Effect of *Bidens pilosa* extract on renal functions and some tumor markers of Ehrlich Ascites Carcinoma bearing mice exposed to \(\gamma\)-radiation.

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**ABSTRACT**

The Ethanolic extract of *Bidens pilosa* (EtBP) was tested in Swiss albino mice transplanted with Ehrlich ascites carcinoma (EAC) and exposed to \(\gamma\)-radiation. EAC mice received intraperitoneal (i.p) 250 mg/kg body weight EtBP for nine days, 24hr after tumor inoculation. Mice exposed to 4Gy \(\gamma\)-radiation 30 min after the first dose of EtBP. Seventy female mice were classified into 6 groups (15 mice in each group) as follows, control, mice treated with EtBP for 9 consecutive days, mice bearing EAC cells, EAC bearing mice treated with EtBP, 24 hour after tumor inoculation, EAC bearing mice and irradiated, and EAC bearing mice treated with EtBP and exposed to \(\gamma\)-radiation. Five animals from each group were sacrificed 18 hr after administration of the last EtBP dose. Blood and ascetic fluid were collected and kidneys were removed for biochemical and histopathological studies. The remaining animals were observed daily for recording survival percentage and body weight. Results showed that treatment of EAC bearing mice with EtBP and/or exposure to \(\gamma\)-radiation increased the survival percentage of the animals and decreased their body weight compared to EAC group. Inoculation of mice with EAC cells resulted in biochemical and histopathological changes leading to kidney damage. Animals of EAC bearing mice with EtBP and/or exposure to \(\gamma\)-radiation significantly restored the elevated levels of serum urea and creatinine, tumor necrosis factor-alpha (TNF-\(\alpha\)), metalo matrix protein (mmp-2 and mmp9), also the elevated level of lipid peroxidation (MDA) in kidneys tissue, compared to EAC group. On the other hand, a significantly decline was observed in glutathione (GSH) and super oxide dismutase (SOD) contents in kidney tissue of EAC group. Treatment of EAC bearing mice with EtBP and/or
exposure to γ-radiation resulted in increase GSH and SOD in kidney tissue and increased caspase-3 in ascetic fluid, comparing to EAC group. It could be concluded that EtBP through its antioxidant properties may be have both radio protective effect on normal tissue cells and radio sensitizer on tumor cells. The biochemical results were supported by the histopathological examination of kidney tissue. There was amelioration in pathological structure, to a great degree, towards normal intact histological structure. In conclusion, Ethanolic extract of *Bidens pilosa* exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing mice.

**Keywords:** Ethanolic extract of *Bidens pilosa*, Ehrlich ascites carcinoma, γ-radiation, kidney functions, antioxidants, antitumor activity, radio sensitizer, Caspase-3, TNF-α, mmp-2 and mmp-9.

**INTRODUCTION**

Ionizing radiation affect on both normal and neoplastic tissues, the nature and extent of such effect can be modified by chemical agents such as radio sensitizers, radio protectors and chemotherapeutic agent (1). The effect of radiation on tumor tissue can be optimized by adding radio sensitizing agents, in order to achieve a greater degree of tumor damage than expected from the use of either treatment alone (1). The combination therapy of tumors treatment with radiation and different anticancer drugs like 5-flurouracil (2), mitomycin C (3), etoposide (4) and carboplatin (5) have been tried clinically. However the side effect of such modes of treatment are severe and have resulted in the occurrence of secondary malignancies (1).

Researchers have been looking for anti-tumor agents in natural products to develop novel therapeutic agents for cancer (6). The herbs drugs have gained attention and popularity because of their negligible toxicity and possibly with a ray of hope that they may replace some of the available anti-neoplastic drugs that are highly toxic.

A benefit of ionizing radiation as a therapeutic tool is a possibility to apply its loco regionally, 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 number of 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 (1).

Several plant products have been tested for anticancer activity and some of them are now available as a drug of choice. One of the best approaches in search for anticancer agents from plant resources is the selection of plants based on ethno medical leads and testing the selected plants efficacy and safety in light of modern science (7).

