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IMPACT OF CARDANOL BASED A - NAPTHALAMINE LIGAND WITH FOUR SELECTED METAL COMPLEXES ANTICANCER POTENTIAL AGAINST THE HELA CELL LINES

Chemistry

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ABSTRACT

The usage of schiff base complex against new remedial agent's therefore it has been recently received more attention as a strategy to combat infections caused by cancer cells. The current study focusing a Schiff bases complex named as Cardanol based α- Napthalamine as a new therapeutic constituent, currently catching the interest of medical chemists. The cardanol Schiff bases are extensively developed from CNSL and its five different metals complexes in the area of medicinal science. Cardanol is a remarkable tetradentate alcohol that was synthesised and analyzed with its Schiff base ligand molecules as well as its complexes with Cu, Co, Ni, and Zr. Schiff base ligand molecules as well as its complexes with Cu, Co, Ni, and Zr.. The sterochemistry and binding mode of the experimental complexes were determined using a variety of physicochemical and spectroscopic techniques, such as elemental and thermal analyses, molar conductance and magnetic susceptibility measurements, such as 1H NMR, 13C NMR, and HRMS spectrum studies. Additionally, the improvement potential impact of apoptotic cell death on HeLa cancer cell lines was also demonstrated such as in all four experimental combinations. While subjected to DFMPMANA-Cu complex molecule Compared with the control untreated cells, the mainstream of the HeLa cells are transformed from spindle to star-shaped, some of which become prepared for damage and irregular shrink morphology. However, treated cell lines showed the morphological changes are directly proportional to a concentration of a sample dosage and duration of the action dependent. Apart from the present research clearly indicated that amongst the four experimental complexes such as cobalt, nickel, copper and zirconium, copper complex possessed significant anticancer potential against the Hela cell lines.

KEYWORDS

Cardanol, DFMPM, α- Napthalamine, Schiff base, HeLa cancer cell

Introduction

Schiff bases are versatile organic compounds which are widely used and synthesized by condensation reaction of different amino compound with aldehydes or ketones known as imine. Schiff base ligands are considered as privileged ligands as they are simply synthesized by condensation .Compounds containing an azomethine group (-CH=—), known as Schiff bases are formed by the condensation of a primary amine with a carbonyl compound (Gupta and Sutar, 2008). Very recently Sreenu et al. (2022) proposed about the Schiff bases of aliphatic aldehydes are relatively unstable and are readily polymerizable while those of aromatic aldehydes, having an effective conjugation system, are more stable described (Wail Al Zoubi, 2013). Schiff base compounds and their metal complexes have been extensively investigated due to their wide range of biomedical applications (Laila et al., 2022). Previously, It has for medications to treat numerous infectious and non-infectious diseases is at an all-time high right now, and advancement in this area is inevitable given the profound impact it has on the lives of all members of the human race. An earlier approach (Issac et al., 2011) reported that cardanol .The primary goal of the current study is to highlight the study affect experimental ligands and complexes under in vitro biological screening. The susceptibility of the pathogenic microorganism has been evaluated using the produced ligand and complexes against eight bacterial species and four fungal organisms in order to locate the most potent therapeutic molecule. In contrast, the focus of this research has been mostly on the cardanol-based DFMPMANA metal ligand and complexes (Shilpa et al., 2005).Since no one research was done the similar ligand with four selected complex so the current work was framed this objective such as anticancer activity of DFMPMANA ligand and its experimental complexes.

