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Original Article

Synthesis and Characterization of Mercury Complex Derived from Trimesic Acid

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ABSTRACT

The effects of mercury complexes on human body and cells vary depending on the extent of exposure and their pharmacological form. **Objectives:** To characterize mercury complex and then investigate the effects on cellular interaction via cell death. **Methods:** The synthesis of the mercury complex was carried out, and its characterization was done by FTIR, elemental percentage and powder X-ray diffraction (PXRD). The complex was analyzed through atomic force microscopy (AFM) and by microscopy imaging its surface morphology and cellular interaction were also studied. **Results:** The presence of the mercury-complex results in cell death in concentration and time dependent manner. **Conclusions:** The synthesized mercury-complex has the ability to harm cells.

INTRODUCTION

At present, many studies have been conducted about the application of biocompatible compounds and process of synthesizing compounds that contain organic ligands, or chemicals derived through live things [1]. Many scientists quickly conjure up images of toxicity and hazardous substances when they hear the word "heavy metals," as do members of the public with any scientific education [2]. Few people are even aware of the fact that a growing number of chemicals, some of which are employed as medications to treat or detect disease, include a heavy-metal ion playing a crucial function [3, 4]. To enhance the performance of molecules within cells and enzymes, a few small amounts of heavy metals are necessary [5]. A number of heavy metals (micronutrients: Ni, Co, Cu, Mo, Zn, Mn, and Fe) are essential for plants and animals when they are found in growth media at low concentrations [6]. They don't get harmful until a certain concentration is reached. For many years, mercury has long been recognized as one of the poisonous elements that results in cell dysfunction and, as a result, seriously harms the cerebral cortex, the kidneys, central nervous system and environment [7-12]. In spite of negative impacts, heavy metal-based complexes have been widely utilized in healthcare for many decades [13]. Although very hazardous, mercury has been incorporated into a variety of human-supporting technology. As an example, consider working out how mercury enters into an ecosystem using the three mercury isotopes, Hg (198, 200, 202). Whereas Hg-303 (mercury's

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radioactive isotopic form) is prepared by Hg-202, Hg-303 is utilized for gamma radiation calibration [14-16]. Earlier research on the immunotoxic and carcinogenic effects of mercury complexes was done by Shenker et al. in 1993 [17]. In light of the aforementioned information, the present research was focused on characterization of synthesized mercury complex derived from trimesic acid. Mercury is a potent metal that is both genotoxic and phototoxic [18, 19]. Mercuric ions have a propensity to create covalent connections with cells, which makes them genotoxic. X-ray-induced cell death and the damage caused by HgCl2 are quite comparable. Unlike those brought on by X-rays, single-strand fractures brought on by HgCl2 are not easily healed. It has been demonstrated that HgCl2 causes true single-strand breaks rather than alkali-labile sites [20]. HgCl2 has been found to deplete reduced glutathione and produce superoxide radicals in intact cells, much like X-rays do. Mercury has been demonstrated to be tightly bound to cells because it resists being extracted using chelating agents and high-salt solutions [21, 22]. Mercury chloride (HgCl2) is particularly effective in damaging DNA in mammalian cells, according to earlier studies [21, 23]. Mercury complexes have not been shown to be cancerous or mutagenic in many systems despite their ability to cause death of DNA cells [24]. Some of the methods through which mercury causes cell death have been examined [25]. New information has also been revealed regarding the impact of methyl-HgCl on DNA in maintained fibroblasts and cultured nerve cells [26]. Yuan et al., and Kim et al., research demonstrates that the Hg complex cause death to cells, and their findings are comparable to the CH3-HgCl2 complex due to its strong reactivity and ability to produce extremely noticeable harm to cells on low concentration [27, 28]. The idea that the mercury ion might cause oxygen radicals to form in cells has also been supported. Although the exact process by which this happens has not been determined, it has been previously demonstrated that it happens at 37°C which is slight less than 40°C [29, 30]. Cantoni et al., also reported that exposure of cells with HgCl2 showed fast and continuous single strand breaks in DNA and it was dependent on duration and concentration of Hg used. Toxicity of HgCl2 under equivalent exposure resulted to cell death [31].

In current work, a new mercury complex based on TMA was utilized for cellular interaction investigation and cell death.

