Tumoricidal Effect of Gamma Irradiation and Water Extract of Harjal Plant Leaves (Hayne) on Ehrlich Carcinoma in Female Mice

N. Hanafi

Radiation Biology Dept., National Centre for Radiation Research and Technology (NCRRT), P.O.Box:29Nasr City, Egypt.
E-mail: Nhanafi58@yahoo.com
Received: 28/05/2012. Accepted: 04/07/2012.

ABSTRACT

The present study aims to evaluate the antitumor effect of gamma irradiation and hot water extract of harjal (Solenostemma argel) plant leaves on Ehrlich carcinoma (EC) in female mice. The effect of Harjal plant leaves extract (HPE) on Ehrlich ascite carcinoma cells and growth of transplantable EC were studied. HPE was administrated orally 3 times per week for 3 weeks to the experimental animals 24 hr and 7 days after EC inoculation at the dose level 15 mg/kg body weight. Experimental animals were exposed to 6 Gy of γ-radiation 7 days after tumor inoculation (ATI). Histopathological examination, apoptotic and necrotic detection in EC tissue were studied. Oxidative stress markers (MDA and GSH levels SOD and CAT activities) for EC tissue were also examined. HPE activates tumor cell death after two weeks. γ-irradiation and HPE either alone or combined significantly decrease tumor size and showed wide zones of apoptotic tumor cells in tumor tissue. γ-irradiation and HPE either alone or combined produced a non significant change in oxidative stress markers of EC tissue. Histopathologically, γ-irradiation and HPE represented large areas of apoptosis, hydropic degeneration and nuclei debris in tumor tissue sections. In conclusion, γ-irradiation and HPE represent antitumor activities either alone or combined.

Keywords: Chemosensitivity of HPE, tumor size, histopathology, apoptosis and necrosis, oxidative stress markers.

INTRODUCTION

Cancer has become an important topic in medicine since it is a major cause of death in both the developed and developing countries and it is now
only secondary to that of myocardial infarction \(^1\). Modern surgery has significantly reduced the cancer mortality. Also, the use of additional treatment such as radiotherapy and chemotherapy has resulted in no more than 5\% reduction in the number of deaths \(^2\). Many plants extracts have shown various biological activities like immunopotentiating and antitumor activities. Therefore, there is a continuing search for better control and preventive methods to reduce cancer mortality and related side effects. Many investigations are now being carried out to discover naturally occurring compounds, which can suppress the process of carcinogenesis \(^3\).

Radiation therapy is considered to be one of the most popular and important tools to cure cancer through the free radicals which interact with critical targets like DNA and membranes bring about irreversible damage leading to cell death \(^4\). However, radiation therapy of cancer as the elevated antioxidant status of irradiated tumors is likely to limit the effectiveness of radiation dose and adversely affect the therapeutic gain \(^4\). Some serious implications as the increased radiation-damage of the distant normal organs are likely to adversely affect the therapeutic gain \(^5\).

Natural products from plants have been valuable sources for anticancer drug discovery \(^6\). Chemical and pharmacological studies of various extracts or compounds purified from the herbs were found to increase myocardial blood blow, reduce radiation damages and purify blood quality \(^7,8,9\). Harjal \((Solenostemma argel)\) is a shrub occurring in wild state in Egypt, and Sudan. The herb of the plant is locally called El Hargal \(^10\). The natives prepare decoctions from the leaves and branches for treatment of various colics and pains \(^10\).

The Ehrlich ascites tumor is a spontaneous murine mammary adenocarcinoma \(^11\) adapted to ascites form \(^12\) and carried in outbred mice by serial intraperitoneal (i.p.) passage.

For developing countries the use of endogenous medicinal plants as cures against cancers is attractive. This study was therefore designed to study the effect of \(\gamma\)-radiation exposure either alone or combined with HPE (hot aqueous extract) to evaluate their antitumor activity against Ehrlich carcinoma (EC) bearing in female mice.

