuals living with cancer is continuing to expand. Due to the toxic and adverse side effects of synthetic drugs, conventional treatments are being failed to provide a fullest cure for cancer. Herbal medicine has made a retort to improve the fulfillment of our present and future health needs. These herbal medicines are the main source of newer chemotherapeutic agents against cancer.

Antioxidant Assay

The preliminary phytochemical screening of the drug powder and aqueous extract revealed the presence of Alkaloid, Flavones, Phenols, Sugar, Quinine, Tannin and Coumarins. DPPH is a stable free radical and the test was performed to access the ability of aqueous extract of *Nerium indicum* Linn to reduce DPPH radical to its corresponding hydrazine. Aqueous extract of the plant was allowed to react with DPPH radicals and its antioxidant potentials were determined by a decrease in its absorbance at 517nm. From Fig 1 it was shown the aqueous extract of test drug scavenge the DPPH radicals. At low concentration of aqueous extract (20µg/ml) showed 20.12% of DPPH scavenging activity where as high concentration (100µg/ml) showed 81.8% of DPPH scavenging activity. The IC50 value was found to be 58µg/ml respectively.In the $ABTS^+$ assay technique $ABTS^+$ cation is produced by incubating the $ABTS^+$ with potassium persulphate. The test is based on the principle that the ability of the extract to scavenge the $ABTS^+$ cation by measuring

the decrease in the absorbance at 700nm. In the $ABTS^{+}$ radical assay, 56.86% scavenging activity was found at the concentration 100µg/ml. The IC50value was found to be 97µg/ml.

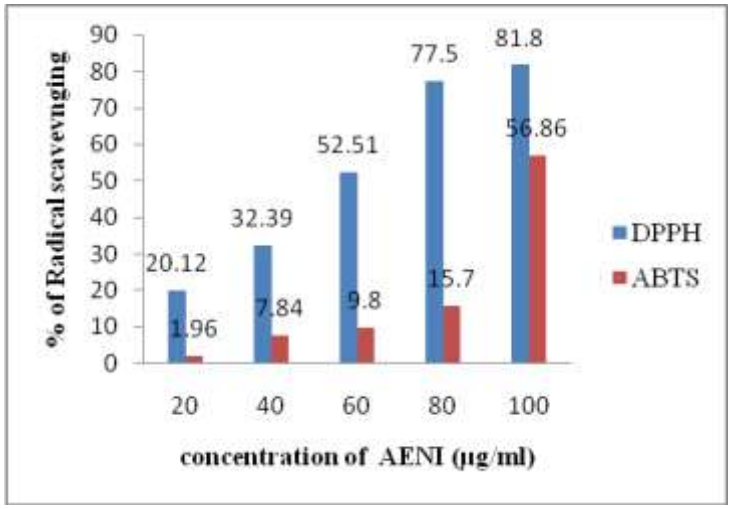

Fig 1: DPPH Radical and ABTS Scavenging potentials of AENI

Fe (III) reduction is often used as an indicator of electron capacity activity, which is an important mechanism of antioxidant activity. In the reducing power assay, the presence of antioxidant in the test drug the produced Fe^{3+} reacts with $FeSo_4$ to form colored complex. Increase absorbance indicated an increase in reducing ability. The IC_{50} value for aqueous extract was found to be 1875µg/ml. Nitric oxide (NO) is produced in the endothelial cells and involved in the regulation of various physiological processes. Excess concentration of Nitric oxide is accompanied with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrate and proxy nitrite anions, which act as free radicals. In this study the extract competes with oxygen to react with nitric acid and thus inhibits the generation of the anions.

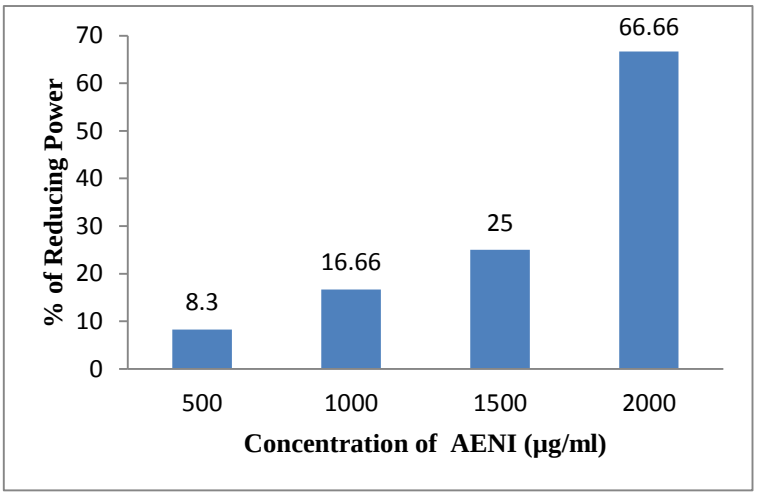

Fig 2:Effect of AENI on Reducing Power Assay

Fig 3 showed the *Nerium indicum* Linn extract at low concentration (100µg/ml) showed 6.66% and high concentration (500µg/ml) showed that 53.33% of no inhibition respectively. The IC_{50} value of aqueous extract was found to be 450µg. The superoxide anion radicals are produced in biological system due to the metabolic pathways. Superoxide radical is potent toxic radical which can damage the cellular molecules leads to the development of various pathological conditions. The Fig 4 was noticed that the AENI is capable of inhibiting the superoxide radical production. The IC_{50} values were found to be 760µg/ml.