*Bidens pilosa* is a herbaceous plant widely distributed in Africa, America, China, and Japan. It is used in traditional medicines as anti-inflammatory and in the treatment of various diseases, including hepatitis and diabetes (8). Nakama *et al.* (2011) (6) evaluated the effects of *Bidens pilosa* and investigated the molecular pathways responsible for anti-adult T-cell leukaemia effect. Previous studies showed that *Bidens pilosa* inhibited the proliferation of HTLV-1 infected T-cell lines by arresting cell cycle and induced apoptosis of HTLV-1 infected T-cell lines (6). Previous studies have demonstrated the cytotoxic activity of *Bidens pilosa* extracts against Hela and KB carcinoma cell lines (9).

*Bidens pilosa* is a worldwide distributed annual and perennial herbs that became rampant in Taiwan only over the past two decades (10). The whole plant and aerial parts are being used in various folk medicines (or as a popular ingredient in herbal tea) for their anti-inflammatory, antiseptic, liver-protective, blood-pressure lowering and hypoglycemic effects (11). The nutritional, phytochemical, antioxidant and antibacterial activities of the acetone, methanol and water extracts of the leaves of *Bidens pilosa* album were investigated by Adedapo *et al.* (2011) (12). The proximate analysis showed that, the leaves of the plant contained appreciable percentage of moisture content, ash content, crude protein, crude lipid, crude fibre and carbohydrate, and the elemental analysis in indicated that the leaves contained sodium, potassium, calcium, magnesium, iron, zinc, phosphorus, copper, manganese, and nitrogen. The chemical composition in mg/100 g d.w. showed the presence of alkaloid, saponins, and phytate.

The two main active constituents in *Bidens pilosa* are polyacetylenes, which inhibit various pathogenic organisms and flavonoids, which are used for reducing inflammation. The polyacetylenes can also manifest an anti-inflammatory action, probably mediated by a different mechanism than the
flavonoids. *Bidens pilosa* also has fried lane triterpenes and essential oils which may contribute to the observed therapeutic action of the herb. Analyses of various species of *Bidens pilosa* have been conducted in several countries. Although there is some variation in the levels activity of the different species of *Bidens pilosa*, probably due to different levels of active constituents, the general properties appear similar (13). These phytochemical components have been found to be responsible for the various medicinal activities of *Bidens pilosa* and these activities include antimalarial activity due to the presence of acetylene and flavonoid (14), chemoprotective activity of ethyl acetate and butanolic fractions (15), protects liver from cholestatic disease (16), anticancer properties by photo-activated polyacetylenes (9), infection-inhibiting and anti-inflammatory properties (17,18), treatment of headache in Zulu land by inhibiting cyclooxygenase, an activity associated with the flavonoid components (19).

MATERIAL AND METHODS

**Preparation of the Bidens pilosa extract:**

*Bidens pilosa* was kindly supplied by National Research Center's garden, Cairo, Egypt. The extract was prepared from dried ground aerial parts. A total of 200 gm of powdered *Bidens pilosa* L. was macerated for 48 hour in 70% ethanol then filtered. The obtained residue was 3-fold extracted with 70% ethanol for 1 hour by mechanical stirring, then extracts were pooled, and concentrated under reduced pressure at 50°C until all the organic solvent was removed. Finally, it lyophilized to obtain the hydroethanolic extract. Animals were administered intraperitoneally with 250 mg/kg body weight per day for 9 consecutive days.

**Tumor inoculation:**

A line of Ehrlich Ascites Carcinoma (EAC), (kindly supplied by the National Cancer Institute, Cairo, Egypt), was maintained and propagated by serial interperitoneal transplantation of EAC in an aseptic environment. Viable EAC cells (10^6) were injected intraperitoneally into each animal in an aseptic condition and the day of tumor inoculation was considered as zero day. All the experiments of tumor bearing mice were conducted 24 hr after the EAC transplantation and that day was considered as the first day.

