Biological evaluation of a new aromatic trinuclear palladium complex with bioactive oxime ligands: In vitro and in vivo effects on mice breast cancer

Accepted 24th September, 2018

ABSTRACT

Oximes are organic molecules, which contain >C=N-OH fragment, and are widely applied as excellent ligands in medical, inorganic and bioinorganic chemistry. A Pd complex ([Pd₃(C₁₂H₈C=NO)₃]₃) has been synthesized and proven to be safe and efficient in the biological systems in this study. The therapeutic effects of the Pd complex on the different tumor cell lines and an animal model of breast cancer have been evaluated. The Pd complex was shown to considerably prevent the rapid growth of the mammary and colon carcinoma cells. The synthesized Pd complex of up to 10 mg/kg dose in mice was safe. Higher doses resulted in minimal hepatocellular toxicity in the preclinical examinations. The average size and weight of the tumor were remarkably lower in the groups exposed to the complex as compared with the control animals. The role of Pd complex in preventing tumor growth models in vitro and in vivo was shown by the results. Strong evidence for the efficiency of the Pd complex in suppressing tumor growth was provided by the results.

Key words: Palladium, breast cancer, optimum dose, mice.

INTRODUCTION

Cancer is one of the most terrible and serious diseases in the world. A rapidly growing area of chemotherapy research is the development of anticancer drugs (Gao et al., 2009). Traditionally, metal based inorganic drugs have been used in chemotherapy (Brown, 2008) since the interaction between transition metal complexes and DNA can often damage the DNA of cancer cells and block their division (Li et al., 1996). A new area of anticancer research based on metallopharmaceuticals was initiated by the outstanding discovery of cisplatin anti-tumoral properties by Rosenberg in 1965 (Rosenberg and Vancamp, 1969). Nevertheless, various side effects, such as nephrotoxicity, emetogenesis and neurotoxicity, have been shown by metallopharmaceuticals during cancer therapy. Palladium (II) complexes are much more unstable as compared with the corresponding platinum (II) complexes due to the shielding effects on the central palladium (II) (Daghriri et al., 2004). To minimize the high instability and fast hydrolysis of palladium complexes in the biological environment (Sebastian et al., 2012; Karami et al., 2015), which may enable cross bindings, inhibit their synthesis and induce apoptosis, chelating ligands have been applied in the synthesis of antitumor agents (Kontek et al., 2011). Furthermore, palladium complexes containing nitrogen donor ligands such as ethylenediamine derivatives, diaminocyclohexane, ammonia, pyridine, quinoline, pyrazole and oxime are among the most promising complexes showing in vitro and in vivo cytotoxicity profiles (Ruiz et al., 2005; Rubino et al., 2011; Karami et al., 2017). Oxime derivatives are interesting compounds owing to their low toxicity to non-target organisms, antibacterial and antifungal properties (Kurtoğlu and Baydemir, 2007; Sun et al., 2010) and high antitumor activity index via DNA intercalation (Grigalevicius et al., 2005; Krstić et al., 2007). In addition, the application of oxime derivatives as ligands for potential metal based drugs makes them biologically...
important compounds. For instance, oxime complexes have reportedly caused biological effects such as the cleavage of nuclear and oxidative DNAs (Hambley et al., 2000). The aims of the present study were to investigate the anticancer properties of a new Pd (II) complex containing a chelating oxime ligand and determine its structure activity relationships (SARs). The toxic effects of different doses of the Pd complex have been evaluated to figure out the toxic doses on mice via clinical observations, hematological/blood chemistry tests and histological experiments. Furthermore, the therapeutic effects of the Pd complex on different tumor cells and a typical animal model of breast cancer were investigated by apoptosis, proliferation and angiogenesis pathways.