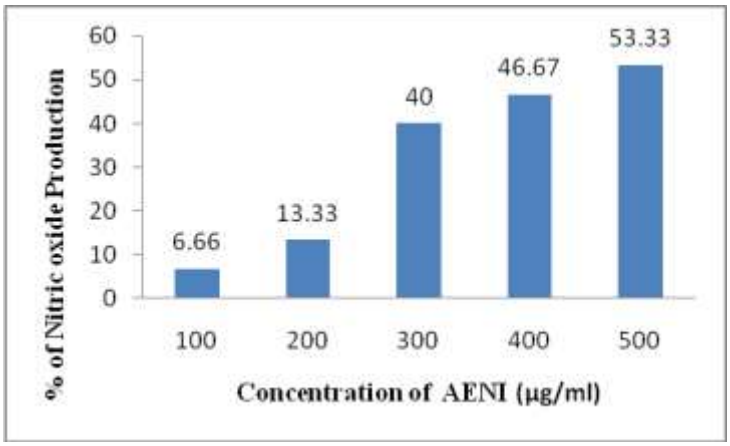

Fig 3:Inhibition of Nitric oxide Production by

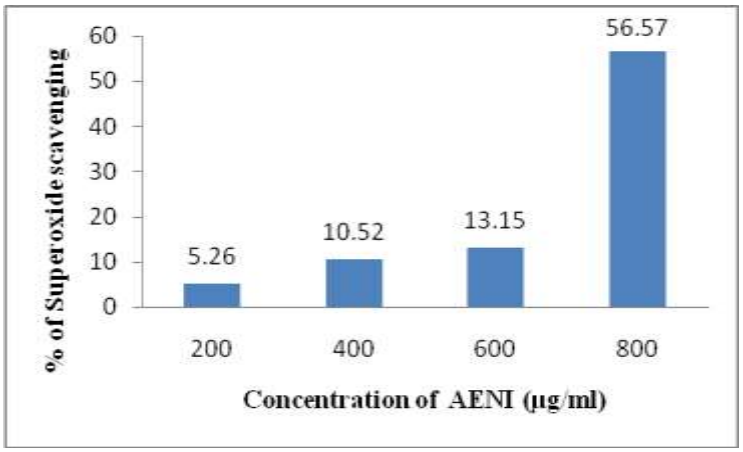

Fig 4: Superoxide scavenging potential of AENI

The trypan blue dye exclusion method based on the principle that, death cell accepts the dye and stain with blue color. The cytotoxic effect of aqueous extract on EAC cell lines were tabulated (Figure 5). The percentage of dead cells statically increased from 100 to 1000µg/ml of AENI and the percentage of viable cellsdecreases with increase in concentration of the plant extract. The

aqueous extract showed 53.4% of cytotoxicity (1000µg) against EAC cell lines. Different concentration of AENI (31.25 to 250µg/ml) was incubated with EAC cell lines for 24 hours and cytotoxicity was determined using MTT assay. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation.

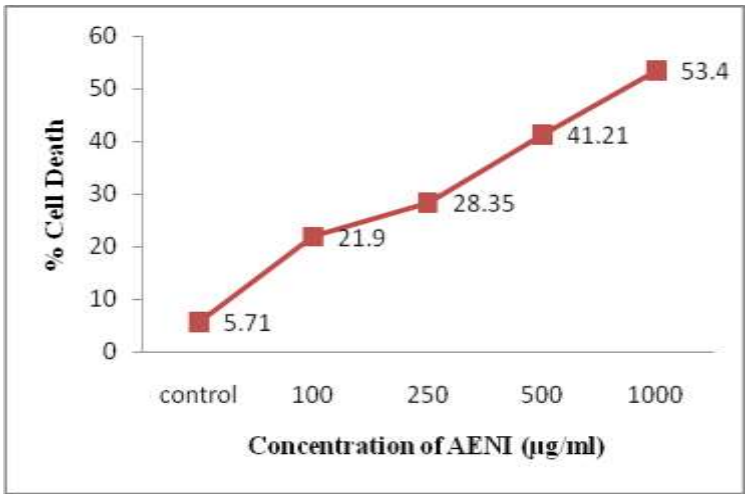

Fig 5: Cytotoxic Effect of AENI on EAC Cells (Trypan blue Method)

