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An electrochemical perspective assay for anticancer activity of calotropis procera against glioblastoma cell line (LN-18) using Carbon nanotubes- graphene nano- conglomerate as a podium

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ABSTRACT

In this article, we report the pre-screening of anti-cancer effect of *Calotropis procera* against glioblastoma cell lines (LN-18) by means of electrochemical methods. Soxhlet assisted extraction (SAE) has been employed to extract the polyphenol contents present in the leaf of *Calotropis procera*. The phytochemical analysis of the extract has been studied and the polyphenol contents were determined using Folin Ciocalteau method. To study the anticancer effects of the aqueous plant extract, a cytosensor (Gr/NT-G/LN-18) was fabricated and its possible mechanism for DNA binding was studied using graphite/ poly(allylamine hydrochloride)/nanotube-graphene composite /polypyrrole /de-oxy ribonucleic acid (Gr/PAH/NT-G/PPy/DNA) modified electrode. The electrochemical characteristics of the proposed Gr/NT-G/LN-18 cytosensor towards the plant extract were evaluated using electrochemical techniques like cyclic voltammetry and differential pulse voltammetry. Scanning electron microscopy (SEM) and energy dispersive analysis of x-ray (EDAX) have been employed to study the physical characterization of the Gr/PAH/NT-G/PPy/DNA modified electrode. These results indicate that the plant extract has an ability to act as an antiglioblastoma against LN-18 cancer cells.

Keywords: Anticancer agents; biological activity; DNA; LN-18 cells; soxhlet assisted extraction.

Introduction

Cancer is one of the awful diseases which are spreading throughout the world. It is the scariest as it can relapse even the regimen or medication **[1]**. Glioblastoma (GBM) or brain cancer is the most malignant, insidious, and problematical to nurse as they have a high tendency to multiply in size within 10-20 days which is conscientious for 4% of death rate caused by cancer. Triumphant treatment of GBM is exceptional and as a consequence of the present usual treatment results in a mean survival of about a year **[2, 3]**. Attempts have been made for effective antitumor activity against GBM. The varieties of anticancer agents that are available are the consequent of natural sources such as plants (eg., Vinblastine etc), marine organisms (eg., Tamoxifen, Bicalutamide etc) and micro-organisms (e.g., Bryostatins, Apratoxin A etc) **[4]**. Plants have been antiquated as medicines since many years. The medicinal properties of plants confide on the phytochemical constituents that sequel in a distinct physiological agility against human body. The vital active elements of plants are alkaloids, tannin, flavonoid, phenolic compounds, polysaccharides etc. **[5]**. Amongst these, a number of them are suspected to have the capability of antioxidant effects. This antioxidant property is able to commence several biological functions, including antimutagenicity, anticarcinogenecity, etc **[6]**. *Andrographics paniculata*, Soybean, *Dendrosicyos socotrana*, *Biorhythms sensitivum*, *Cassia auriculata*, *Lanata camara*, *Vitex trifolia* etc and many more number of plants are acknowledged to possess anticancer ability **[7]**.

Calotropis procera (Milk weed, Aak) is a shrub belonging to the family Asclepiadaecae. The plant was

considered as toxic, whereas it has a wide variety of medicinal properties used against ailments like chronic inflammatory disease, in the treatment of cold, cough, pathological hemorrhoids, ulcers, gastroenteritis, cardiovascular diseases, Hansen's disease, rheumatism and disorders of skin, spleen, liver and abdomen **[8-10]**. The latex of this plant, publicized to contain anticancer, antiulcer, antihyperglycemic and anti-inflammatory activities **[11]** while the alcoholic root extract was also documented to contain anti-cancer property **[12]**. In addition, the plant has also reported to suppress the growth of tumor in Breast cancer **[13]**.

It is important to select and employ a stable and rapid method to assay the anti-cancer activity. The *in-vivo* and *in-vitro* screening of the natural extracts or synthesized drugs are widely studied on whole animals' and cancer cell lines have been the authentic model **[10, 11]**. These methods of screening are expensive, ethical and time constriction. There is an alarming necessity for the development of analytical techniques that can solve the above mentioned problems. Electrochemical methods are one such tool which can substitute the expensive methods for the screening of anticancer properties. The electrochemical methods are gaining popularity due to its sensitivity, low cost and relatively short analysis time contrast to the other methods **[14]**. Capecitabine, organogermalactones, tirapazamine, berbine etc. are some of the anticancer compounds or drugs used for the electrochemical determination of anti-cancer activity **[14-17]**. There are some interesting reports on studying the interaction of the immobilized cancer cells with drug for the detection of anticancer activity by utilizing the electrochemical methods **[18, 19]**.