**MATERIALS AND METHODS**

**Materials, instrumentation and physical measurements**

The human colon cancer cells (CT26), mouse mammary (MC4-L2) carcinoma cell lines and normal human fibroblast cells (MF1-P130) were obtained from Pasteur Institute of Iran (Tehran, Iran). 9-fluorenone, hydroxylamine hydrochloride and palladium (II) chloride were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). The highly pure solvents used in the syntheses and physical measurements were supplied by Merck Chemical Co (Germany). An FT-IR JASCO 680 spectrophotometer was used to record infrared (IR) spectra (400−4000 cm−1) using KBr pellets. A Varian Cary 100 UV-Vis spectrophotometer was used to record the UV-Vis spectra using a 1 cm path length cell. A Bruker spectrometer was used to record NMR spectra at 400.13 MHz (1H), 100.61 MHz 13C−{1H}. A LecoCHNS-932 apparatus was used to perform elemental analyses.

**The study design**

Four series of experiments were carried out including (i) synthesis of the Pd complex, (ii) effects of the Pd complex on the viability of mammary and colon carcinoma cells, (iii) the acute and chronic toxicity of the Pd complex and (iv) the therapeutic effects of the Pd complex on an animal model of breast cancer.

**Animals**

All experiments were confirmed by the international guidelines of the Weatherall report and Institutional Animal Care and Use Committee (IACUC) of Tehran University of Medical Sciences. All 6–8 old inbred BALB/c mice, obtained from Iran Pasteur Institute, were maintained in large group houses under 12-h dark and light cycles and were provided with food and water ad libitum.

**Synthesis and spectroscopic characterization of the Pd(II) complex**

PdCl2 (1.75 g, 10 mmol) and LiCl (0.85 g, 4 mmol) were dissolved in methanol (80 mL). The resulting solution was then heated to 50°C and maintained at that temperature for 1 h. The solution color changed from orange to black during this period. The corresponding 9-fluorenone oxime (2.4 g 10 mmol) dissolved in methanol (60 ml) and anhydrous sodium acetate (0.8 g, 10 mmol) were added to a solution of Li2PdCl4 in methanol. Subsequently, the solution was stirred for 2-3 days at ambient temperature (Figure 1). The corresponding trinuclear palladium complex precipitated upon the addition of water (30 mL) was filtered off and left for crystallization. Slow evaporation of the filtrate over a 15 day period yielded orange crystals of X-ray quality. The crystals were filtered and washed using methanol.

Yield: 64%. Anal. Calc. for C27H18N2O2Pd2: C 63.1; H 3.2; N 5.1%. Found. C 61.3; H 3.3; N 5.6%. Anal. Calc. for C27H18N2O2Pd2: C 63.1; H 3.2; N 5.6%. Found. C 61.3; H 3.3; N 5.6%. IR (KBr pellet, cm−1): v (s, C=N) = 1602, v (bs, OH) = 3370, v (m, N−O) = 1064, v (m, C=Haverage) = 3062. 1H NMR (400.13 MHz, CDCl3, ppm): δ=10.87 (d, 6H, H=), 7.83 (d, 6H, H=), 7.72 (d, 6H, H=), 7.45 (d, 6H, H=), 7.64 (t, 6H), 7.55 (t, 6H), 7.16 (t, 6H), 6.73 (t, 6H), 13C−{1H} NMR (100.61 MHz, CDCl3, ppm) 130.44,128.09, 127.87, 123.75, 122.51, 120.22,119.66.

**MTT assay**

MC4-L2, CT26 and MF1-P130 normal fibroblast cells were grown in Dulbecco's modified Eagle's medium (DMEM; GibCO, USA), which contained 10% fetal bovine serum (FBS; GibCO, USA) at a temperature of 37°C in humid atmosphere. MTT [3-[4, 5-dimethylthiazol-2-yl] 2,5-phenyltetrazolium bromide) assay was used to measure the cell viability. 10 mM solution of the Pd complex in methanol was used to prepare the Pd complex solution (100 µM) in DMEM. The percentage of methanol in the final solution was below 0.4% v/v. Identical cell numbers (1 × 10⁶ cells) in 200 µl of DMEM containing 10% FBS were seeded in triplicate on 96-well plates, followed by overnight incubation. Cells were then treated with different Pd complex concentrations (200, 300, 400, 500 and 600 µM) for MC4-L2 and MF1-P130 cell lines and various Pd complex concentrations (1, 10, 20, 30, 40, 50 and 100 µM) for CT26 cell line for 24, 48 and 72 h. 20 µl of 5 mg/ml MTT were then added to each well and incubation was continued for an additional 4 h after which 200 µl of dimethyl sulfoxide were added. A 96-well plate reader (TECAN, Switzerland) was then used to determine the relative cell viability at 570 nm. Measurements were carried out and the required
concentration for a 50% inhibition of viability (IC\textsubscript{50}) was graphically determined using the standard graph obtained by plotting the drug concentration in X axis and the relative cell viability in Y axis (Johari-Ahar et al., 2016).