**MATERIALS AND METHODS**

Healthy Adult female Swiss albino mice (6–8 weeks old) weighing 20–25g purchased from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Cairo) were used in this study. The animals
were maintained on a commercial standard pellet diet and tap water *ad libitum*. Animal maintenance and treatments were conducted in accordance with the National Institute of Health Guide for Animal, as approved by Institutional Animal Care and Use Committee (IACUC).

**Radiation facility:**

Whole body gamma irradiation of animals was performed using a Canadian $^{137}$Cs Gamma Cell-40 at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt; at a dose rate of 0.61 Gy/min. Mice were exposed to 6Gy whole body $\gamma$- radiation delivered as a single dose.

**Tumor Transplantation:**

A cell line of Ehrlich Ascites Carcinoma (EAC) was used in this study. The parent line was supplied from the Egyptian National Cancer Institute (NCI), Cairo University. The tumor line was maintained in the experimental female Swiss albino mice by weekly intraperitoneal injection of 2.5 millions cells per mouse. The EAC cells were counted before intraperitoneal injection using the bright line haemocytometer and dilution was done using physiological sterile saline solution. The desired numbers of cells were injected in a volume of 0.2 ml. To asses Ehrlich solid tumour, 0.2 ml EAC cells ($2.5 \times 10^6$ cells/mouse) were inoculated intramuscularly in the right thigh of the lower limb of female mouse.

**Preparation of Harjal plant water extract**

Harjal plant was obtained from local market. The leaves of Harjal (*Solenostemma argel*) were free from the other parts of the plant and crushed using an electric blender (Moulinex). 10 g of the crushed leaves were extracted with 100 ml of distilled water in water bath ($80^\circ$C) for 90 min. The volume was adjusted to 100 ml by passing hot water through the residue. The resulting straw colored infusion was filtered through a muslin cloth. The HPE was administered orally to experimental animals at the doses of 15 mg/kg body weight/day x 3/week for three weeks.

**Cytotoxic activity of HPE**

To study the cytotoxic activity of HPE extract on Ehrlich ascites carcinoma cells (EACC), 10 animals of female Swiss albino mice were transplanted with EACC and each mouse was day per day forced to ingest orally via a stomach tube about 0.2 ml of hot water HPE extract at dose level 15 mg/kg body weight/day. After one week samples of EACC were taken and the
number of EACC was calculated for each animal in each week

**Tumor size monitoring**

The growth of solid Ehrlich carcinoma in the lower limb of female mouse was monitored throughout the experiment. The tumor size being measured regularly twice or thrice weekly using Vernier callipers and represented in terms of tumor size. The tumor size was estimated using the following formula: Tumor size (mm$^3$) = $0.52 \ A \ B^2$, where $A$ is the major axis and $B$ is the minor axis$^{(13)}$. The mean tumor size with the corresponding standard error was calculated in each experimental group. 3 weeks ATI experiment was terminated. At the termination of the experiment, all the animals were sacrificed.

**Experimental design:**

Hot aqueous extract of HPE and 6 Gy of $\gamma$- irradiation were tested to evaluate their antitumor activity against Ehrlich carcinoma (EC). HPE was administered day after day orally to experimental animals 3 times/ week for 3 weeks. $\gamma$-radiation exposure was done 7 days ATI. The animals were randomly divided into 6 groups.

- **G1:** Mice bearing EC.
- **G2:** Mice bearing EC exposed to $\gamma$- radiation 7 days ATI.
- **G3:** Mice bearing EC treated by HPE 24 hours ATI.
- **G4:** Mice bearing EC treated by HPE 7 days ATI.
- **G5:** Mice bearing EC treated by HPE 24 hours ATI and exposed to $\gamma$- radiation 7 days ATI.
- **G6:** Mice bearing EC treated by HPE and exposed to $\gamma$- radiation 7 days ATI. Twenty four hours after the last treatments all mice were sacrificed.