MATERIALS AND METHODS

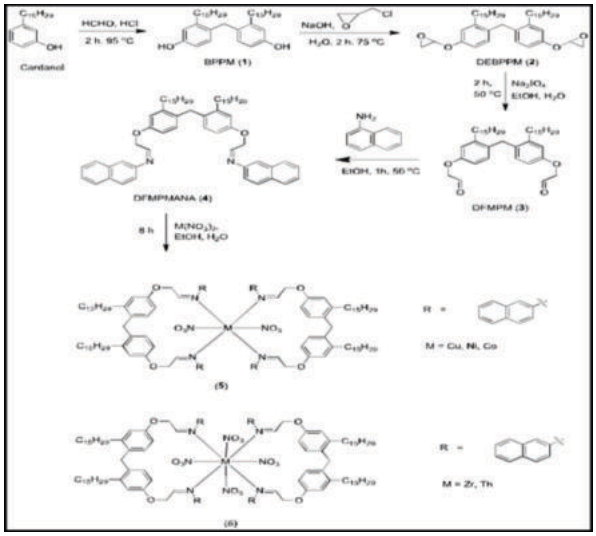
Synthesis of cardanol consisting α- Napthylamine Schiff based ligand

As per the earlier method stated (Isac et al., 2011), the cardanol (497 mmol, 150 g) has been taken together with formaldehyde (8 mL) in the presence of HCl (20 mL) is kept for 2 hrs at 95 ºC to yield a red colour liquid (85%) the bis (3-pentadecenylphenol) methane (BPPM). Further, this BPPM (100 mmol, 61.5 g) with epichlorohydrine (18.6 mL) in the presence of NaOH (200 mmol, 8 g) kept for 2 hrs at 75 ºC offered a red colour liquid (yield: 90%) the diglycidyl ether of bis (3-pentadecenylphenol) methane (DEBPPM). To this DEBPPM (100 mmol, 72.8 g) with sodum periodate (185 mmol, 42.8 g) dissolved in water for 2 h at 50 ºC to yield a red colour liquid (85%) the di-α-formylmethoxy bis (3-pentadecenylphenyl) methane (DFMPM) to the prepared (DFMPM) aldehyde (10 mmol, 7 g) has been heated in a beaker for 30 minutes, further α- napthylamine (20 mmol, 2.86 g) is dissolved in 20 mL of ethanol, then the mixture is refluxed at 90 ºC for 1 h. Further the reaction mixture is poured into a beaker of ice cubes and received a black semisolid precipitate. This black semisolid precipitated product (DFMPMANA) is then filtered and dried to give a yield of 94% in weight.

Synthesis of cardanol consisting α-Napthylamine Schiff based metal complexes

At the molar ratio of 1:2, the aqueous solution of metal nitrate salt of Cu (II) (2.5 mmol, 0.6025 g), Co (II) (2.5 mmol, 0.7275 g), Zr (IV) (2.5 mmol, 0.8475 g), and sulphate salt of Ni (II) (2.5 m. mol, 0.61 g) have slowly been added to a mixture of Cardanol based Schiff base ligand (DFMPMANA) (5 mmol, 4.75 g) which is dissolved by stirring with 30 mL of ethanolic solution. The final mixture is refluxed for 9 h. at 85 ºC and is filtered and the filtrate has been kept at room temperature further the precipitate is washed with ethanol and diethyl ether to remove the impurities and is finally dried in a desiccator to get the stable product.

Synthetic route for cardanol based α-Napthylamine ligand and five experimental metal complexes



Anticancer Activity of experimental sample of schiff based metal complexes

From the present result simply denoted significant anticancer activity possessed against the HeLa cell lines because this experimental complex showed perfected cell death as well the arrested the uncontrolled manner of cell growth when compared with control cancer cell lines beyond dosage and the time interval . In order to find experimental sample of schiff based metal complexes indicated that totally all the treated cells are visibly seen the apoptotic cell death through the MTT assay. Meanwhile, the control cell lines are tightly the uncontrolled cell division, but after treated cells are prominent appearance it was demonstrated initially the detachment or devised on both cell volume of cytoplasm and nucleoplasm. Mainly cytoplasmic surface decreased directly proportionate nucleoplasm and its organelles of chromatin threads are reoriented while the cell division of S1 phase. As a result the whole cells are shrunken; finally the whole cells are devised from the actual attachment of the substratum so the individual cells are disunion from the origin of the organelles locality. Therefore this current research work has been expressed specifically withered all the HeLa subjected cell lines are remarkable apoptotic cell death takes place.