Cell viability (%) = Mean OD/Control OD × 100%

Dosing procedure

To study the acute and chronic toxicity of the Pd complex in two different acute and chronic phases on sixty male BALB/c mice, a dosing procedure based on the explained protocol was performed (Alizadeh et al., 2015). 1000, 200, 100 and 50 mg/kg doses of the Pd complex were injected in the acute toxicity phase. After 24 h, the animals were euthanized and the blood samples were collected for the hematological/biochemical analyses. The dose was adjusted according to the average toxicity and the last tolerated dose for the new group of mice in the case of 24 h adverse reactions. Toxicity signs included abnormal blood hematological/blood chemical indices and body weight changes.

Chronic toxicity was studied using 2, 10 and 50 mg/kg doses of the Pd complex for 7 consecutive days according to the results of acute toxicity. All animals were euthanized after 7 days and the blood samples were collected for the hematological/biochemical analyses. The dose, which showed no adverse reactions during 24 h, was referred to as the survival dose. The survived animals were weighed every day and euthanized on the eighth day.

Hematology and blood chemistry tests

Using 100 mg/kg ketamine and 10 mg/kg xylazine, animals were decapitated under general anesthesia and the blood samples were collected and added into ethylenediaminetetraacetic acid (EDTA) and heparin coated tubes for hematology and clinical chemistry tests, respectively. An animal blood counter (Celltac, Nihon Kohden, Tokyo, Japan) was used to measure total leukocyte count (WBC), erythrocyte count (RBC), hemoglobin (HGB) and platelets (PLT). A CCX system (CCX WB, Nova Biomedical, USA) was used to determine creatine (Cr), glucose (Glu) and plasma urea nitrogen (Urea). In addition, an autoanalyser System (Autoanalyser Model Biotecnica, BT 3500, Rome, Italy) (Alizadeh et al., 2015) was used to measure direct bilirubin (Bill D), total bilirubin (Bill T), serum total protein (TPr), alanine transaminase (ALT), aspartate transaminase (AST) and plasma alkaline phosphatase (ALP).

Tumor transplantation

MC4-L2 cells were grown into solid tumor in female BALB/c mice flank. MC4-L2 tumor from the mice bearing breast cancer was cut into pieces below 0.2–0.3 mm\textsuperscript{3} and subcutaneously transplanted into mice right flank under anesthesia using 100 mg/kg, i.p ketamine and 10 mg/kg, i.p xylazine. The tumors were visible two weeks following tumor implantation (Farhanji et al., 2015).

Therapeutic effects of Pd complex on an animal model of breast cancer

Twenty four mice with breast cancer were randomly divided into three different groups: (1) control, (2) treated with 2 and (3) 10 mg/kg Pd complex. Two weeks through tumor implantation, the Pd complex was intraperitoneally injected for 14 days from day 14 up to day 28. Furthermore, normal saline was given in the control animals.

Using a digital vernier caliper (Mitutoyo, Japan), tumor volume was measured two times a week and reported by the following formula (Isanejad et al., 2016):

\[ V = \frac{1}{6} (\pi LWD), \]  

where \( V \) = volume, \( L \) = length, \( W \) = width, and \( D \) = depth.
Table 1: The acute toxicity effects of pd complex on the hematology and biochemistry parameters.