This study demonstrates pre-screening of anti-cancer effect of *Calotropis procera* using simple electrochemical methods. The electrochemical methods can be a used as an alternate for the screening of anticancer properties of drugs. In the present work, we have extracted the phytochemicals constituents of *Calotropis procera* by Soxhlet assisted extraction method. The phytochemical extract was analyzed for anticancer property against LN-18 cells using electrochemical methods. Its possible DNA binding mechanism was also examined. To the best our knowledge, electrochemical anticancer detection of aqueous extract of *Calotropis procera* against LN-18 cancer cell line has not been reported so far. The development of cell line biosensor and DNA biosensor used in this study was simple to fabricate. Electrochemical techniques such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were employed to characterize and to study the effect of plant phytochemicals on the developed biosensor. The surface characterization of the modified electrodes was examined using scanning electron micrograph.

Experimental

Reagents and materials

DNA from herring sperm was procured from HiMedia, India. Multiwalled carbon nanotubes (MWCNTs) and poly (allylamine hydrochloride) (PAH), Dulbecco's Modified Eagle's Medium (DMEM) were obtained from Sigma aldrich. Tris HCl, Gallic acid, Folin ciocalteu (FC) reagents were purchased from Loba Chemie, India. Pyrrole was obtained from Spectrochem, India. Silica thin layer chromatography (TLC) plates were obtained from Merck, USA. PK-3 electrode polishing kit containing 1 µm aqueous polishing diamond and 0.05 µm polishing alumina was obtained from BAS Inc. Tokyo, Japan. Phosphate buffer saline was prepared from stock solution of 0.1 M KH_2PO_4, 0.1 M K_2HPO_4 and 0.1 M KCl. pH was adjusted using 0.5 M HCl and 0.5 M NaOH. The NT-G composite was prepared following a procedure as reported elsewhere **[20]**. The obtained multiwalled nanotube graphene (NT-G) composite was dispersed in 8 µL of 5 wt% Nafion solution along with 1 mL of pure water and sonicated for 10-15 min. All other chemicals used were of analytical reagent grade unless otherwise mentioned and used without further purification. All solutions were prepared with pure water.

Electrochemical measurements

All electrochemical experiments were carried out with Versa stat 3 (Princeton Applied Research, USA). The field emission scanning electron microscope (FE-SEM), model Hitachi S-4800II with accelerating voltage of 5 kV was utilized to observe the surface morphologies of the electrodes and the elemental analysis of the electrodes were determined by energy dispersive analysis of x-ray (EDAX). All experiments were done in a conventional three electrode electrochemical cell with bare Graphite (Gr) or LN-18 cell modified or DNA modified electrode as working electrode, saturated calomel electrode as reference electrode and platinum wire as auxiliary electrode.

Cell culture

LN-18 cells were cultured in DMEM media and were maintained at 37ºC in 5 % humidified CO_2 incubator. Trypsin- ethylene-diaminetetraacetic acid (EDTA) was then added to remove the cells from the bottom and place them in incubator. The LN-18 cells were centrifuged to 1000 rpm for 20 s and were used for the electrochemical studies.

Extraction of phytochemicals constituents

The taxonomical identification of the plant sample (*Calotropis procera*) was done with the help of professional Botanist. Based on these taxonomical characteristics, plant material was collected from the Local area. A voucher specimen has been deposited in the herbarium of the Botany department, NMKRV College for our future reference. The fresh leaves of about 1 kg were cut, cleaned with water and air dried. The dried plant material weighed about 750 g was ground to powder and stored in airtight bottles until its use. The powdered plant material was weighed and about 50 g was taken and the active components was extracted by Soxhlet method in 300 mL of autoclaved sterilized water for 48 hrs **[21]** and then filtered. To, the best of our knowledge, Soxhlet assisted extraction method tends to yield a sterilized product. The extracted chemical constituents exhibited long term stability when stored at room temperature. Further the shelf-life can be extended if stored at lower temperature (4ºC). The plant extract (PE) of SAE is denoted as SAPE. The filtered extract was filled in airtight bottle and stored in

refrigerator which was used for the electrochemical detection of anticancer properties.