DNA Fragmentation Assay

Experimental cancer cell lines such as HeLa (cervical cancer) were purchased from the National Centre for Cell Sciences (NCCS), Pune, India. The purchased cells were preserved in the logarithmic level of growth in DMEM medium accompanied with 10% (v/v) heat-inactivated fetal bovine serum, 100 µg/mL penicillin, and 100 µg/ mL streptomycin. The samples were preserved at 37 °C in a 5% CO2–95% air humidifed incubator. Morphological variations of PI-stained HeLa cells after treated with using 25µg/ml of the experimental ligand with complexes of cardanol consisting α-Napthylamine Schiff based metal complexes for 24h. HeLa cells were separately plated at a density of 5×104 cells/well into a 6-well chamber plate. At >90% confluence, the cells were treated with experimental Schiff based metal complexes for 7, 14 and days to 21 days (1week to 3weeks). The cells were washed with PBS fixed in methanol: acetic acid (3:1, v/v) for 10min and stained with 50µg/mL PI for 20 min. For the nuclear analysis, the cell plates were washed with PBS and stained with 5µL of AO (100 µg/ mL) and 5 µL of EB (100 µg/mL). The morphological variations in the stained cells, including apoptotic nuclei (condensed chromatin, fragmented nuclei, and intensely stained), were perceived by fluorescence microscopy. Further, electrophoresis analysis were carried out for HeLa cell lines using a get tank consisted of Ethidium bromide with one percentage of Agarose gel at 1hour in 90V by reported method.

Anticancer activity of experimentally synthesized α- napthylamine ligand and metal complexes

(i) DNA fragmentation assay of DFMPMANA ligand and its experimental complexes

DNA fragmentation results were observed after 7, 14, and 21 days of exposure to the experimental ligand DFMPMANA (Fig-1). After seven days, cancer cells that were given 50 and 100 g of treatment did not have a harmful effect nor did they cause any additional cancer cells to die. A 100 µg experimental cancer cell was also fragmented after 14 days, with the first band starting at 1050 bp and the second band at 575bp. Similar to this, 50 and 100µg treated cells after 21 days of treatment distinctly demonstrate that the fragmentation occurs at the level of 600 bp base pairs. Three regions of the polypeptide, including 1150, 1050, and 820 bp, were fragmented in the 100 g-treated cells.

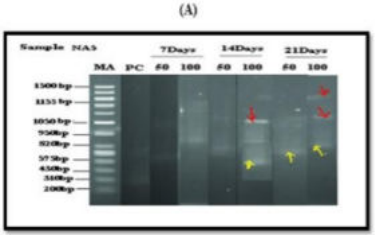


Fig: 1 DNA fragmentation of experimental ligand DFMPMANA on HeLa cancer cell lines

(ii) DNA fragmentation assay of DFMPMANA-Co complex

Fig-2 showed that the experimental complex DFMPMANA-Co and its DNA fragmentation activity after exposure with the stipulated period of days (7, 14, and 21). The lowest concentration of experimental complex for 50 and 100 µg/ml had no detrimental effect on any other cancer cell death when cancer cells were given a seven-day treatment. Additionally, fragmentation begins between 1000 and 950 bp after experimental cancer cells are exposed to 50µg for 14 days. Fragmentation occurs at 950 bp for 100 g. Similar to this, 50 and 100 µmg treated cells after 21 days of treatment clearly demonstrate that the fragmentation occurs on the base pairs level of 900 and 420 bp. After that, cells exposed to 100 g of the polypeptide see fragmentation on its 1050 bp and 575 bp. Finally, the overall result of the DNA fragmentation demonstrated that whenever there is an increase in concentration and treatment period, probably the DNA fragmentation is also increased,