**Biochemical analysis of oxidative markers**

After cervical decapitation of mice, samples of EC tissue were washed with 0.9% NaCl solution and stored at -20°C till biochemical oxidative markers analysis. Reduced glutathione (GSH) concentration was measured by the method of Moron et al.$^{(14)}$. Malondialdehyde (MDA) level was measured by the method of Beuege and Aust$^{(15)}$. However the activity of super oxide dismutase (SOD) was assayed by the method of Kakkar et al.$^{(16)}$. Meanwhile Catalase (CAT) activity was assayed according to the method of Maehly and Chance$^{(17)}$.

**Histopathological observations**

Samples of tumor tissue were fixed in 10% formalin and embedded in paraffin. Sections of tumor tissue were stained with Hematoxylin and Eosin (H & E) and examined under light microscope.
Apoptosis and necrosis detection.

For apoptosis and necrosis detection according to Bank \(^{18}\) paraffin tumor tissue sections on positive slides were stained in 5 μg/ml of propidium iodide and 50 μg/ml of acridine orange in phosphate-buffered saline and examined under fluorescence microscopy. The early apoptotic cells had yellow chromatin in nuclei that were highly condensed or fragmented. Apoptotic cells also exhibited membrane blebbing. The late apoptotic cells had orange chromatin with nuclei that were highly condensed and fragmented. The vital regions were stained in green and the necrotic regions had bright orange chromatin in round nuclei.

Statistical Analysis

Statistical analysis for the obtained results was carried out with the aid of the SPSS computer software program.

RESULTS

Chemosensitivity of HPE extract.

As shown in Table (1) when HPE was administrated to mice the numbers of EACC in tumor-bearing mice were significantly reduced. A non significant change in viable cell number was recorded after one week. However HPE appear to activate EACC tumor cell death possibly after two weeks.

Table (1): Effect of the extracts on the number of viable cells in transplanted mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>One week (- (\times 10^6))</th>
<th>Two weeks (- (\times 10^6))</th>
</tr>
</thead>
<tbody>
<tr>
<td>EACC</td>
<td>83 ± 3.90</td>
<td>479 ± 22.51</td>
</tr>
<tr>
<td>EACC +HPE</td>
<td>81 ± 2.92</td>
<td>47 ± 1.69</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D and mean of three replicates.

Tumor size

Mice-bearing EC were administered by gavage with HPE either 24 hr. or 7 days of tumor cells inoculation and/or \(\gamma\)- radiation exposure up to day 21. Antitumor effect was assessed by time interval measurements of changes in tumor size during the experimental time course. Fig. (1) shows that gavages of mice with HPE either 24 hr (G3) or 7 days ATI (G4) or exposure to \(\gamma\)- radiation 7 days ATI (G2) caused a continuous regression of tumor size recording 317.000±28.40 mm\(^3\), 274.200±27.00 mm\(^3\) and 274.200±10.47 mm\(^3\), respectively compared with control level 608.75±54.70 mm\(^3\) on the 13\(^{th}\) day ATI. A more pronounced regression in tumor size was recorded when experimental animal’s were administrated with HPE either 24 hr (G5) or 7 days ATI (G6) combined
with exposure to 6 Gy of γ-radiation 7 days ATI. The tumor size reached 204.850±18.85 mm³ and 157.933±15.16 mm³, respectively compared to 1188.25±95.00 control tumor sizes (G1) after 21 days of tumor inoculation.