Preparation of the Gr/NT-G/LN-18 cells and Gr/PAH/NT-G/PPy/DNA modified electrode

An electrode was prepared by drilling a Teflon bar to 0.5 mm diameter and introducing a Graphite (Gr) cylinder into it. An electrical contact was made with a copper wire through the centre of the Teflon bar. Prior to modification, the Gr electrode was polished to get a mirror shining surface by means of PK-3 electrode polishing kit. It was then ultrasonicated for several minutes and rinsed with pure water to remove adhered impurities.

For the fabrication of cytosensor- the cleaned Gr electrode was drop-casted with 5 µl of NT-G composite and dried out for 20 min at 28 ºC. 1 ml of LN-18 cells was centrifuged to 1000 rpm for 20 s and the supernatant was discarded. Then, the pellet was redispersed with 1 mL of PBS. From this 10 µL was taken into eppendorf tube to which 10 µL of 0.4% trypan blue solution was added and swirl slowly. 10 µL of this mixture is dropped into the hemocytometer and the numbers of viable cells were counted.

The numbers of cells were calculated as follows- Cells/mL= the average count per square × dilution factor $\times 10^4$ which was found to be 3×10^{-6} cells/ mL and Total cell number = cells/mL × original of the fluid from which sample was removed. This was found to be 3×10^{-7} total cells.

Finally LN-18 glioblastoma cells were grown on the surface of the modified electrode in an incubator for 2 days for proper attachment of the cancer cells to the surface. The approximate number of cancer cells grown on 6 mm of the Gr/NT-G was found to be 300 total cells. Thus obtained electrode was denoted as Gr/NT-G/LN-18 electrode.

Similarly, to fabricate the DNA biosensor, the cleaned Gr surface was wrapped with PAH, a polycation by dipping the electrode in the PAH solution (2 mg mL^{-1} of 0.2 M NaCl) **[22]** for about 20 min to make the negatively charged Gr surface positive and for effortless affixing 10 µL of NT-G composite was drop-casted onto the Gr/PAH electrode surface. Subsequently, electro-polymerization of polypyrrole (PPy) was performed on the above obtained Gr/PAH/NT-G electrode. As mentioned earlier PPy acts as an immobilizing matrix for DNA. The electro-polymerization bath was prepared according to a reported literature **[23]** with slight modification of using acetonitrile. Briefly, The electrode was subsequently immersed in 10 mL of acetonitrile solution containing 0.1 M pyrrole and 0.1 M $MgCl_2$. Acetonitrile increases the amount and quality of electro polymerization of PPy **[24]**. CV was performed on the electrode surface with a potential range from – 0.3 to 0.80 V (versus SCE) at a scan rate of 50 mV s^{-1} for 20 cycles. Thus obtained Gr/PAH/NT-G/PPy electrode was washed gently with water to remove any unadsorbed particles. The electro-polymerized electrode was then subjected to oxidation by employing chronoamperometry in 20 mM $MgCl_2$ and 10 mM Tris-HCl buffer (pH 7.2) at 0.5 V (versus SCE) for 600 s. Galvanostatic electrodeposition was implemented for immobilization of DNA (60 µg mL^{-1} in 0.1 M PBS of pH- 7) **[25]** at 0.8 V for about 600 s. The electrode was incubated at – 4°C for overnight. The as obtained electrode is denoted as Gr/PAH/NT-G/PPy/DNA throughout this manuscript.

Results and discussion

The preliminary investigation of plant phenolics such as alkaloids, flavonoids, saponins, steroids, tannins, triterpenoids, phenols, anthraquinones, proteins, quinones present in the aqueous extract was evaluated using standard protocols **[5, 9]**. The results revealed that the aqueous extract of SAPE contains falvonoids, saponins, steroids and anthraquinones. It is noteworthy to mention that these phytochemical agents have been reported to contain anticancer activity **[26-30].**