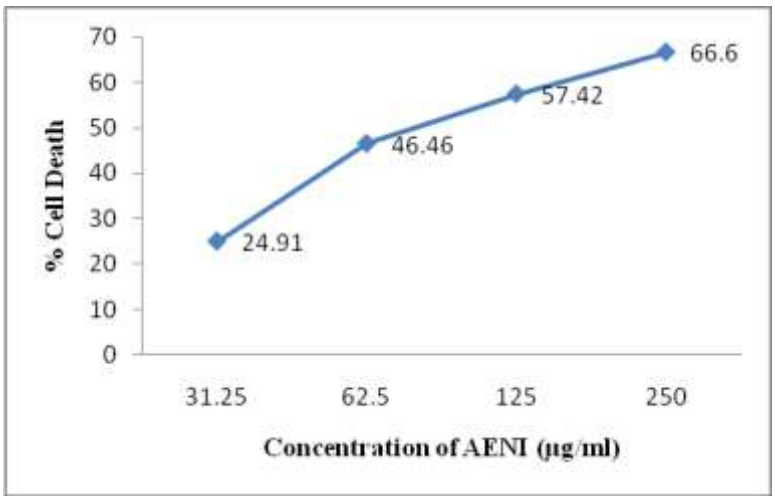

Fig 6: Cytotoxic Effect of AENI on EAC Cells (MTT ASSAY)

The yellow color tetrazolium MTT (3- (4, 5 dimethyl thiazolium-2) – 2, 5 di phenyl tetrazolium bromide) is reduced by metabolically active of cells by the action of mitochondrial dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The intracellular purple formation can be solubilized and quantified by spectrophotometric method. The plant extract (AENI) might have induced apoptosis in the EAC cell resulted in the loss of mitochondrial function which is evident from the decreased production of formazon salt. 66.6% of cytotoxicity was observed at the concentration 250µg/ml and the IC50 value was found to be 105µg/ml.

CONCLUSION

The present study stating that the aqueous leaf extract of *Nerium indicum* Linn showed a significant antioxidant and *in vitro* anti-tumor activity against EAC cells. Further in-depth study, will results in the arrival of safe efficacious anticancer drug from *Nerium indicum* Linn.

REFERENCE

[1] Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB, *Cancer is a preventable disease that requires major lifestyle changes*, Pharm Res, 25(9), 2097-116(2008).

[2] Tolar J and Negli JP, *Tran placental and other routes of cancer transmission between individuals*,J.Pediatr.Hematol.Oncol, 25(6), 403-4, (2003).

[3] Madhuri S and Pandey G, *Some anticancer medicinal plants of foreign origin,Current Science,* 96 (6) 779-783 (2009).

[4] Cragg G. M., Newman D. J. *Plants as a source of anti-cancer agents*,J. Ethnopharmacol,100, 72–79, (2005).

[5] Lawal RA, Ozaslan MD, Odesanmi OS, Karagoz ID, Kilic IH,Ebuehi OAT, *Cytotoxic and antiproliferative activity of Securidacalongepedunculataaqueous extract on Ehrlich ascitescarcinoma cells in Swiss albino mice*, International Journal of Applied Research in Natural Products, 5 (4), 19-27,(2012).

[6] Gamble JS, *Flora of Presidency of Madras,* Botanical Survey of India, II, 1088, (1997).

[7] Brindha P, Sasikala and BhimaRao,*Pharmacognostic studies on Coleus AromaticusBenth*, Indian Borage, B.M.E.B.R, XII, 17-31, (1981).

[8] Gyamfi MA, Yonamine M, Aniya Y, *Free radical scavenging activity of medicinal herb of Ghana: Thonningia sanguine on experimentally induced liver injuries*, Gen Pharmacol, 32, 661-667, (1999).

[9] Manmohan sing, ArindamPul, Hemendrapratap sing, sushilkumarDubey and Kalpesh gaur, *Evaluation of Reducing Power assay of ChalconeSemicarbazones*,J.chem.Pharm.Res, 3(3), 639-645, (2011).

[10] Robert RE, NicolettaPellegrini, Anna Proteggente, AnanthPannala, Min Yang, Catherine Rice-Evans, *Antioxidant activity applying an improved ABTS radical cationdecolorization assay,Free Radical biology & Medicine,* 26, 1231-1237, (1999).

[11] Sreejayan N and Rao MN, *Free radical scavenging activity* by curcummoids,J.PharmPharmaco, 47, 105, (1997).

[12] Halliwell B and Gutteride JMC, *Role of free radicals and catalytic metal ions in human disease: an overview, Methods enzymol,* 186(1), 85, (1990).

[13] Gothoskar SV and Ranadive KJ, Anticancer screening of SAN-AB, *An extract of marking nut Semicarpusanacardium.Indian* J ExpBiol, 2, 58-62, (1971).

[14] Sheeja KR, Kuttan G and Kuttan R, *Cytotoxic and antitumour activity of Berberin,* Amala Res Bull, 17, 73-6 (1997).

[15] Sridharan G, Brindha P, Raja R and AmuthaPriyaR, *in-vitro and in-vivo Cytotoxic Effect of Salvia leucanthacav. against EAC cell lines,* Int J Pharm PharmSci,4(2), 143-146, (2012).

[16] Bernhard Svejdal, Victor Aguiriano-Moser, Sonja Sturm, HaraldHoger, Elisabeth Ingolic, VeronikaSiegl,HermannStuppner and RoswithaPfragner, *Anticancer Activity of novel plant extracts from* ***Trailliaedoxagracilis****(w. w. smith & forest) in human carcinoid krj-i cells,*Anticancer Research, 30, 55-64, (2010).