**Fig. (1): Effect of HPE and/or γ–radiation on tumor size of Ehrlich carcinoma.**

**Effect of HPE and/or γ–irradiation on oxidative markers parameters.**

As shown in Table (2) the levels of lipid peroxidation in tumor tissue were not significantly changed when experimental animals were treated with HPE either after 24 h. (G3) or 7 days (G4) of tumor inoculation compared to tumor MDA level. However the exposure of the tumor bearing mice to 6 Gy γ-radiation 7 days ATI (G2) predict a significant increase (P < 0.05) in lipid peroxides of tumor tissue 21 days ATI. Combined treatment of tumor bearing mice with HPE and γ-radiation exposure caused a no significant change in lipid peroxides of tumor tissue either for G5 or G6. Gavage administration of HPE 24 hr. ATI or 7 days ATI either alone or combined with γ-radiation exposure did not significantly change GSH level and CAT activity. However γ-radiation exposure either alone (G2) or combined with HPE administration predict some ameliorative effect in SOD level compared to that of the control tumor tissue level.

**Table (2): Effect of HPE and/or γ–irradiation on oxidative markers parameters of tumor tissue in mice bearing EC.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (µg/ g tissue)</th>
<th>GSH (µg/ g tissue)</th>
<th>CAT (µmol/ g tissue)</th>
<th>SOD (µg/ g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>111.542 ± 4.86</td>
<td>67.703 ± 2.67</td>
<td>26.666 ± 2.70</td>
<td>4.708 ± 0.10</td>
</tr>
<tr>
<td>G2</td>
<td>145.928 ± 3.13a</td>
<td>73.668 ± 1.92</td>
<td>21.018 ± 1.19</td>
<td>3.640 ± 0.10a</td>
</tr>
<tr>
<td>G3</td>
<td>102.575 ± 3.25</td>
<td>70.643 ± 2.98</td>
<td>25.643 ± 2.08</td>
<td>4.303 ± 0.15</td>
</tr>
<tr>
<td>G4</td>
<td>105.380 ± 2.24</td>
<td>71.335 ± 3.52</td>
<td>23.835 ± 1.75</td>
<td>4.715 ± 0.15</td>
</tr>
<tr>
<td>G5</td>
<td>108.660 ± 2.37</td>
<td>73.750 ± 1.87</td>
<td>23.575 ± 1.78</td>
<td>4.228 ± 0.22a</td>
</tr>
<tr>
<td>G6</td>
<td>117.018 ± 2.12</td>
<td>67.778 ± 1.92</td>
<td>25.728 ± 2.37</td>
<td>3.923 ± 0.12a</td>
</tr>
</tbody>
</table>

Results are presented as the mean±SE.

Superscript a: significant from Ehrlich group at P ≤ 0.05.
Histopathological examination of the Ehrlich carcinoma

Histopathological examination of EC tissue under light microscope shows aggregation of the tumor tissue cells spread within the muscular tissues. EC tumor tissue shows groups of large, round and polygonal cells, with pleomorphic shapes. Hyperchromatic nuclei and binucleation were also observed in tumor cells. Mitosis, muscle invasion and coagulation necrosis represent disappearance of stainable nuclei of tumor cells were also noticed (Fig. 2 A). Treatment of female mice bearing EC 24 hr. ATI for 3 weeks with HPE represents large areas of nuclear karyorrhexis, nuclear karyolysis and hydropic degeneration in tumor tissue sections (Fig. 2 B). Another same effect was observed in tumor tissue section when the experimental animals gavages with HPE on the 7th day ATI (Fig. 2 C & C*).

Fig (2): Photomicrographs of sections in EC. A: Section in normal EC. shows large, round and polygonal tumor cells (↑). B: Section in EC treated with HPE 24 hr ATI represents hydropic degeneration (blocked arrow) and nuclear debris (▲). C&C*: Sections in EC treated with HPE 7 days ATI. (H&E. X400)
Highly degenerative effect was detected in tumor tissue section extirpated from animals exposed to 6 Gy of γ-radiation on the 7th day ATI (Fig. 3 D). Tumor tissue section shows great areas of apoptosis in addition of some pyknotic nuclei. In the tumors extirpated from animals treated with HPE 24 h. ATI for 3 weeks and exposed to 6 Gy of γ-radiation on the 7th day ATI extensive regions of apoptosis were observed. In addition to the presence of remnant tumor cells contained pyknotic nuclei were detected (Fig.3 E & E*). In the other hand in the group of experimental animals treated with HPE on the 7th day ATI and exposed to 6 Gy of γ-radiation, apoptotic tumor cells and nuclear debris were observed (Fig.3 F&F*).