SEM and EDAX were performed to characterize the structural and chemical composition of Gr/PPy/DNA, Gr/PAH/NT-G and Gr/PAH/NT-G/PPy/DNA electrodes. Inset of **Fig. 1A** shows the electro-polymerized DNA grown on the positively charged polycation PPy modified Gr electrode. From the figure, it is clear that the electrodeposition of DNA forms a uniform surface of double helical structure throughout the electrode surface. The inset image depicts the helical structures entwined together to form a flower-like pattern. The filaments which are crumpled together in large and quite supple loops may be due to its end-to-end aggregations. From the SEM image of Gr/PAH/NT-G composite (Inset **Fig. 1B**) it can be witnessed that the individual or pairs of carbon nano-ribbons recline jumbled on the surface of the Gr/PAH layer. The appearance of the craggy like structures, in and around the ensquared regions suggests that the graphene has torn out from the walls of the CNTs giving an evidence of development of NT-G composite and its adsorption on the PAH modified bare Gr electrode surface. The proposed Gr/PAH/NT-G/PPy/DNA biosensor (Inset **Fig. 1C**) demonstrates that the NT-G composite unite with each other as tiny granules which can be visualized throughout the surface as dark spots, whereas long filaments of DNA were intertwined. It could be predicted that the DNA may have interacted with Π- Π stacking of grapheme **[31]** assisting in good binding and the covalent bonding between the carboxyl groups of CNT **[32]**. Further, to validate the binding of DNA onto NT-G composite, FTIR was recorded using Jasco FTIR 1400 (Japan) and is as illustrated in **Fig. 1D**. As observed, the FTIR spectrum exhibits three characteristic bands resultant to the presence of DNA. A typical peak at 1216 cm^{-1} assigned to C-N group whereas a peak at 2616 cm^{-1} corresponds to C-H bond and a C=C band centered at 1496 cm^{-1} correlating to the presence of aromatic group. The existence of MWCNTs was witnessed by the presence of a carboxylic group (C=O stretching at 1732 cm^{-1}) and a peak at around 2832 cm^{-1} corresponds to COOH**.** From this it can be confirmed that the DNA has carpeting NT-G composite indicating the successful immobilization and modification of the electrode.

EDAX was employed to study the elemental composition of modified electrodes. The carbon, oxygen, nitrogen, sodium and phosphorous are the major constituents of DNA (**Fig. 1A**). This also appends well with SEM indicating the electrodeposition of DNA. **Fig. 1B** shows the existence of NT-G composite in PAH films. The carbon

and oxygen were the major elements of NT-G composite as tabulated in the inset of **Fig. 1**. The nitrogen groups results from reactions of ammonia with the oxygen functionalities, during the annealing process of synthesis of NT-G composite **[20]**. The fluorine content in the NT-G composite may be due to presence of nafion **[33]**. The same can also be perceived in the Gr/PAH/NT-G/PPy/DNA biosensor (**Fig. 1C**), along with the peaks of C, O, and N relate to the combination of NT-G composite and DNA. Whereas the K and Na peaks may arise due to the presence of DNA. This also gives us a confirmation for successful development of the Gr/PAH/NT-G/PPy/DNA electrode.