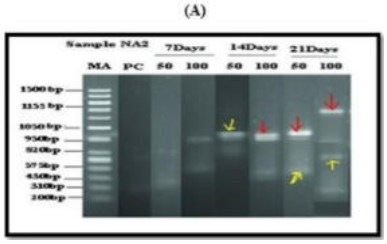


Fig: 2 DNA fragmentation of experimental complex DFMPMANA-Co on HeLa cancer cell lines

DNA fragmentation result has been noticed in 7, 14 and 21 days subjected with the experimental complex DFMPMANA- Ni (Fig. 3). After seven days treated cancer cells fragmentation takes place, 100 µg concentrations show one band fragmentation in the 650 bp range weight of the DNA polypeptide. In addition, after 14 days subjected experimental cancer cell for 100 µg fragmentation starts one at 1050 bp next band at 950 bp. In the same way, after 21 days treatment for 100 µg treated cells clearly show that the fragmentation takes place on the base pairs level of 1050, 720 along with outstandingly DNA fragmentation that is stayed at 420 bp. Finally, the overall result of the DNA fragmentation is shown that whenever concentration and treatment period increase, probably DNA fragmentation on anticancer effect has been observed in this experimental complex on the cancer cells. In difference, the concentration of 50 and 100 µg/ml has had no toxic effect on any other death occurred on the cancer cells. Remarkably this experimental complex after two and three weeks of exposure, two high concentrations has almost completely blocked cell growth of the cancer cell lines. While cell viability is detected by MTT Assay, Stain has been used to detect the chromatin condensation and decreasing cell viability has appeared in a dose dependent and time dependent manner. Finally, DNA analysis reveals typical marker as early as 32 hrs after treatment indicates of apoptosis on the cancer cell lines.

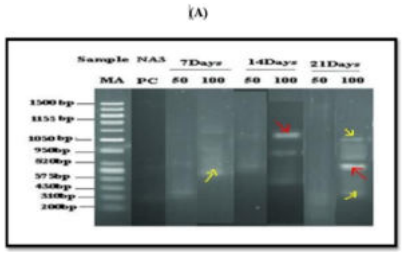


Fig: 3 DNA fragmentation of experimental complex DFMPMANA- Ni on HeLa cancer cell lines

(iii) DNA fragmentation assay of DFMPMANA - Cu complex

DNA fragmentation result has been noticed in 7, 14 and 21 days subjected with the experimental complex DFMPMANA-Cu (Fig. 4). After seven days treated cancer cells with the lowest concentration of experimental complex 25 and 50 µg/ml have had no toxic effect on any other death occurred on the cancer cells. In addition, 100 µg treated cancer cell lines clearly reveal that the DNA polypeptide has been fragmented in two regions one at 700 bp and another at 600 bp. In addition, after 14 days subjected experimental cancer cell for 50 µg, fragmentation starts from 700 bp to 600 bp. For 100 µg, fragmentation starts one at 700 bp next band at 200 bp. In the same way, after 21 days treatment 50 and 100 µg treated cells clearly show that the fragmentation takes place in one place at 200 bp. Then 100 µg treated

cells notice fragmentation on 850 bp and 800 bp of the polypeptide. Finally, the overall result of the DNA fragmentation is shown that whenever concentration and treatment period are increased probably DNA fragmentation on anticancer effect is observed in this experimental complex on the cancer cells. In difference the lowest concentration of experimental complex 25 and 50 µg/ml has had no toxic effect on any other death occurred on the cancer cells. Remarkably this experimental complex after two and three weeks of exposure at three high concentrations, have almost completely been blocked cell growth of the cancer cell lines. Stain has been used to detect the chromatin condensation and decreasing cell viability appeared in a dose dependent and time dependent manner. Finally, DNA analysis reveals typical marker as early as 32 hrs after treatment indicates of apoptosis on the cancer cell lines.