<table>
<thead>
<tr>
<th>Index</th>
<th>Control</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Weight (g)</td>
<td>24.3 ± 2.2</td>
<td>25 ± 3.1</td>
<td>24.7 ± 2.5</td>
<td>23.2 ± 2.5</td>
<td>22.3 ± 2.8</td>
</tr>
<tr>
<td>WBC (1000/mm³)</td>
<td>9.4 ± 2.4</td>
<td>9.9 ± 4.2</td>
<td>11.3 ± 3.9</td>
<td>10.2 ± 1.8</td>
<td>9 ± 0.9</td>
</tr>
<tr>
<td>RBC (Millil/mm³)</td>
<td>8.4 ± 0.7</td>
<td>8.9 ± 0.8</td>
<td>8.4 ± 0.5</td>
<td>8.5 ± 0.7</td>
<td>8.4 ± 0.8</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>12.8 ± 0.6</td>
<td>14.6 ± 0.7</td>
<td>12.6 ± 0.8</td>
<td>14 ± 0.8</td>
<td>13.7 ± 0.5</td>
</tr>
<tr>
<td>Hgb (g/dl)</td>
<td>43.5 ± 3.2</td>
<td>44 ± 1.0</td>
<td>43 ± 1.0</td>
<td>44 ± 1.9</td>
<td>42.5 ± 0.9</td>
</tr>
<tr>
<td>Pt (1000/mm³)</td>
<td>1603 ± 282</td>
<td>1633 ± 172</td>
<td>1467 ± 115</td>
<td>1497 ± 371</td>
<td>1385 ± 244</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>254 ± 24</td>
<td>278 ± 65</td>
<td>235 ± 3.8</td>
<td>251 ± 71</td>
<td>148 ± 21</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>39.4 ± 7</td>
<td>38.4 ± 6.1</td>
<td>31.4 ± 3.8</td>
<td>35.7 ± 7.4</td>
<td>23 ± 3.5</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>150 ± 25</td>
<td>136 ± 34</td>
<td>132 ± 13</td>
<td>130 ± 24</td>
<td>88 ± 29</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>22 ± 2</td>
<td>21.7 ± 3.5</td>
<td>22.4 ± 2</td>
<td>24.7 ± 1.6</td>
<td>23.5 ± 0.7</td>
</tr>
<tr>
<td>Cr (mg/dl)</td>
<td>0.1 ± 0.02</td>
<td>0.1 ± 0.03</td>
<td>0.1 ± 0.05</td>
<td>0.1 ± 0.04</td>
<td>0.1 ± 0.07</td>
</tr>
<tr>
<td>T.P (mg/dl)</td>
<td>1.4 ± 0.8</td>
<td>1.6 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Glu (mg/dl)</td>
<td>145 ± 21.4</td>
<td>138 ± 7.0</td>
<td>157 ± 14</td>
<td>152 ± 17</td>
<td>149 ± 5</td>
</tr>
<tr>
<td>T.Bil (mg/dl)</td>
<td>0.54 ± 0.06</td>
<td>0.4 ± 0</td>
<td>0.3 ± 0.01</td>
<td>0.4 ± 0</td>
<td>0.1 ± 0</td>
</tr>
<tr>
<td>D.Bil (mg/dl)</td>
<td>0.07 ± 0.8</td>
<td>0.08 ± 0.03</td>
<td>0.1 ± 0.07</td>
<td>0.05 ± 0.08</td>
<td>0.1 ± 0.05</td>
</tr>
<tr>
<td>ALB (mg/dl)</td>
<td>1.5 ± 0.5</td>
<td>1.3 ± 0.3</td>
<td>1.4 ± 0.7</td>
<td>1.5 ± 1</td>
<td>1.4 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P<0.05 as compared with the control, the doses of 50, 100, 200 and 1000 mg/kg of pd complex, RBC= Red blood cell, HCT= hematocrit, WBC= White blood cell, Pt = Platelets, Cr = Creatinine, Glu = Glucose, AST = Aspartate transaminase, ALT = Alanine transaminase, ALP = Alkaline phosphatase, ALB = Albumin, T.P = Total protein, T.Bil = Total bilirubin, D.Bil = Direct Bilirubin.