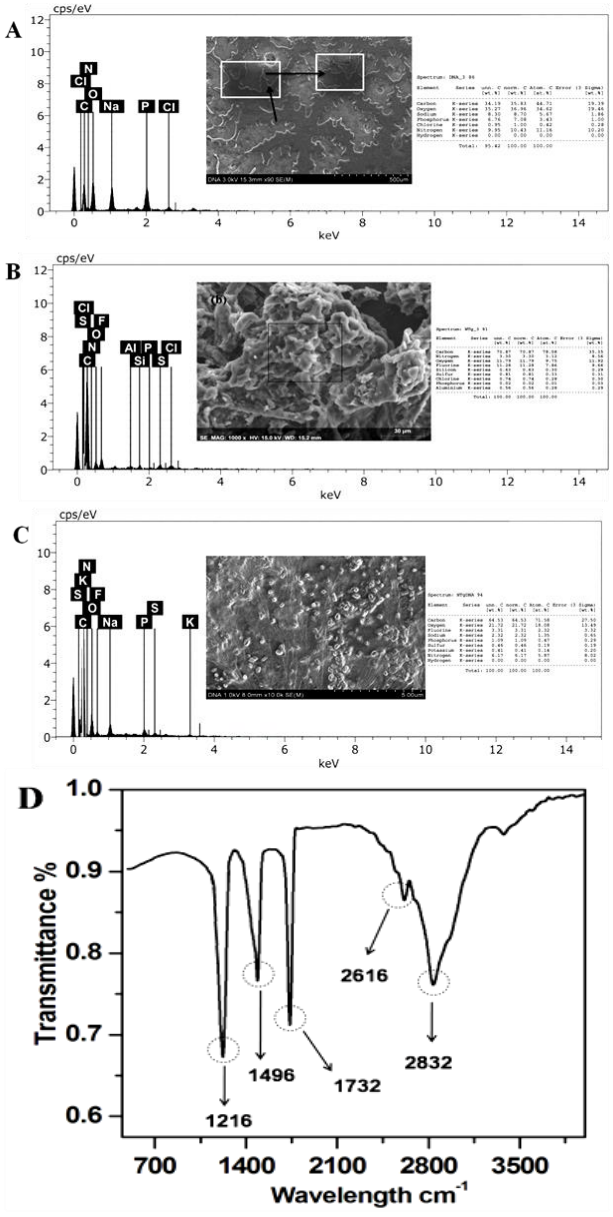

Fig. 1. SEM images of (A) Gr/PAH/PPy/DNA (B) Gr/PAH/NT-G and (C) Gr/PAH/NT-G/PPy/DNA. Inset: EDAX spectrum recorded for the electrodes (A) Gr/PAH/PPy/DNA (B) Gr/PAH/NT-G and (C) Gr/PAH/NT-G/PPy/DNA. Inset- relative elemental composition of the modified electrodes determined from EDAX. (D) FTIR spectra of Gr/PAH/NT-G/PPy/DNA.

Electrochemical characterization of the electrodes was carried out in order to see the effect of each layer on the total charge of the potential surface. CV and impedance studies were carried out to study the electrochemical behavior in presence of 0.1 M PBS containing 5 mM [Fe $(CN)_6]^{3-/4-}$ solution. **Fig. 2A** exhibits CVs recorded for each consecutive modification of Gr/NT-G/LN-18 modified electrode whereas **Fig. 2B** for Gr/PAH/NT-G/PPy/DNA electrode. A well-established redox peaks were observed for the shuttling of [Fe $(CN)_6]^{3-/4-}$ ions from the solution to the electrode surface. The CV of bare graphite (Gr) electrode (**Fig. 2A** curve a) depicts a sluggish electrochemical reaction with peak to peak separation ΔE_p of 187 mV which can be due to the unmodified surface of the electrode that exerts a barrier for the transfer of [Fe $(CN)_6]^{3-/4-}$ ions. On the other hand, in Gr/NT-G electrode, the redox current increased [**Fig. 2A(b)**] which can be attributed to the presence of NT-G composite affording a commendatory of [Fe $(CN)_6]^{3-/4-}$ ions. In contrast, Gr/NT-G/LN-18 cancer cell modified electrode [**Fig. 2A(c)**] resulted in decrease in peak current with concomitant increase in peak separation of 144 mV which may be attributed to the resistance of the cell membrane to [Fe $(CN)_6]^{3-/4-}$ ions as the surface charge of cells is negative due to the presence of phosphate and carboxyl groups. In comparison CV studies of Gr/PAH/NT-G/PPy/DNA electrode (**Fig. 2B**) demonstrates a similar behavior as of bare Gr electrode [**Fig. 2 B(a)**] and Gr/PAH/NT-G [**Fig. 2B(c)**]. Conversely, in Gr/PPy/DNA electrode, peak current decreased [**Fig. 2 B(b)**] because the DNA molecule is negatively charged and it repels the negative [Fe $(CN)_6]^{3-/4-}$ ions with a ΔE_p of 172 mV. Evidently, as seen in **Fig. 2B(d)** the Gr/PAH/NT-G/PPy/DNA biosensor showed a slight decrease in the redox current with ΔE_p of 164 mV which may be due to the combined effects of NT-G and DNA with conductivity and no conductivity respectively which has lead to the moderate peak potential. In contrast the ΔE_p of Gr/PAH/NT-G is higher than that of the other electrodes indicating the more adsorption of the ferricyanide ions compared to other electrodes.