**Radiation source:**

Whole body γ-radiation was performed with a Canadian Cs^{137} Gamma Cell-40 at the NCRRT. Cairo, Egypt, at a dose rate 0.61 Gy/min. Mice were
exposed to a single dose of 4Gy, 30 min after the first dose of the extract treatment.

**Experimental animals:**

Seventy healthy adult female Swiss albino mice weighing 22±2g (obtained from the breeding unit of the Egyptian Organization for Biological Products and Vaccines, Cairo) were used. The animals were maintained on a commercial standard pellet diet and tap water *ad libitum*.

The mice were divided into 6 groups, (15 mice in each)

**group 1:** Normal mice (control).

**group 2:** mice were administered EtBP intraperitoneally 250 mg/kg body weight for 9 successive days (EtBP group).

**group 3:** Mice inoculated with EAC cells (EAC group).

**group 4:** EAC bearing mice and treated with EtBP (EAC+EtBP).

**group 5:** EAC bearing mice exposed to γ- radiation (EAC+IRR).

**group 6:** EAC bearing mice treated with Et.B.P and exposed to γ-radiation (EAC+EtBP+IRR).

After 18 hr fasting from the last dose of EtBP treatment, 5 mice from each groups were sacrificed. Blood samples were collected to separate serum for estimation of kidney functions and tumor markers. Also ascetic fluids were collected for estimation of caspase-3. Kidneys were removed out, then divided into two parts, the first part was homogenized in a bi-distilled water to give 10% homogenate and kept in ice for biochemical studies. The second part of kidney was kept in 10% formalin for histopathological study. The remaining animals (10 in each group) were kept to check and recording their survival percentage and body weight for 45 days.

**Biochemical analysis**

Urea in serum was determined by an enzymatic colorimetric method as described by Parton and Crouch (1977)\(^{(20)}\). Serum creatinine was determined by a colorimetric method as described by Henry, (1964)\(^{(21)}\). Caspase-3 and MMP were measured using Enzyme-linked Immunosorbent Assay Kit, Uscn company. Tumor necrosis factor alpha (TNF-α) was determined using commercially available kits according to manufacturers’ instructions (TNF-α ELISA kit, KAMYA BIOMEDICAL COMPANY). Lipid peroxide
concentrations were determined by measuring the Malonaldehyde (MDA) end product according to the method of Yoshioka et al., (1979)\(^{(22)}\). Superoxide dismutase (SOD) activity was estimated by Nisikimi et al., (1972)\(^{(23)}\) and reduced glutathione (GSH) content were measured according to Beutler et al., (1963)\(^{(24)}\).

**Histopathological examination**

At the time of sacrifice, samples from kidney from each group were fixed in 10% formalin and embedded in paraffin. Sections of tissues were stained with hematoxylin and eosin\(^{(25)}\) (Culling, 1974).

**Statistical analyses**

All data were expressed as mean±SE (standard error). Data were assessed by using a one-way ANOVA model with Dunnett’s adjustments\(^{(26)}\) for the comparison of multiple treatment groups. Values of p< 0.05 was considered significant.

**RESULTS**

**Survival percentage of EAC bearing mice of different groups:**

Survival percentage were recorded continuously along 45 days period after treatment. In both control and EtBP treated groups 90% of animals survived all over the experimental time. On the other hand, none of EAC group survived more than 22 days after tumor inoculation. Treatment of EAC bearing mice with EtBP (EAC+EtBP group) increased the survival percentage compared to EAC group. Exposure of EAC bearing mice to γ-radiation (EAC+IRR group), improved the survival percentage compared to EAC group. The highly increase in the survival percentage observed in the group of EAC bearing mice treated with EtBP and exposed to γ-radiation (EAC+EtBP+IRR group), are shown in figure 1.
Figure (1): Survival percentage of EAC bearing mice groups

% increase of body weight of EAC bearing mice of different groups:

The effects of EtBP on body weight of EAC bearing mice have been calculated as a percentage increase of body weight. The non-treated EAC bearing mice showed a progressive increase in their total body weight. Either EAC bearing mice treated with EtBP and/or exposed to γ-radiation showed an inhibition in the progressive increase in body weights compared to EAC control. The results are represented in figure 2.