Statistical analysis

To compare the groups, analysis of variance (ANOVA) and Tukey’s Post Hoc Tests were performed. Two groups were compared using Student’s t-test. Fisher’s exact test was carried out to analyze the differences in tumor incidence (the percentage of animals with breast cancer). Values shown as mean ± SD. P<0.05 were considered to be statistically significant. SPSS statistical software version 14.0 was used to perform statistical analyses.

RESULTS

Pd complex effects on MC4-L2, CT26 and MF1-PI30 cells

Pd complex remarkably prevented the proliferation of CT26 and MC4-L2 cancerous cells in a dose and time dependent manner as compared with the control group (P<0.05). For MC4-L2 cells, Pd complex IC₅₀ was 600 µM after 48 h (Figure 2A), decreasing to 500 µM in 72 h (Figure 2A). For CT26 cells, Pd complex IC₅₀ was 100 µM after 24 h (Figure 2B), reducing to 50 and 100 µM after 48 and 72 h, respectively (P< 0.05) (Figure 2B). The Pd complex did not affect normal fibroblast cells (Figure 2C).

Pd complex toxicity in mice

Tables 1 and 2 show the main toxicity signs for the Pd complex in the various doses. BUN and Cr plasma levels were determined for the kidney function. However, ALP, T.Bil, D.Bil, GGT, ALB, ALT and AST were performed for the liver function. 50, 100, 200 and 1000 mg/kg doses of the Pd complex were injected in the acute toxicity. ALB, ALT and ALP levels increased after 24 h using 1000 mg/kg doses of the Pd complex (p<0.05) (Table 1).

In the present study, 2, 10 and 50 mg/kg doses of the Pd complex were injected for 7 consecutive days in the chronic toxicity groups and the animals were euthanized at the end of one week. No unfavorable reactions were observed in the hematological and blood chemical examinations up to the 10 mg/kg dose of Pd complex (Table 2). The survived animals (10 mg/kg and lower) were not clinically different from the control animals. A remarkable safety rate was shown by Pd complex doses of up to 10 mg/kg (Table 2). ALT was considerably lower in the animals of the chronic 50 mg/kg Pd complex than the control animals (p<0.05) (Table 2). A complete blood count analysis was carried out to measure the hematological markers. No inflammatory responses were observed in the acute and chronic groups because the total leukocyte counts remained within the normal range (Tables 1 and 2). Liver appears to be the target organ for the toxicity of the Pd complex in high doses in both acute and chronic groups.

Therapeutic effects of Pd complex on an animal breast cancer

Following tumor transplantation, the Pd complex (2 and 10 mg/kg) was given for 14 days up to day 28 to study the
Figure 2: The cytotoxic effects of the Pd complex on CT26 (A), MC4L2 (B) and MF1-PI30 (C) cells.

The results showed that the pd complex had a higher cytotoxicity in high doses. The IC_{50} doses of Pd complex in 72 and 48 h were around 500 and 600 nM, respectively in MC4L2 cell line. However, the corresponding IC_{50} dose was around 50 and 100 nM in 48 and 24 h, respectively in CT26 cell line. The PDd complex had no cytotoxicity against MF1-PI30 cells.
corresponding therapeutic effects on mice breast cancer. In the second week after Pd complex treatment (p<0.05), the average tumor volume was significantly less than that of the control group. As compared with the control group (1.35 ± 0.23 mm³), the mean final tumor volume reached almost 0.65 ± 0.07 and 0.55 ± 0.05 mm³ in groups treated with 2 and 10 mg/kg doses of the Pd complex, respectively (Figure 3).

Metastases

Routine surgery was performed on all animals at the end of the study. No signs of metastasis were observed in the major organs of both the Pd complex and the control groups.