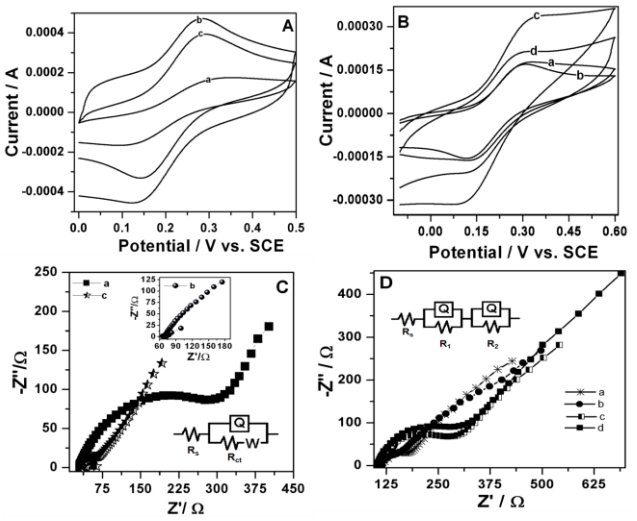

Fig. 2. **(**A) CVs obtained for (a) bare Gr electrode (b) Gr/NTG and (C) Gr/NT-G/LN-18 cancer cell electrodes in 0.1 M PBS containing 5 mM [Fe $(CN)_6]^{3-/4-}$ solution (scan rate 50 mV/s) (B) CV obtained for (a) bare Gr electrode (b) (Gr/PAH/PPy/DNA (c) Gr/PAH/NT-G and (d) Gr/PAH/NT-G/PPy/DNA electrodes with same parameters as that of (A). (C) and (D) Nyquist Plots for electrochemical impedance measurements for the A and B respectively. Inset- equivalent circuit describing the impedance responses for the modified electrodes.

Impedance spectroscopy is a tool enabled for the significant determination of kinetic and diffusion parameters **[34]**. Impedance experiments were performed using the formal potential obtained from the CVs profiles **(Fig. 2 A and B).** The results obtained with CV are supported with the impedance nyquist plots. The electrochemical behavior of transfer of ions at the electrode/ electrolyte interface can be interpreted by fitting the data to the electronic equivalent circuits that relates to the practically obtained impedance spectra. The values obtained from the simulation for fitting the impedance spectra is given in **Table 1**. As shown in **Fig. 2C** and **D**, the nyquist plots exhibits a semicircle part and a linear part. The sigmodial semicircle at higher frequencies corresponds to the electron transfer acquired by the extremely porous electrode/electrolyte interface.

Table 1A. Parameters obtained from the simulation of impedance responses using equivalent circuit of (a) bare graphite electrode (b) Gr/NT-G and (c) Gr/NT-G/LN-18 electrodes in 0.1 M PBS containing 5 mM $[Fe(CN)_6]^{3-/4-}$ solution.

Electrode	R_s (Ω)	R_{ct} (Ω)	Q	W
Bare Gr	33.44	1466	0.0001	5.40E-03
Gr/NT-G	37.38	69.83	0.005	6.63E-03
Gr/NT-G/LN-18	34.66	373.8	0.006	6.32E-03

Table 1B. Parameters obtained from the simulation of impedance responses using equivalent circuit of (a) bare graphite electrode (b) Gr/PPy/DNA (c) Gr/PAH/NT-G and (d) Gr/PAH/NT-G/PPy/DNA electrodes in 0.1 M PBS containing 5 mM $[Fe(CN)_6]^{3-/4-}$ solution.

Electrode	R_s (Ω)	R^1 (kΩ)	Q_1 (μF cm^{-2})	n_1	R^2 (Ω)	Q^2 (μF cm^{-2})	n_2
Bare Gr	1.37	116.5	3000	0.8	49.3	0.48	0.8
Gr/PPy/DNA	1.67	117.6	0.769	0.8	2887	2.3	0.4
Gr/PAH/ NT-G	0.15	113.9	0.581	0.8	1503	2.9	0.8
Gr/PAH/NT-G/PPy/DNA	0.19	115.5	0.03	1	2382	1.6	0.8