METHODS

Metal salt (HgCl2) and ligand (1, 3, 5-benzene tricarboxylic acid or TMA), solvents (ethanol, methanol, DMF) tetrahydrofuran (THF) and trifluoroacetic acid (TFA) were used in the synthesis of Hg-complex. The detector of elements Vario Micro Cube, made by Elementar Germany, was used for CHNS percentage. On a Bruker Tensor 27 FT-IR spectrometer with a Diamond-ATR module, FTIR spectra were captured in the 4000-400cm-1 region. An XPert PANlytical Powder diffraction Machine mounted on Cu Kα (I=1.54 Å) radiation was utilized to check the X-ray diffraction pattern of the synthesized Hg-complex. The images of the surface morphology of complex were captured by electron microscope.

Synthesis of Complex

First of all, metal salt solution was prepared by weighing HgCl2 (135 mg, 0.2 mmol) and dissolving in deionized H2O (2ml). Now the ligand solution was prepared by dissolving (105 mg, 0.2 mmol) trimesic acid in deionized H2O (5ml). The ligand must be put on hot plate and stirred at 200 °C for 5-10 minutes. In order to avoid re-crystallization of ligand (TMA), 1 ml THF was added in ligand solution. Both metal and ligand solutions were mixed and sonicated for 15 minutes to get clear solution. After filtration, when the filtrate was allowed to stay for ten days, the solvent evaporated, and off-white rod-like crystals were recovered. After recovering the crystals using filter paper, they were washed with a THF to H2O solution (V: V=10:90) and allowed to dry at room temperature (Yield: 60%).

Cell Culture Preparation

The cell lines were developed and obtained from University of Veterinary and Animal Sciences (UVAS) Lahore.

RESULTS

CHNS Determination

For Mercury complex (C18H10HgO12) its molecular weight was 618.55amu. Analytically calculated percentage values of C, H, N were 31.53, 3.82, 6.02 % and the C, H, N percentage values actually found were 31.29, 3.46, 1.61. The theoretically calculated percentage values of ligand trimesic acid (TMA) were (C, 46.2%, H, 17.00%). The %age values of ligand's C & H have reduced in the mercury complex, indicating that the ligand has bonded to a metal ion.

Fourier-Transform Infrared (FTIR) Spectroscopy of Complex

Mercury complex with peak arrangements is demonstrated as follows: 740 cm-1 (sharp), 1255.38 cm-1 (broad), 1322 cm-1 (sharp), 1539cm-1 (broad), 1637 cm-1 (sharp), and 2847.11 cm-1 (medium and broad). To check the presence of a carboxyl group (-COOH) broad peak at 2847 cm-1was indicated and to represent bonding of a ligand (TMA) with metal, peak at 1637 cm-1was represented. These reduced C=O values show that ligand coordination is involved in the production of the mercury complex. The stretching vibration of the carboxyl group's C=O bond on the aromatic ring must be responsible for the occurrence of the particular peak at 1322 cm-1. On the other hand, the peaks at 3688 cm-1 and 3578 cm-1 show the ethanol's -OH group (Figure 1).

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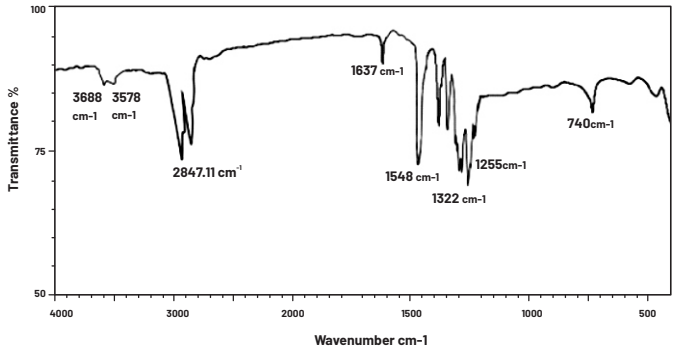

Figure 1: Mercury-complex FTIR Spectrum

The theoretical shape of the mercury-complex is square planar geometry. Mercury is coordinated in [Hg (TMA)2] by two oxygen atoms [O (14), O (15)] of one ligand (TMA) and two oxygen atoms [O (23), O (24)] of another ligand (TMA) (Figure 2). The proposed complex has a molecular mass of 618.55 amu. The complex's empirical formula is C18H10HgO12.