Figure (2): percentage increase of body weight of EAC bearing mice.

The body weight of normal animals was 20±2 gm at the beginning of the experiment and reached 25 ± 2 at the end of the experimental period.

Serum level of Urea and creatinine of different groups:

Data represented in Table 1, showed that administration of healthy mice with EtBP showed insignificant change in serum urea and creatinine levels, while inoculation of EAC cells into mice, resulted in an elevation in serum urea
(22%) and creatinine (20%) compared to normal control. Also a remarkable increase in urea (33%) and creatinine (56%) were shown in EAC bearing mice that exposed to γ- radiation. Administration of EtBP to EAC bearing mice alone or with irradiation showed a remarkable improvement in urea and creatinine levels compared to EAC group.

Table (1): serum level of urea and creatinine of different group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>62.70± 1.30</td>
<td>0.89±0.01</td>
</tr>
<tr>
<td>EtBP</td>
<td>61.54± 0.82 (-1.8%)</td>
<td>0.87±0.03 (-2.2%)</td>
</tr>
<tr>
<td>EAC group</td>
<td>76.58± 0.9a (22%)</td>
<td>1.07±0.02a (20%)</td>
</tr>
<tr>
<td>EAC+IRR</td>
<td>83.58±2.2ab (33%)</td>
<td>1.39±0.09ab (56%)</td>
</tr>
<tr>
<td>EAC+EtBP</td>
<td>69.6±2.1ab (11%)</td>
<td>0.99±0.02ab (11.2%)</td>
</tr>
<tr>
<td>EAC+EtBP+IRR</td>
<td>67.63±2.1ab (7%)</td>
<td>0.93±0.02b (4%)</td>
</tr>
</tbody>
</table>

Each value represents mean± SE (n=5). %: percentage change from control
a Significant against control
b Significant against EAC group

GSH, MDA contents and level of SOD of kidney tissues of different groups:

In Table 2, the results showed that inoculation of EAC cells in mice induced a significant increases in the level of MDA (18%) compared to control level, accompanied by drastically decreased in the levels of GSH (-30.8%) and antioxidant enzyme SOD (-33.8%) in the kidney tissue, in comparison with level of control group. Also EAC bearing that exposed to γ-radiation showed a remarkable increase in MDA (31.7%) and a significant decrease in GS (-28.3%) and SOD (-41.4%) compared to normal control. On the other hand a significant decrease in MDA level and a significant increase in SOD and GSH were shown in EAC bearing mice treated with EtBP alone or in combination with radiation, compared to EAC group.
Table (2): Levels of MDA, SOD and GSH content in kidney tissues of different mice groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA μmol/g tissue</th>
<th>SOD μg/gm tissue</th>
<th>GSH mg/gm tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>72.8±0.89</td>
<td>25.42±0.9</td>
<td>20.02±1.2</td>
</tr>
<tr>
<td>EtBP (0.4%)</td>
<td>73.11±1.3</td>
<td>26.57±1.9 (4.5%)</td>
<td>21.24±1.1 (6%)</td>
</tr>
<tr>
<td>EAC group</td>
<td>85.91±1.8* (18%)</td>
<td>17.59±1.3* (-30.8%)</td>
<td>13.25±0.9* (-33.8%)</td>
</tr>
<tr>
<td>EAC+IRR</td>
<td>95.87±2.1*ab (31.7%)</td>
<td>18.23±1.5* (-28.3%)</td>
<td>11.73±0.5*ab (-41.4%)</td>
</tr>
<tr>
<td>EAC+EtBP</td>
<td>83.76±1.9*ab (15%)</td>
<td>19.87±1.9* (-21%)</td>
<td>16.87±0.6a* (-15.7%)</td>
</tr>
<tr>
<td>EAC+EtBP+IRR</td>
<td>81.86±1.5*ab (12%)</td>
<td>21.39±1.4* (-15.8)</td>
<td>18.91±0.9b (-5.5%)</td>
</tr>
</tbody>
</table>