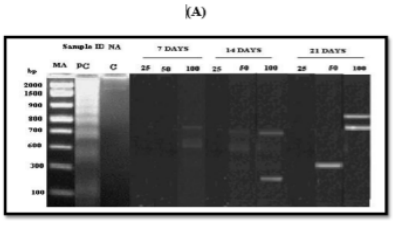


Fig: 4 DNA fragmentation of experimental complex DFMPMANA- Cu on HeLa cancer cell lines

(iii) DNA fragmentation assay of DFMPMANA-Zr complex

DNA fragmentation result has been noticed in 7, 14 and 21 days subjected with the experimental complex DFMPMANA- Zr (Fig. 5). After seven days treated, cancer cells the fragmentation takes place for 100 µg treated cancer cell lines clearly reveal that the DNA polypeptide has been started to fragment one at 700 bp and another at 600 bp. In addition, after 14 days subjected experimental cancer cell for 50 µg, fragmentation starts from 700 bp and at 650 - 550 bp. For 100 µg, fragmentation starts one at 650 bp next band ranged between 400 bp to 350 bp. In the same way, after 21 days treatment, 50 and 100 µg treated cells clearly show that the fragmentation takes place on the base pairs level of 650 bp along with outstandingly DNA fragmentation has been stayed from the 400 bp level to 250 bp. Then 100 µg treated cells have noticed fragmentation takes place on 750 bp, 600 bp and 400 to 300 bp of the polypeptide. Finally, the overall result of the DNA fragmentation is shown that whenever concentration and treatment period increase, probably DNA fragmentation on anticancer effect has been observed in this experimental complex on the cancer cells. In difference, the lowest concentration of experimental complex 25 and 50 µg/ml has had no toxic effect on any other death occurred on the cancer cells. Remarkably this experimental complex after two and three weeks of exposure three high concentrations has almost completely blocked cell growth of the cancer cell lines. While, cell viability is detected by MTT Assay. Stain has been used to detect the chromatin condensation and decreasing cell viability appeared in a dose dependent and time dependent manner. Finally, DNA analysis reveal typical marker as early as 32 hrs after treatment indicates of apoptosis on the cancer cell lines.

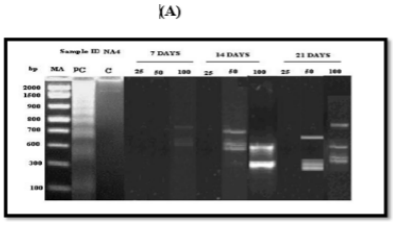


Fig:-5:- DNA fragmentation of experimental complex DFMPMANA- Zr on HeLa cancer cell lines

Finally, the overall result of the DNA fragmentation is shown that whenever concentration and treatment period increase, probably DNA fragmentation on anticancer effect is also increasing in this experimental complex on the cancer cells. In difference, the lowest concentration of 25 and 50 µg/ml has had no toxic effect on any other death occurred on the cancer cells. Remarkably, this experimental complex after two and three weeks of exposure five high concentrations has almost completely blocked cell growth of the cancer cell lines. While cell viability is detected by MTT Assay. Stain has been used to detect the chromatin condensation and decreasing cell viability appeared in a dose dependent and time dependent manner. Finally, DNA analysis reveals typical marker as early as 32 hrs after treatment indicates of apoptosis on the cancer cell lines.

Morphological changes of cancer cells in response to the DFMPMANA experimental ligand

Apart from the result of the MTT assay, it is found that when the Hela cancer cell lines are treated with the experimental ligand of the DFMPMANA, it is determined by light microscopical modules denoted (Fig. 6). After final experiment, the treated cells deliberately express that it is mild to potentially vibrant cell death has been noticed on random manner. Though, morphological features of each HeLa cell line are exhibited, the hallmark phenomenon of the cell cycle during the time of cell growth are arrested during the phase of G1 and S phase. As a result of this, the frequently mitotic divisions are restricted; and so, condensation as well as shrinkage of the cell takes place totally, trilobed structure, of nucleus and irregular level of spindle fiber formation.