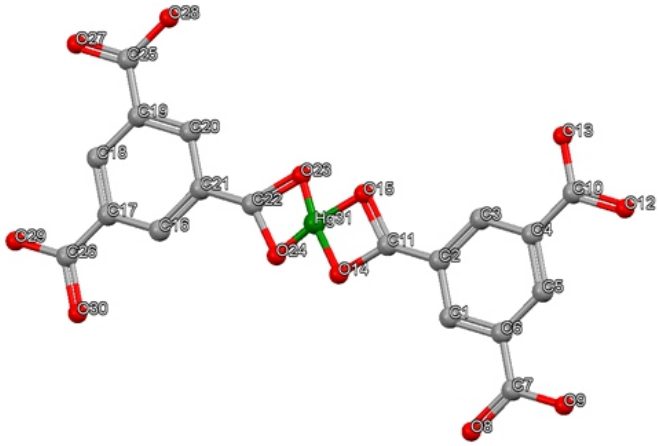

Figure 2: Structure of mercury-complex [Hg(TMA)2]

Powder X-Ray Diffraction (PXRD)

Mercury-complex's powder X-ray diffraction analysis was investigated using XPertPANIytical Powder's Cu Kα (I=1.54 Å) irradiation. Before PXRD examination, the material thoroughly dried then ground into tiny particles [32]. Mercury-complex XRD patterns demonstrate that the complex is homogenous in phase, without any other components or undetectable contaminants. Figure 3 shows PXRD spectrum of mercury-complex. The complex exhibits peaks for diffraction at 2θ = 20.5°, 21.75°, 26.5°, 33.18° and 48.85 which were ascribed to literature peaks in contrast to library references, founded on face-centered cubic arrangements of the synthesized Hg-complex [33, 34]. It corroborated the structure of mercury-comprising compound.

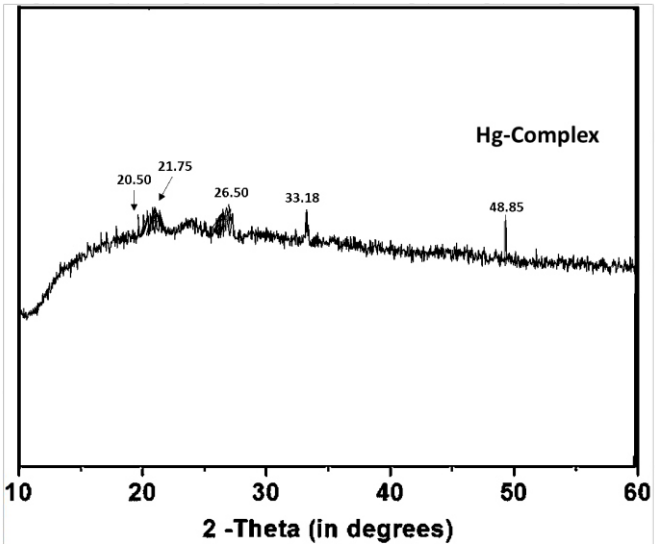

Figure 3: Powder X-Ray diffraction of Hg-complex

Microscopic Imaging

An electron microscopic analysis of the prepared mercury-complex's morphological structures was conducted. Images were captured at magnifications of 200–500 and in the 2–5 mm range. Images show the uniform dispersion of rod-like crystals; due to their low energy at the surface, these crystalline structures are not heavily agglomerated. There were few bigger crystals due to minimal aggregation or overlaying of tiny particles. The microscopic images clearly display a distribution of smaller, irregularly sized crystals, as well as a uniform rod-like crystal structure and flakes-like architecture. The outcomes of our microscopic examination are listed in figure 4.

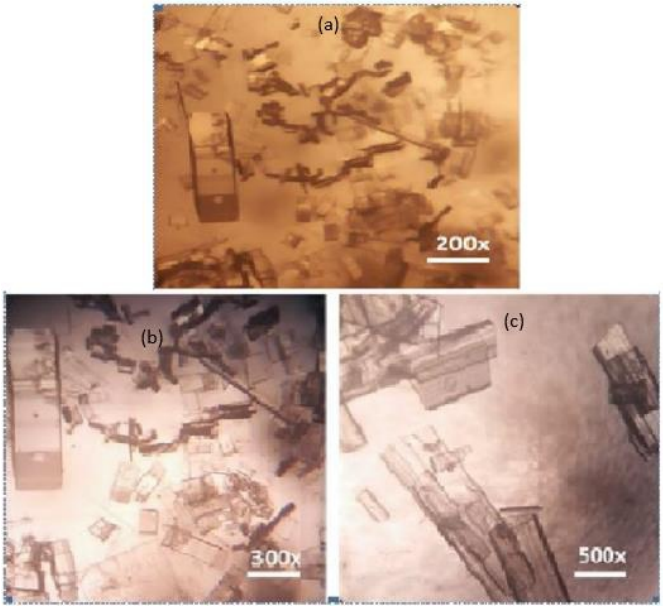

Figure 4: Mercury-complex scanning for microscopy images at range 2-5 mm with magnification of 200x(a)300x(b)and 500x(c)