**Apoptotic and necrotic examination of the Ehrlich carcinoma**

Apoptotic and necrotic examinations of the EC tissue section under a fluorescent microscope represented green viable tissue cells with no zones of necrosis (orange cells) or apoptosis (yellow cells) (Fig. 4 A&B). Treatments of female mice bearing EC 24 hr. ATI for 3 weeks with HPE represented no zones of necrosis but with high and wide zones of apoptotic cells (Fig. 4 C&D). Less effect was observed in tumor tissue sections when experimental animals were
gavage with HPE on the 7th day ATI represented a viable green tissue cells with moderate sporadic apoptotic cells (Fig. 4 E) and multiple vacuoles (Fig. 4 F).

Exposure of the experimental animals to 6 Gy of $\gamma$-radiation on the 7th day ATI showed viable green tissue cells and sporadic apoptotic yellow cells (Fig. 5 G&H) after 3 weeks from radiation exposure. In the tumors extirpated from animals treated with HPE 24 hr. ATI for 3 weeks and exposed to 6 Gy of $\gamma$-radiation on the 7th day ATI represent extensive wide zones of apoptotic cells with presence of some vacuoles (Fig. 5 I&J). While in the group treated with HPE on the 7th day ATI and exposed to 6 Gy of $\gamma$-radiation, no zones of necrosis (orange cells) were observed but wide zones of apoptotic cells were observed (Fig. 5 K&L).

Fig. (4): Fluorescent photomicrographs of sections in EC. A&B: Sections in normal EC represent viable tissue (green cells). C & D: Sections in EC treated by HPE 24 hr ATE. represent wide zones of apoptotic cells (yellow cells ➲) E&F: Sections in EC treated by HPE 7 days ATI represent apoptotic cells (yellow cells ▲) and multiple vacuoles(↑).
Fig (5): Fluorescent photomicrographs of sections in EC. G&H: Sections in EC subjected to \(\gamma\)-radiation 7 days ATI show sporadic apoptotic yellow cells (↑). I&J: Sections in EC treated by HPE 24 hr ATI and subjected to \(\gamma\)-radiation 7 days ATI show zones of apoptotic cells (→) and some vacuoles (▲). K&L: Sections in EC treated by \(\gamma\)-radiation and HPE 7 days ATI represent wide zones of apoptotic cells (curved arrows).

**DISCUSSION**

In recent years, there is an increasing awareness that certain naturally occurring compounds in plants and other sources, have protective effects against environmental mutagens/carcinogens and endogenous mutagens \(^{19}\). It was shown that plant like HPE which is a sharp occurring in wild state in Egypt noticed to have several glycosides used in treatment of various colics and pains \(^{10}\). In the present study a pronounced delay in EC tumor volume was recorded when experimental animal’s gavages with HPE either 24 hr or 7 days ATI which is advantages with the finding of Plaza et al. \(^{20}\) whom reported that HPE contain 15-keto pregnane glycosides. The effect of these compounds on the vascular endothelial growth factor (VEGF) induced in Kaposi’s sarcoma cell proliferation was tested and the results indicated that all the compounds reduced the cell proliferation in a dose dependent manner. Also Nassr-Allah et al. \(^{21}\) reported that hot water extract of HPE significantly reduced Ehrlich ascites carcinoma cells induced tumor growth and delayed animal death by 29 days.