Legends as in table 1

Caspase-3 concentration in ascetic fluids of EAC bearing Mice groups:

The concentration of Caspase-3 in the ascetic fluid of different EAC bearing mice groups were shown in Figure(3). Treatment of EAC bearing mice with EtBP or exposure to γ-radiation showed a significant increase in caspase-3 compared to the level of EAC group (12% and 10% respectively), and a remarkable increase in caspase-3 were shown in ascetic fluid of mice that treated with EtBP and exposed to γ–radiation (30%).

![Caspase-3 concentration in ascetic fluids of EAC bearing mice groups](image)

Fig (3): Caspase-3 concentration (ng/ml) in ascetic fluids of EAC bearing mice groups.
Each value represents mean± SE (n=5).
* significant at p < 0.05 compared to EAC control.
Serum levels of TNF-α, MMP-2 and MMP-9 of different Mice group:

As shown in Table 3, the matrix metalloproteinase specific activity measured in serum of mice in different groups showed changes in the activity of both MMP-2 and MMP-9. A significant increase in both mmp2 (64%) and mmp9 (24%) was recorded in EAC group as compared to normal control. Also, exposure of EAC bearing mice to γ-radiation showed a significant increase of mmp-2 (34%) and mmp-9 (21%), compared to control level, and significant increase were shown in mmp-2 and mmp-9 (41% and 22% respectively) in sera of EAC bearing mice treated with *Bidens pilosa* extract. Treatment of EAC bearing mice with both EtBP and exposure to γ-radiation ameliorated the increased level of mmp-2 and mmp-9 (22.5% and 14.5% respectively). The levels of TNF-α showed a significant increase (54.5%) in sera of EAC group as compared to normal control. Also EAC group that exposed to γ-radiation or treated with EtBP showed a significant increase in TNF-α as compared to control group (26% and 21%) respectively. Treatment of EAC bearing mice with EtBP and γ-radiation showed a remarkable improvement in TNF-α (15.8%) compared to control level.

Table (3): Serum levels of MMP-2 MMP-9 and TNF-α of different Mice groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MMP-2</th>
<th>MMP-9</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>28.96±0.6</td>
<td>109.02±1.5</td>
<td>13.20±0.3</td>
</tr>
<tr>
<td>EtBP</td>
<td>28.67±1.3 (-1%)</td>
<td>106.29±0.9 (-2.5%)</td>
<td>12.44±0.4 (-5.7%)</td>
</tr>
<tr>
<td>EAC group</td>
<td>47.54±1.8a (64.2%)</td>
<td>135.29±1.5a (24%)</td>
<td>20.4±0.6a (54.5%)</td>
</tr>
<tr>
<td>EAC+IRR</td>
<td>38.83±1.3ab (34%)</td>
<td>132.5±1.8a (21%)</td>
<td>16.68±0.4ab (26%)</td>
</tr>
<tr>
<td>EAC+EtBP</td>
<td>41.1±1.5ab (41%)</td>
<td>133.5±1.7a (22%)</td>
<td>16.2±0.5ab (22%)</td>
</tr>
<tr>
<td>EAC+EtBP+IRR</td>
<td>35.47±1.3ab (22.5%)</td>
<td>124.8±1.7ab (14.5%)</td>
<td>15.29±0.3ab (15.8%)</td>
</tr>
</tbody>
</table>

Legends as in table 1

Histopathological studies:

Photographic section of the kidney of normal mice and those treated with EtBP showed the normal histopathological structure of renal parenchyma (photo A and B, respectively). On the other hand, kidney of EAC control group showed a vacuolar degeneration of endothelial lining glomerular tufts as well as epithelial lining renal tubules (photo C). When EAC bearing mice exposed to γ-radiation, histopathological sections of kidney showed atrophy of glomerular
tufts in some regions (photo D1) while in other regions showed no pathological changes (photo D2). Administration of EtBP to EAC bearing mice showed vacuolations of endothelial lining glomerular tuft and epithelial lining renal tubules (photo E). A photographic sections of kidney of EAC bearing mice treated with EtBP and exposed to radiation showed focal tubular necrosis completely replaced by inflammatory cells infiltration and vacuolation of epithelial lining renal tubules and perivasculitis congestion of the glomular tuft (photo F).