Atomic Force Microscopy

Atomic force microscopy is unquestionably the most effective and adaptable microscopic method for studying different

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complexes. Adhesive capacity, electromagnetic factors, and mechanical features are only a few of the impacts that AFM may use to assess and specify mercury complex [35]. Using AFM5500M, the prepared mercury complex's surface structure was investigated. The mercury complex's surface image is displayed in figure 5. With computed depth of designated design scale in actual units, the measured phase topographical data of the chosen section is shown as a surface plot. The area that was evaluated is 5 × 5 µm2. With relative depth measurements in µm as determined by the numerical computation, the phase profile is shown in this instance as a surface plot. The variation between the observed and calculated phase profiles, on the other hand, is unaffected by the size or placement of larger structures. It displays the synthesized material's roughness. The great compactness of the mercury complex was demonstrated by these images.

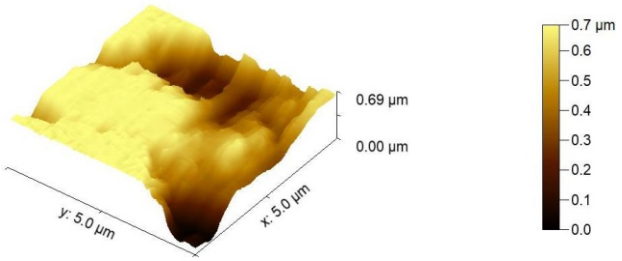

Figure 5: Scanning of mercury-complex by AFM. A surface projection of the phase pattern is used to depict the phase topography of the specified segment as determined by phase technique, showing the exact phase shift that was estimated in microns

Cellular Interaction Study

The complex was examined for cellular interaction research in order to look into the effects of the mercury-complex on cells. With such a reason, both the lack of as well as addition of the mercury-complex, cells had been grown, with different concentrations after three days. Presence of Hg-complex is correlated with cell mortality, cell overlapping, and shrinking margins as opposed to the lack of a complex. There may be a correlation between high Hg accumulations by cultures and the decrease in cell proliferation at high Hg concentrations. Cells may then need to use additional energy in order to deal with the elevated mercury content throughout the tissues [36]. After three days of exposure to the mercury complex, the findings of the cellular interaction investigation are shown in Figure 6, which revealed a significant amount of extra harm as well as cell death. As presence of Hg-complex exists, numerous cell populations die even if there were an increase in clustering as compared to the control. The number of cells dying increases as Hg-complex levels rise and the number of living cells falls drastically.

Live/Dead Cell Assay

To evaluate how the Hg-complex affects the viability of cells, live/dead tests were carried out using fluorescence imaging for three days, either in the absence or presence of mercury complex at concentrations of 1µg/ml, 10µg/ml, and 50µg/ml. A cell assay with varying mercury complex concentrations is described in Figure 6 (A, B, C, D). It showed an experiment using live or dead cells and varying Hg-complex concentrations. The green dots depict the live cells where as red dots is the background.

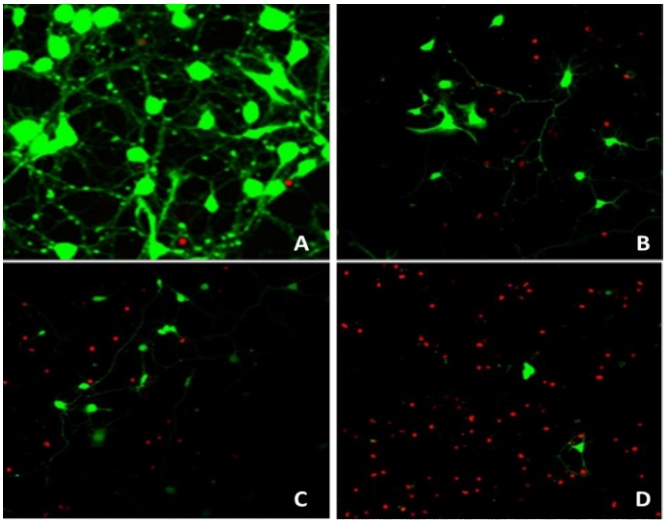

Figure 6: Live/Dead cell assay; A) Control without Hg- complex and B) Cell culture death 1 µg/ml, C) and 10 µg/ml and D) 50 µg/ml; with Hg- complex

The graphical representation of the cell assay at various concentrations is shown in figure 7.