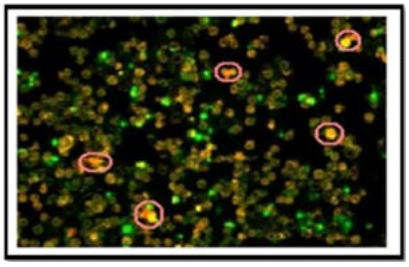
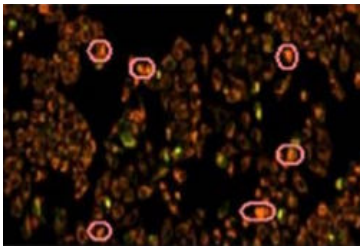


Fig: 6- MTT assay of experimental ligand DFMPMANA on HeLa cancer cell lines

Morphological changes of cancer cells in response to the DFMPMANA-Co experimental complex

When the cancerous HeLa cells cell lines are treated with DFMPMANA-Co complex cell lines reveal the specific features of cell contraction, and withering takes place on the centre region of the experimental complex digested cells. It has also been observed as a demonstrated lobulated appearance in apoptotic cell death along with other changes visible through light microscope such as rounding and partial detachment of sample applied cells (Figure 7).

Fig: 7:- MTT assay of experimental complex DFMPMANA-Co on HeLa cancer cell lines



(a)Morphological changes of cancer cells in response to the DFMPMANA-Ni experimental complex

Similarly, DFMPMANA-Ni treated cancer cell lines show clearly with its morphological view compared with untreated HeLa cell lines (control). In this experimental sample, treated cell lines have expressed the randomly minimum proliferation efficiency, that is simply denoted with the specific characteristics of slowly dividing cells at G-0 phase that means lowest nuclear - to - cytoplasm ratio, prominent broken nucleus and reduced range of mitosis division also exhibit large apoptotic cell death, has been observed in Fig. 8. (B).

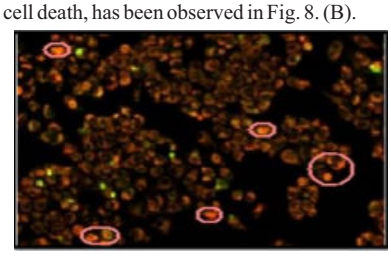


Fig: 8 MTT assay of experimental complex DFMPMANA-Ni on HeLa cancer cell lines

(a) Morphological changes of cancer cells in response to the DFMPMANA-Cu experimental complex

Compared with the control untreated cells, the mainstream of the HeLa cells are transformed from spindle to star-shaped, some of which become prepared for damage and irregular shrink morphology (Fig. 9). Meanwhile, their growth is repressed in a concentration of sample dosage dependent manner. These morphological changes are typically leading to the initial point of apoptosis phenomenon when subjected to DFMPMANA-Cu complex molecule. Consequently, sample treated cell lines diminished the sequences of the cells ordering, reduction the cell capacity and chromatin condensation compared with normal cell lines.

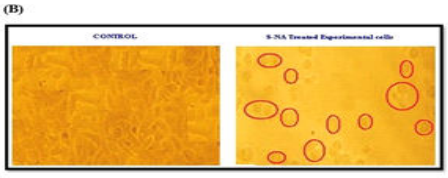


Fig: 9 MTT assay of experimental complex DFMPMANA-Cu on HeLa cancer cell lines

Morphological changes of cancer cells in response to the DFMPMANA-Zr experimental complex

Correspondingly, another experimental ligand- complex of DFMPMANA-Zr treated Hela cancer lines have expressed the following cell morphology such as cell contraction, negotiating, fractional impartiality and lobulated appearance as well as the aggregation of the treated cells prominently shown depend on the dose dependent range. Subsequently, sample treated cell lines reduce the series of the cells arranging, decrease of cell capacity, loss of reliability of membrane which results in twisted and vesicle shape of the membrane and chromatin condensation compared with normal cell lines (without treated HeLa cells) Fig. 10.