Fig (4): Photographic of kidney sections of mice under studied. 
A: normal control  
B: normal mice treated with EtBP  
C: EAC control  
D: EAC bearing mice exposed to radiation  
E: EAC bearing mice treated with EtBP.  
F: EAC bearing mice treated with EtBP and exposed to γ-radiation. (H&E x400).
DISCUSSION

In the present study, EAC bearing mice treated with EtBP and /or exposed to γ-radiation resulted in increase in the survival percentage and inhibition of the progressive increase in the body weight, compared to EAC control. The dependable principle for assessing the importance of any anticancer drug includes the prolongation of the life span of animals. It is evident that EtBP and irradiation increased the survival percentage and inhibited the progressive increase of body weights of EAC-bearing mice by restricting the activity of the EAC cells.

In the present study, urea and creatinine levels, which are considered as makers of kidney function, were significantly elevated in serum of mice of EAC control indicating renal impairment. These alterations induced by toxic conditions, reflected metabolic cellular dysfunction of these tissues. An elevation of urea may be attributed to an increase in nitrogen retention or excessive protein breakdown. It was demonstrated that, the presence of tumors in human body or experimental animals is known to affect many functions of vital organs. Our studies have demonstrated that EtBP with irradiation have been able to inhibit the proliferation of EAC cells in mice.

Either EAC bearing mice and those exposed to γ-radiation showed significant increases in the level of MDA accompanied by decreasing GSH content and SOD activity in the kidney tissue in comparison with normal control group. Administration of EtBP in EAC bearing mice either alone or in combination with radiation, ameliorate the changes, compared to EAC control. Lipid peroxidation process plays a key role in tumor growth invasiveness. Accordingly, the decrease of total antioxidants value concomitant with increase in TBARs (MDA level) concentration observed in the present study could be considered as onslaught of free radicals resulted from carcinomous tissues. Extensive lipid peroxidation results in membrane disorganization by pexroidizing the highly unsaturated fatty acids, which in turn alters the ratio of other polyunsaturated fatty acids leading to a decrease in the membrane fluidity which may be sufficient to cause cell damage. While, Zhang et al., (1996) attributed the acceleration in lipid peroxidation contents to the peroxidation of the membrane unsaturated fatty acids due to free radical propagation concomitant with inhibition of bio-oxidative activities. Bidens pilosa plays an important role in preventing the generation of reactive oxygen species (ROS), it has modulator effect on iron-induced lipid peroxidation due to their
polyphenolic contents, strong reducing power and superoxide radical scavenging activity.\(^\text{36}\)

On the other hand, the free radical scavenging system SOD is present in all oxygen-metabolizing cells and their function is to provide a defence against the potentially damage reactivities of superoxide and hydrogen peroxide. Sun \textit{et al.}, (1989)\(^\text{37}\) reported a decrease in SOD activity in EAC bearing mice which might be due to loss of Mn SOD activity in EAC cells leading to a decrease in total SOD activity in the kidney. The inhibition of SOD activities as a result of tumor growth was also reported by Marklund \textit{et al.}, (1982)\(^\text{38}\). Similar findings were observed in the present investigation with EAC bearing mice. The administration of EtBP increased the SOD levels, which may indicated its antioxidant and free radical scavenging property.