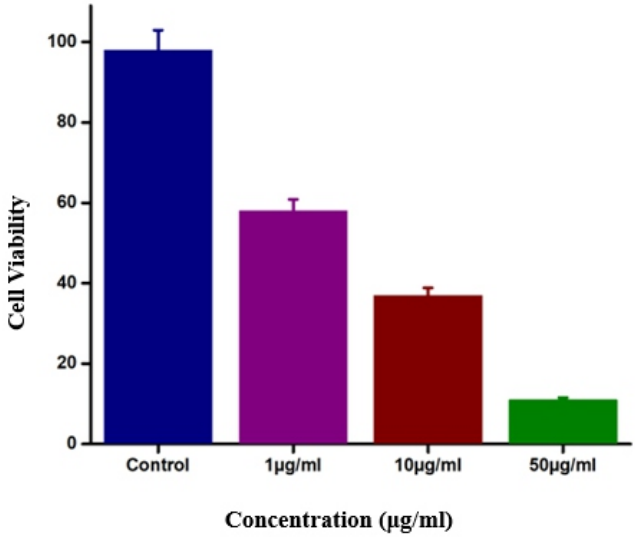

Figure 7: Live/Dead cell assay graph; Control without Hg-complex and 1 µg/ml and 10 µg/ml and 50 µg/ml; having presence of Hg-complex

DISCUSSION

FTIR spectra depicted the bonding of ligand with metal mercury, while X-Ray diffraction analysis demonstrated peaks for diffraction. Atomic force microscopy represented the high compactness of Hg-complex synthesized. The harmful effects of Hg-complex on cell culture were validated by experiments on cells. Studies were conducted on the effects of the Hg-complex at various concentrations. The impacts of Hg-complex on cell cultures were intensified when the concentration of the Hg-complex increased. The mercury complex put damaging impacts on cell growth and development, which

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might be employed to further studies on cellular interaction. There is no doubt that the mercury compound might inhibit the cell's expansion. Images depicting cell death and growth could be confirmed with a fluorescent microscope. Fluorescence signals may be found in the interior space of the cells, indicating dispersion within the cells of mercury complex. Cell viability graph showed that as the concentration of Hg-complex increased from control to 50µg/ml, the cell viability decreased which represented that Hg-complex increased amount put more damaging effects on cell. The outcomes of the current research are consistent with a study by Cantoni et al., which showed that the ability of Hg-complex to target the targeted region of cells caused more cell death as concentration and duration rise [26, 37, 38]. Therefore, considering its dangerous character as well, the best investigation should be done to determine whether the mercury-complex may be best used for medical therapy [9, 39]. By regulating its development in response to changes in concentration and time, the mercury complex may cause harm to the targeted cells under precise and regulated conditions by increasing its time and exposure. Our research based on cellular interaction study indicates that this new mercury compound can cause cell damage.

CONCLUSIONS

In summary, the present work was focused to look into how Hg-complex, which is made from trimesic acid, caused death to cells. The Hg -complex was synthesized using sonication, its characterization was done by FTIR, elemental percentage, powder X-ray diffraction (PXRD), microscopic imaging, atomic force microscopy and possible impacts in the growth and death of cells were studied by cellular interaction. It is recommended that complex based on mercury could be used as affordable and environmentally friendly agent and research on cells demonstrated the effectiveness of the Hg-complex on cells. Its harmful impacts were minimal at low concentrations, however as soon as the concentration of mercury-complex was increased; cell viability showed that it totally caused the death of cells. Mercury complex is a non-biodegradable and biocompatible complex that put damaging impacts on cells. Multiple parameters, that include type of cells applied for cellular interaction study, the concentration of synthesized mercury complex and its molecular weight (MW) put impacts on cell damage. With an increase in concentration and duration, Hg-complex demonstrated its detrimental impacts on cells.

Authors Contribution

Conceptualization: JHS, SS
Methodology: JHS
Formal analysis: JHS, SS, RR, AA
Writing-review and editing: JHS, RR, AA
All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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