Also the exposure to \(\gamma\)-radiation 7 days ATI caused a continuous delay of tumor volume due to radiation oxidative stress induction which was reflected
by the enhanced levels of peroxidative damage, DNA fragmentation, LDH activity and nitric oxide levels (4). A benefit of ionizing radiation as a therapeutic tool is the possibility to apply it locoregionally thereby preventing systemic toxicity. However like chemotherapeutic agents ionizing radiation does not affect all target cells, which can lead to severe side effects in the surrounding tissue after the therapy. In addition there are large numbers of human malignant tumor cells that respond poorly to ionizing radiation. However, radiation dose to the tumor can not be increased as needed because of the normal tissue toxicity in the radiation field. Hence there is a need for chemical agents which upon contact with tumor cells increase their sensitivity to radiation thus minimizing large doses of radiation and also spare normal tissue from the combined toxic effects (22). In the present study more pronounced delay in tumor volume was recorded when experimental animals gavage by HPE and γ-irradiation in which the antitumor synergistic effect between radiation exposure and HPE supplementation were detected.

Induction of apoptosis in tumor cells, a form of physiological death in unwanted or dysfunctional cells, is an appealing therapeutic approach (23). Treatment of female mice bearing Ehrlich carcinoma tumor 24 hr. or 7 days ATI for 3 weeks with HPE represented wide zones of apoptotic cells in tumor tissue. The results reported here that hot water extracts of HPE may have an immuno-modulatory potential associated with the content of phenolics, including flavonoids stimulating antiproliferation of tumor cells and appears to involve apoptosis-induced cell loss (21). Also the exposure to 6 Gy of γ-radiation on the 7th day ATI represent sporadic apoptotic yellow cells in tumor tissue due to induction and repair of DNA damage, cell cycle disturbances, programmed cell death, alterations in gene expression and signal transduction pathways (24, 25). Induction and repair of DNA damage are central among the several molecular targets for modifying cellular radiation responses and most often correlated well with cell death (26, 27). Also radiation exposure produces peroxidation on biological membranes. Peroxidation brings about changes in the structure and functions of tumor membranes (28). This oxidative damage of membranes is also closely linked with radiation induced apoptosis (29).

Gavage treatment of animals bearing EC by HPE either after 24 hr. or 7 days ATI combined with γ-radiation exposure recorded wide zones of apoptotic cells in tumor tissue which predict the synergistic effect of HPE and γ-radiation exposure in resulting tumor regression.
The results of functional tests together with histological observations suggest that either γ-radiation exposures or Ehrlich tumour inoculation treatments leads to serious changes in histology of mice organ tissues (30). The increased formation of lipid peroxides and associated reactive oxygen species leads to damage in membrane integrity and other pathological changes.

Lipid peroxidation, an autocatalytic free radical chain propagating reaction, is known to be associated with pathological conditions of a cell. Malondialdehyde (MDA), the end product of lipid peroxidation, was reported to be higher in cancer tissues than in non diseased organ (31). In the present study HPE treatment either after 24 h. or 7 days of tumor inoculation predicts a non significant change in lipid peroxidation level of tumor tissue. However the exposure of the tumor bearing mice to 6 Gy γ-radiation predict a significant increase in lipid peroxides in tumor tissue context with the findings of Agrawal et al. (4) whom reported that Radiation induced oxidative stress in Ehrlich solid tumor in mice.

The previous revealed that γ-radiation induced oxidative stress as reflected by the enhanced levels of peroxidative damage in Ehrlich solid tumor in mice (4) and HPE was an immunomodulatory components containing phenolics, including flavonoids play a role in oxidation inhibition (21). So the combined treatments of tumor bearing mice with HPE and γ-radiation exposure resultant a non significant change in lipid peroxides in tumor tissue.

A non significant change in GSH and CAT levels in tumor tissue were predicted after gavages administration of HPE 24 hr. ATI or 7 days ATI either alone or combined with γ-radiation exposure predict a non significant change in GSH and CAT which explain the more pronounced delay in tumor volume. However γ-radiation exposure either alone or combined with HPE administration predict some ameliorative effect in SOD level compared to control tumor tissue level context with the finding of Agrawal et al. (4).