Glutathione, a potent inhibitor of neoplastic process play an important role as an endogenous antioxidant system. The present results revealed depletion in GSH contents in kidney of EAC control. The depletion in GSH may be attributed to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by Ehrlich ascites cells or to the diminished activity of glutathione reductase due to the deficiency or inactivation of glucose-6-phosphate dehydrogenase, the main supplier for NADPH which is necessary to change oxidized glutathione to it’s reduced form\(^\text{39}\). EtBP reduced the elevated levels of lipid peroxidation and increased the GSH content in Ehrlich bearing mice. This could be attributed to the phytochemicals of its contents which increases the induction of antioxidant status enzymes.

Treatment of EAC bearing mice with EtBP and /or exposure to \(\gamma\)-radiation resulted in a significant increase in the concentration of Caspase-3 of the ascetic fluid, as compared to EAC group. The caspase increases in apoptosis could be interpreted in the view of the three pathways (extrinsic, intrinsic as well as a perforin/granzyme). The apoptotic process requires specific triggering signals to begin a cascade of energy dependent molecular events. Each pathway has its own initiator caspase which in turn activate executioner caspase\(^\text{40}\). The extrinsic signaling pathways that initiate apoptosis involve transmembrane receptor-mediated interactions. These involve death receptors that are members of the tumor necrosis factor. It appeared that Biden pilosa stimulates caspase-3 dependent apoptosis by down-regulating the expression of the Inhibitor of Apoptosis (IAP) family of proteins\(^\text{6}\).

In the present study, the level of TNF-\(\alpha\) showed a significant increase in
sera of EAC group mice as compared to control. Administration of EtBP to EAC bearing mice showed a significant decrease when compared with the EAC group level. Exposed Ehrlich ascites–bearing mouse to γ-radiation slightly improve the above mentioned increase in TNF-α when compared to that of EAC group. Cytokines like TNF-α has been recognized as an important host defense cytokines that affects tumor cells. TNF-α plays an inhibitory role in cancer progression, it is rational to hypothesize that blocking its activity may result in increases cancer incidence\(^{(41,42)}\). TNF-α receptor gene superfamily\(^{(40)}\), members of the TNF-α receptor family share similar cysteine-rich extracellular domains and a cytoplasmic domain of about 80 amino acids called the death domain\(^{(43)}\). This death domain plays a critical role in transmitting the death signal from the cell surface to the intracellular signaling pathways\(^{(44)}\).

The matrix metalloproteinases specific activity were measured in sera of mice in different groups. A significant increase in both mmp-2 and mmp-9 in EAC control group was recorded, as compared to normal control. While the results showed an amelioration in the mmp-2 of other groups as compared to EAC control, and a remarkable significant decrease was observed in the level of mmp-9 of EAC bearing mice group that treated with EtBP and exposed to γ-radiation.

The matrix metalloproteinases (MMPs) are a family of zinc-dependent proteases that play a major role in proteolytic degradation of structural components of extracellular matrix\(^{(45)}\). MMPs, like most proteolytic enzymes, are synthesized as inactive zymogens. Therefore, the activation of pro-MMPS represents a step in the regulation of MMP activity\(^{(46)}\).

The results of the present study, suggested that MMP-2 and MMP-9 could be involved in the configuration of invasive carcinoma of no special type\(^{(47)}\). The apoptotic modulation and subsequent suppression of cancer growth observed in the present work could be attributed to disturbances in the MMP system. The activity of MMPs is specifically inhibited by tissue inhibitor of metalloproteins (TIMP). The inhibitory effects of TIMPs on tumor progression are not only due to their ability to inhibit MMP activity, but also because of their ability to directly modulate the cell growth and apoptosis of tumor cells, as well as host endothelial cells\(^{(48)}\).

Plant derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells and antitumor activity in experimental animals\(^{(49)}\). The lowering of lipid peroxidation and increase in levels of SOD
and GSH in EtBP treated group indicates its potential as an inhibitor of EAC induced intracellular oxidative stress. We proposed that the additive and synergistic antioxidant activity of phytochemicals such as flavonoids, triterpenoids, steroids, etc, present in *Bidens pilosa* are responsible for its potent antitumor activity which can be inferred from the increase in the survival time of EAC tumor bearing mice, inhibition of the tumor growth and improvement of tumor markers.