This is because of the accumulation of opposing ions are drastically affected by the pore structures **[35]**. The linear part of slope is the low frequency range indicating diffusion- limited process. The electronic circuit which fits to the data obtained to the cancer cell modified electrode and DNA modified electrode was R_s (Q_1 (R_1W)) and R_s (Q_1 (R_1 (Q_2R_2))) respectively. R_s contributes to solution resistance, "R^1 and R^2" are charge transfer resistance, Q_1 and Q_2 are constant phase elements (CPE) for two electron transfer processes, n is the number of electrons transferred. Q_2 is a double-layer capacitance evolved at the interior pores in a frequency not affected by the porosity of the electrode surface, whereas Q_1 is the double-layer capacitance developed at the outer pores of the electrode surface and W is the Warburg resistance **[36]**. The decrease in R_1 observed in Gr/NT-G composite compared to Gr may be attributed to the ability of NT-G composite to pass electrons through it. When LN-18 cells were allowed to grow on the NT-G modified electrode, the R_{ct} increased remarkably which can be attributed to LN-18 cells that upset the interfacial electron transfer. The ability of the electrode to resist the $[Fe(CN)_6]^{3-/4-}$ ions leads to increase in R_s and R_1. This can be interpreted as the Gr/PAH/NT-G composite, (**Fig. 2D**) with high peak current showed decrease in R_1 and R_s. Whereas the R_1 and R_s values of the Gr/PPy/DNA and Gr/PAH/NT-G/PPy/DNA increased implying that DNA hinders the electron transfer at the electrode surface. The CPEs increase when the electrode surface acts as a conductor allowing the movement of the ions to pass into it. From the data obtained and as seen in the table the Q_1 (μF cm^{-2}) of Gr/PAH/NT-G (0.76) > Gr/PAH/NT-G/ PPy/DNA (0.58) > Bare Gr (0.4) > Gr/PPy/DNA (0.03). The Q_2 acts just like a resistor. The 'n' values of CPEs are smaller than 1 representing that the electrode surface is rough and porous. From the results obtained and from the **Table 1**, it can be inferred as the successful modification of biosensors.

The total polyphenol content (TPC) of the plant extract was measured using standard spectrophotometric Folin-Ciocalteau method **[37]** using Gallic acid as standard. The calibration curve of absorbance vs. concentration of standard was used to calculate the TPC (Figure not shown). Results were expressed as mg Gallic acid equivalents per liter of SAPE (mg GAE/L). The TPC present in the sample of 20 μL of SAPE was found to be 500 mg GAE/L. 10 μL of *Calotropis procera* PE was drop casted onto the cleaned bare Gr electrode surface and was allowed to dry. The dried electrode was dipped in a 0.1 M PBS pH 7 and CV was carried out with a potential range from 1 to –1 V and after successive readings –0.5 to 0.7 V was set for the electro-catalytic characterization of the PE. In which, the *Calotropis procera* PE modified electrode (**Fig. 3A**) was found to give one anodic peak (a^1 at 0.4 mV) and two cathodic peaks (C^1 at –0.2 mV and C^2 at 0.1 mV). From this, it can be attributed that the *Calotropis procera* PE may contain hydroxyl groups which tend to undergo oxidation in the electrochemical reaction. This anodic peak at 0.345 V can be ascribed to the presence of oxidation centers present in polyphenolic molecules or to the 3', 4'-dihydroxyl moiety at B ring (catechol moiety) **[38]**. The cathodic peaks may also indicate the presence of catechol group.

Based on these results we carried out DPV studies using platinum (Pt) as a working electrode in 0.1 M PBS (pH 7). The typical DPV voltammograms shown in **Fig. 3B** exhibits an oxidation peak which tends to increase linearly upon increasing the concentration of SAPE in the range of 1 μM to 8 μM and the current response of PE is almost constant with the increasing the PE contents. The results demonstrate that the PE contains electro-active molecules which are helpful for the electrochemical redox reactions of the PE. For the regression plot of I_p vs SAPE concentration the slope is 5.31 μM, intercept is –0.01 μA and correlation coefficient is 0.997.

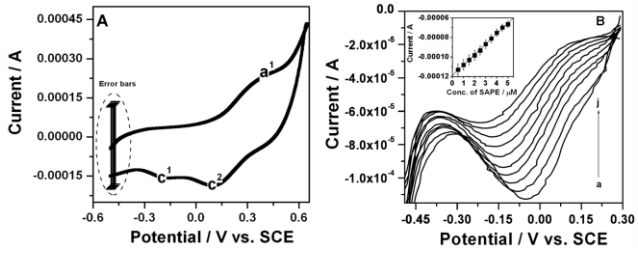

Fig. 3. (A) Electrochemical response of plant extract modified electrode in 0.1 M PBS. (B) DPV studies of increasing concentration of SAPE. The error bar represents the standard deviation.