Histologically treatment of female mice bearing Ehrlich carcinoma tumor 24 h. or 7 days ATI for 3 weeks with HPE represented large area of apoptosis, hydropic degeneration and nuclear debris in tumor tissue sections. Ariens et al. (32) reported that tissue toxicity usually manifests itself, especially in the histological prepration, in the form of cell degeneration accompanied by formation of large vacuoles, accumulation of fat and tissue necrosis which predict the toxicity of HPE on tumor tissue (21). Exposure to 6 Gy of γ-radiation revealed highly degenerative effect with great areas of apoptosis in addition of
some pyknotic nuclei. The increased formation of lipid peroxides associated with reactive oxygen species leads to damage in membrane integrity and other pathological changes\(^{(30)}\).

Due to the synergistic effect of HPE and $\gamma$-radiation exposure in resulting tumor regression, tumors extirpated from animals treated with HPE 24 h. or 7 days ATI for 3 weeks and exposed to 6 Gy of $\gamma$-radiation on the 7\(^{th}\) day ATI extensive areas of apoptosis, presence of remnant tumor cells contained pyknotic nuclei and nuclear debris were recorded.

The available data from the studies indicate that HPE (15 mg/kg body weight) has a tumorcidal action against Ehrlich carcinoma. Combined treatments of mice bearing Ehrlich carcinoma with HPE and radiation exposure enhances the oxidative stress against tumor tissue. This may offer potential therapeutic benefit, which warrants clinical study for application in cancer radiotherapy.

**REFERENCES**


التأثير المتلف لأشعة جاما واستخلاص الماني من أوراق نبات الحرج (هاين)
لورم إيرليك في إناث الفنار
نعمان حنيف أحمد
قسم بحوث بيولوجيا الإشعاع - المركز القومي لبحوث وتقنية الإشعاع

تهتم الدراسة الحالية بتقييم تأثير 6 جرائ من أشعة جاما و المستخلص الماني لأوراق نبات الحرج (هاين) المستضد لورم إيرليك السرطاني في إناث الفنار. حققت فنار التجارب عن طريق الفم بالمستخلص الماني لحفل بجرعة مقدارها 15 مجم/كجم بعد 24 ساعة و 7 أيام من زرع ورم إيرليك. وتم تعرض فنار التجارب لأشعة جاما بعد 7 أيام من زرع ورم إيرليك. من خلال تلك الدراسة تم الكشف عن التأثير الكيميائي، المستخلص الماني للحفل على خلايا ورم إيرليك وفحص التركيب الهيستوئولوجي ونوبة أنسجة ورم وإجراء دراسة هستوئولوجية وقياس مستوى إنزيمات الضغط التاصدي لنسبة الورم. المستخلص الماني لأوراق نبات الحفل ينشط خلايا الورم بعد أسبوع ويسجل منفردا أو معتمدا مع التعرض لأشعة جاما إنتاج كيرفي حجم الورم ويظهر في أنسجة الورم مناطق واسعة متشابهة بخلايا الورم المبرمجة موتا. سجل التعرض لأشعة جاما والمستخلص الماني لأوراق نبات الحفل تغيير غير معنوي لإنزيمات الضغط التاصدي بنسبة الورم بفنان التجارب. هستوئولوجيا أشعة جاما و/أو المستخلص الماني لأوراق نبات الحفل أوضحت مساحات كبيرة من الموت المبرج للخلايا وتنكس هيدروبكي وأنقاض نوية في مقاطع أنسجة الورم.

المستخلص: أوضحت الدراسة تأثير أشعة جاما و/أو المستخلص الماني لأوراق نبات الحرج المضاد لتكوين ورم إيرليك السرطاني وتطوره.