The biochemical results are supported by the histopathological examination of mice bearing EAC and administrated with EtBP and/or exposed to γ-radiation. There was a significant amelioration towards normal intact histological structure. In conclusion, Ethanolic extract of *Bidens pilosa* exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing mice.

REFERENCES


تأثير مستخرج عشب الأبر الإسباني على وظائف الكلى وبعض دلالات الأورام

في الفئران الحاملة لخلايا أرخ السرطانية والمعرضة كلياً لأشعة جاما.

خان القباني* - سحر إسماعيل **

المصادر: المركز القومي لبحوث وتكنولوجيا الإشعاع - قسم البحوث الصحية - قسم البيولوجيا الإشعاع.

تأثر الإشعاع على الأورام يمكن أن تتزايد باستخدام مواد يمكن أن تزيد الضرر على الأورام أكثر من مواد أو تستخدم كلاً على حد سواء. تم اختبار تأثير المستخرج الكحولي لعشب الأبر الإسباني على فئران حاملة لأورام خلايا أرخ السرطانية والمعرضة كلياً لأشعة جاما. تراجعت الفئران الحاملة لورم بمستخرج الكحولي للأبر الإسباني بدرجة 25% تم جر عكسي في الفئران هذا، حيث تم علاج الأورام، وتم علاج الفئران بمستخرج الكحولي. تم حسن الفئران بجرعة واحدة يوميا لمدة ثمانية أيام متتالية بعد التشعيب.

صنفت الفئران إلى ستة مجموعات:

المجموعة الأولى: مجموعة التحكم السليمة.
المجموعة الثانية: مجموعة معقولة بالمستخرج الكحولي.
المجموعة الثالثة: فئران حاملة لأورام معقولة بالمستخرج الكحولي.
المجموعة الرابعة: فئران حاملة لأورام معرضة للإشعاع.
المجموعة الخامسة: فئران حاملة لأورام معقولة بالمستخرج والإشعاع معاً.
المجموعة السادسة: فئران حاملة لأورام معرضة بالإشعاع للأشعة السينية والأشعة السينية مع الإشعاع.

تم دمج 6 فئران من كل مجموعة بعد 18 ساعة من إعطاء الجرعة الأولى. تجمعت عينات الدم وسائل الدم وأزيلت الكلى للدراسات البيوكيميائية والباثولوجية. وتركب باقي الفئران للاختبارات الفعلية مع إعطاء الجرعة الثانية وقياس نسب البقاء على قيد الحياة وزن الجسم الفئران الحاملة لأورام.

اجرت هذه الأغذية للتحقق من تأثير مستخرج عشب الأبر الإسباني والإشعاع على خلايا أرخ السرطانية. فعالة تم تحميل الفئران الحاملة لورم مستخرج عشب الأبر الإسباني مع/أو العرض للإشعاع. في حين نسب الفئران المتبقي على قيد الحياة والاضافات وزن الجسم للمقارنة ب الفئران الحاملة لأورام. إما تقليل الفئران حلبيا ملتحي إلى تغييرات بيوكيميائية وباثولوجية، سبباً تلف الكلى. تراجعت نسب الفئران الحاملة لورم مستخرج العشب الإسباني مع/أو العرض للإشعاع لأشعة جاما إلى تحسن في عيوب الكلى المقادس في مصل الدم (البويهاريا والكيرباتين) وذلك من الدهون اليرسكس والجلوداتون أنزيم السوبر أكسيد ديسميتانت المقادس في نسيج الكلى. كما لحظ تحسن في دلالات الأورام المقدسة في مصل الدم وذلك بالمقارنة بالمجموعة الحاملة للورم.

ومن هذا يمكن إعتبار المستخرج الكحولي للأبر الإسباني من خلال خصائصه المضادة للكسدة كم corridور للأورام بالإضافة إلى أنه قد يكون لها فائدة محتملة في تعزيز العلاج الإشعاعي.