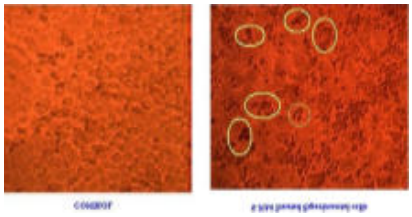


Fig: 10 MTT assay of experimental complex FDMPMANA-Zr on HeLa cancer cell line

Discussion

In order to protect human life, anti-pathogenic complexes are highly required and recommended. As a result, numerous complexes and ligands are being examined worldwide to determine their biological activities, and research (Kotwal et al. (2010) ascertains that metal complexes are more active when compared to their corresponding ligands. Since 1798, the organisation complexes have been researched, and substantial advancements in inorganic and organic chemistry have been made with regard to the purpose for the synthesis of pharmaceutically relevant chemicals, explanation, and application of this huge group of metal complexes (Saranya et al., 2004) and (Chellaian and Madhavan, 2008). The main focus of this coordination has been on creating second-order mixtures that definitively represent the complexes created by the first direction. Any chemical element can serve as the core atom of a ligand, with the second coordination sphere (ionisation sphere) appearing outside of the brackets. Meanwhile, the ligands can be ions, atoms, or neutral molecules that can function as donors (Silverman, 1992). Mono-/polydentate ligands can be neutral molecules or mono-/polyatomic anions with one or more unshared electron pairs; the latter ones form complexes with cyclic structure known as chelates. Numerous pharmacological compounds act as ligands and chelating agents under in vivo and in vitro contingencies (Martin et al., 2010).

One or more unshared electron pairs that can act as ligands are present in the molecules of the medicinal drugs. In reality, many of the fundamental building blocks of living things (such as amino acids, peptides, proteins, hormones, lipids, and carbohydrates) may behave as ligands because their molecules contain donor atoms like nitrogen, oxygen, sulphur, and phosphorus (Shukla et al., 2013). It is common knowledge that numerous pharmacological ingredient compounds function as ligands in both in vitro and in vivo settings. It is important to remember that these ligands will compete for a specific metal ion in vivo with a range of different ligands, therefore extrapolating this in-vitro behaviour should be done cautiously. It should always be taken into consideration that the drug ligand molecules' orientation and the capacity to bind the receptors have a significant impact on the therapeutic efficacy (Shilpa et al., 2015). Thus the matter of fact is that, there are many ways to use these metal complexes in the biomedical field, including introducing them into the body to treat metal ion deficiencies, using the ligands as antidotes for various metal intoxications, and procuring pharmacotherapy effects by blocking metal ions that are absolutely essential for some enzymatic systems (Ramachandrula et al., 2016). Metal ions have a significant role in both the critical processes of living things and in the study and control of medicinal drugs and this id accomplished through the creation of complexes which can be detected using a wide range of of physicochemical techniques, including those of spectroscopy, chromatography, microscopy, etc.

Conclusion

A novel cardanol-based Schiff ligand and mixed ligand complexes with DFMPMANAs are successfully synthesised and thoroughly tested against clinical pathogens in a molar ratio of 1:2 . However, the current research highlighted the four experimental complexes, DFMPMANA-Cu showed a more potent effect of apoptotic cell death on HeLa cancer cell lines, hence its stronger and highly effective anticancer drug. for the reason that the DNA fragmentation as well as the MTT assay demonstrated that the cardanol-based Schiff base and mixed ligand complex of DFMPMANA-Cu, hence the present study showed that four complexes DFMPMANA-Cu represents one of the best anticancer drugs against the dominant cancer cells of HeLa cell lines. Thus, the current results were showed that [DFMPMANA-Cu] complex is a promising molecule for the development of new therapies associated with schiffbase Cardanol derived α- Napthalamine ligand, it could be control the growth of an uncontrolled growth of related to cancer HeLa cell lines.

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