The CV of Gr/NT-G/LN-18 cytosensor is as shown in **Fig. 4A**. The CV demonstrates a quasi-irreversible process with an oxidative peak at around 0.6 V and a reduction

peak at 0.05 V. The anodic peak is due to the existence of guanine present in cytoplasm of the living cells [**39**]. This CV studies revealed the established behavior of LN-18 cell lines indicating its successful immobilization on the NT-G composite. The effect of scan rate was also determined and is as shown **Fig. 4B** as the scan rate increased from 10 mV s^{-1} to 100 mV s^{-1} the peak current also increased. Further from the calibration plot of square root vs peak current, the electrode system indicates a diffusion controlled process. To examine the effect of PE against LN-18 glioblastoma cells, CV studies were carried out in 0.1 M PBS with increasing concentration of PE. As the concentration of SAPE increased the peak current decreased indicating that it has the ability to suppress the viability and proliferation of LN-18 cells. **Fig. 4C** displays the DPV signals of the Gr/NT-G/LN-18 in presence of increasing concentration of PE. As the concentration of the PE increased, the peak current decreased notably and had a propensity toward a constant value after the concentration had reached 25 µgmL^{-1}. This decrease in peak current suggested that SAPE could restrict the viability of LN-18 cancer cells. From these results it can be attributed that the decreasing peak current depended on the SAPE concentration, which indicated that electrochemical techniques could be used as anticancer screening test.

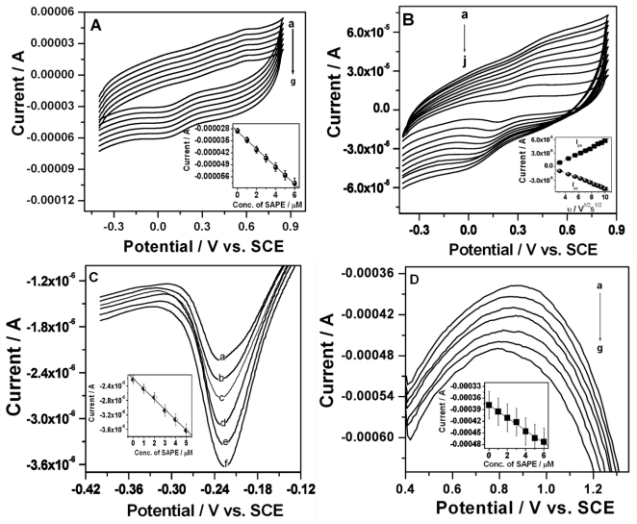

Fig. 4. (A) CV of LN-18 cells treated with varying concentrations of SAPE. Inset- Linear plot of reduction current as a function of concentration. (B) Effect of scan rate on the cytosensor (a-j: 10 mV s^{-1} to 100 mV s^{-1}) in 0.1 M PBS. (C) DPV of the cytosensor with varying concentration of SAPE. Inset- Linear plot of reduction current as a function of concentration of SAPE. (D) Effect of varying concentration of SAPE on DNA modified electrode studied using DPV. The error bar represents the standard deviation.

The possible mechanism of SAPE binding to DNA was also studied. Anticancer drugs interact with DNA preventing the proper relaxation of DNA. The discovery of anticancer drugs requires a characterization in which the compounds can target DNA. Clinically DNA binding compounds corresponds to anticancer molecules which can take part either by binding or by modifying the DNA [**40**]. We have carried out DPV studies to detect the anticancer properties of PE's. After subsequent trail and errors 0.4-1.2 V was set as the potential window. As shown in **Fig. 4D**, the peak current at 0.8 V decreased with increasing concentration of PE's (1-7 µM). The peak potential is in good consistent with DNA sensor [**41**]. The decrease may be due to the SAPE molecules intercalating DNA or in other words the ROS in SAPE species getting oxidized at the DNA surface is difficult and is also arduous getting the reduced ROS species out of the DNA. The PE molecules act like adsorbed molecules inside the DNA cavities and it does not require any electrochemical activation energy to get reduced inside the DNA. This results in surplus anions production at the DNA surface due to the reduction of SAPE molecules or ROS leading to the decrease in peak current.

Conclusion

The present work demonstrates a simple method of pre-screening of anticancer effects of *Calotropis procera*. Furthermore, Folin ciocalteau spectrophotometric analysis of *Calotropis procera* plant extract was carried out to evaluate the polyphenols content. The electrochemical experiments revealed that fabricated Gr/NT-G/LN-18 cancer cell and DNA biosensor interacts efficiently with the phytochemical constituents of the SAPE. The DNA binding properties of the PE demonstrates its beneficial characteristics towards cancer therapy. The developed electrode was simpler and the screening technique using electrochemistry is much simpler compared to conventional methods which are expensive, ethical and time consuming. In addition, taking the intercalating properties of *Calotropis procera* of PE into consideration it can be declared that it has potential application and can be incorporated in developing anticancer drug.

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