DISCUSSION

The toxicity and therapeutic effects of the Pd complex on BALB/c mice breast cancer were evaluated in the present study. Toxicity is a vital factor in the safety of the metal particles in biomedicine arena. Appropriate strategies regarding the selection of suitable compositions to develop biocompatible and efficient metal carriers may be established by understanding the metal particle toxicity. Application of nanoparticles can open new frontiers in the treatments of breast cancer. Determination of non-toxic and safe doses of nanoparticles is a definitive step towards the clinical application of this technique. Administration of the Pd complex may cause liver toxicity in high doses in both the acute and chronic groups based on the hematological and chemical parameters. The findings of previous studies showed that the direct interaction with this element may lead to toxicity responses (Goossens et al., 2006). Significant renal tubular dysfunction and kidney toxicity were observed by Fontana et al. (2015) upon IV administration of 12 µg/kg Pd nanoparticles in the female Wistar rats (Fontana et al., 2015). In addition, higher Pd complex dosages can change the expression level of the pro-inflammatory cytokines including IL-1α, IL-4, IL-6, IL-10, IL-12, GM-CSF and INF-γ in the mammalians (Iavicoli et al., 2015). Our findings regarding the acute and chronic toxicities of the Pd complex may prove the low toxicity of the nanostructure in vitro conditions.

The Pd complex was observed to remarkably suppress the growth of the colon and the mammary carcinoma cells in vitro. More importantly, the complex considerably increased the tumor suppressing effects in both cell culture and a typical animal model of breast cancer. Based on the results of this study, the Pd complex suppressed the breast tumor development in both 2 and 10 mg/kg doses as compared with the control group. The safety and efficiency of the Pd nanoparticles in breast cancer treatment have been reported by (Shanthi et al., 2015). Pd nanoparticle prepared from Syzygium aromaticum aqueous extracts significantly decreases the development of cervical tumor and tumor weight in the xenograft mice (Jacob et al., 2016).

The main objective of this study was the development of

### Table 2: The chronic toxicity effects of Pd complex on the hematology and biochemistry parameters.

<table>
<thead>
<tr>
<th>Index</th>
<th>Control</th>
<th>2</th>
<th>10</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Weight (g)</td>
<td>24.3 ± 2.2</td>
<td>25 ± 3.1</td>
<td>24.7 ± 2.5</td>
<td>23.2 ± 2.2</td>
</tr>
<tr>
<td>WBC (1000/mm³)</td>
<td>9.6 ± 2.6</td>
<td>13.4 ± 4.3</td>
<td>9.8 ± 2.9</td>
<td>12.2 ± 4.2</td>
</tr>
<tr>
<td>RBC (Millin/mm³)</td>
<td>8.5 ± 0.8</td>
<td>9.0 ± 0.8</td>
<td>8.7 ± 0.6</td>
<td>9.0 ± 0.7</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>12.7 ± 0.8</td>
<td>14.3 ± 0.2</td>
<td>13.7 ± 0.7</td>
<td>14.3 ± 0.6</td>
</tr>
<tr>
<td>Hgb (g/dl)</td>
<td>43.5 ± 3.8</td>
<td>41.3 ± 2.5</td>
<td>40.3 ± 1.5</td>
<td>41.7 ± 1.5</td>
</tr>
<tr>
<td>Plt (1000/mm³)</td>
<td>1503 ± 322</td>
<td>1516 ± 146</td>
<td>1434 ± 133</td>
<td>1404 ± 204</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>166 ± 28</td>
<td>154 ± 16</td>
<td>190 ± 18</td>
<td>179.3 ± 20</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>39.4 ± 7</td>
<td>31.6 ± 17.7</td>
<td>33 ± 7.3</td>
<td>22 ± 2.7</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>150 ± 25</td>
<td>169 ± 30.3</td>
<td>163 ± 26.2</td>
<td>135 ± 22.4</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>22 ± 2</td>
<td>20 ± 2.7</td>
<td>24 ± 20.6</td>
<td>20.6 ± 2.5</td>
</tr>
<tr>
<td>Cr (mg/dl)</td>
<td>0.1 ± 0.02</td>
<td>0.1 ± 0.01</td>
<td>0.1 ± 0.02</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>T.P (mg/dl)</td>
<td>1.4 ± 0.8</td>
<td>1.5 ± 0.2</td>
<td>2 ± 0.9</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Glu (mg/dl)</td>
<td>145 ± 22</td>
<td>134 ± 15</td>
<td>126 ± 24</td>
<td>125 ± 31</td>
</tr>
<tr>
<td>T.BIL (mg/dl)</td>
<td>0.54 ± 0.07</td>
<td>0.40 ± 0.1</td>
<td>0.45 ± 0.2</td>
<td>0.40 ± 0.2</td>
</tr>
<tr>
<td>D.BIL (mg/dl)</td>
<td>0.10 ± 0.04</td>
<td>0.2 ± 0.09</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.04</td>
</tr>
<tr>
<td>ALB (mg/dl)</td>
<td>1.5 ± 0.5</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.5</td>
<td>0.8 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P<0.05 as compared with control, the doses of 50, 100, 200 and 1000 mg/kg of Pd complex, RBC= Red blood cell, HCT= hematocrit, WBC= White blood cells, Plt= Platelets, Cr= Creatinine, Glu = Glucose, AST = Aspartate transaminase, ALT = Alanine transaminase, ALP = Alkaline phosphatase, ALB = Albumin, T.P = Total protein, T.BIL = Total bilirubin, D.BIL = Direct Bilirubin.
Figure 3: Effects of Pd complex on tumor growth of mice breast cancer. Administration of both 2 and 10 mg/kg Pd complex inhibited breast tumor growth. The volume of the tumors was significantly smaller than that of the control on days 24, 27 and 30 after tumor implantation. * p<0.05 as compared with the control.

an applicable strategy for cancer treatment. Chemotherapy is still one of the initial modalities for cancer treatment. A new class of trinuclear pharmacologically applicable palladium oximes has been synthesized. Since chelating ligands minimize the high lability and fast hydrolysis of palladium complexes in biological media, they exhibit good clinical effects as cytotoxic agents on the therapy of various cancers. Complexation increases the potency of the cytotoxic agents. Therefore, the addition of ligands enhances the biological activity of metal salts. Metal ion positive charge is suggested to be partially shared with the donor atoms. In addition, there is electron delocalization over the whole chelate ring system containing the center anion. This effectively influences the complex solubility. However, chelation makes the metal salts more powerful and potent cytotoxic agents. Such factors as solubility, conductivity, dipole moment and cell permeability mechanism (influenced by the presence of metal ions) may be responsible for this increased biological activity. Nevertheless, the application of chelating ligands in the synthesis of trinuclear palladium oximes improved their stability, which was further enhanced by the generation of cyclopalladated compounds. Stirring Li₂PdCl₄ with 9-fluorenone oxime and sodium acetate in MeOH for 2-3 days at ambient temperature yielded [Pd₃(C₃H₆C=NO)₆] palladium oxime complex in one step (Figure 1). IR spectroscopy can be used to monitor the coordination mode of the ligand. The typical bands in the IR spectrum of the complex at 3370, 3062 and 1064 cm⁻¹ are associated with ν (OH), ν (C-H aromatic) and ν (N-O), respectively. The band associated with the oxime complex in ν (N-O) of the free ligand (997 cm⁻¹) was shifted to higher wave numbers and ν (C=N) stretch at 1602 cm⁻¹ shifted to lower wave numbers (as compared with the free ligand) as a result of the N-coordination of the oxime (Karami et al., 2015). Pd complex formation has been further confirmed by detection of the peaks in IR, ¹H and ¹³C NMR spectra of the complex (Figures S1-S3 in the Supporting Information). The NMR spectrum of complex is in line with the proposed structure. The Pd complex electronic spectra shows bands of around 236, 258 and 364 nm due to intra-ligand (ILCT) transitions of (n-π*) and (π-π*) types and metal to ligand charge transfer (MLCT), respectively.

Conclusion

In conclusion, the synthesized Pd complex has been shown to be effective in suppressing tumor growth both in vitro and in vivo. The synthesis and characterization of a new series of trinuclear palladium complexes and the pharmacological properties of the complexes in DNA/protein binding interactions have been carried out. In addition, 2 and 10 mg/kg daily doses of the Pd complex are suggested to be suitable for 2 weeks in the treatment of breast cancer. The findings show that the Pd complex is a
promising drug delivery system for cancer therapy.

ACKNOWLEDGMENT

This study was supported by a grant from Tehran University of Medical Sciences.

REFERENCES


Submit your manuscript at: http://www.academiapublishing.org/ajmp

Cite this article as: