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Research Article
Effect of Honey Bee Venom on Cancer in Rats Model

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Abstract

The present study aimed to evaluate the tumor growth inhibiting effects of bee venom in rats (*in vivo*) and in tumor cell cultures (*in vitro*). Growth inhibitory effect of bee venom (*in vitro*) administered in various concentrations (0, 0.01, 0.1, 1, 10 and100 μg mL^{-1}) to eight different types of cancer cell lines were estimated by counting their viable cell number after 72 h of treatment. Results indicated that the growth inhibition by bee venom was dose dependent in all cell lines. The highest death cell was observed in HEPG2 (liver cancer), A549 (lung cancer) and HEP-2C (larynx cancer) cell lines treated with bee venom, while the lowest one was occurred with HCT116 cell line (colon cancer). Intraperitoneally administration of bee venom (0.28 and 0.56 mg kg^{-1}) to rats (*in vivo*) significantly reduced the body weight, ascites tumor volume, Packed Cell Volume (PCV), viable tumor cell, tumor cell count number and increased Mean Survival Time (MST). Aspartate aminotransferease (AST) and alanine aminotransferease (ALT), alkaline phosphatase (ALP) and total proteinand albumin levels significantly ($p \leq 0.05$) decreased in ehrlich ascites carcinoma bearing rats treated groups with 0.28 and 0.56 mg kg^{-1} of bee venom (G5, G6) and 5-fluoruracil (G4). Histopathological investigation of peritoneum, liver, kidney and lung was performed. The results indicated that the studied bee venom can be used as effective anticancer agent.

Key words: Bee venom, anticancer, rats, cancer cell lines

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cancer is the major public burden in all developed and developing countries. A total of 1,638,910 new cancer cases and 577,190 deaths from cancer are projected to occur in year 2012 (Siegel *et al*., 2012). In all types of cancer, genetic alterations give rise to changes in expression, activation or localization of regulatory proteins in the cells, affecting the signaling pathways that alter their response to regulatory stimuli and allow the unrestricted cell growth. Various therapies have been used for treating cancer such as chemotherapy, radiotherapy, immunotherapy and gene therapy (Baskar *et al*., 2012) but still there is an urgent need of finding a better natural safe way to treat cancer with little effect on normal cells. Anticancer drug developments from natural biological resources are ventured throughout the world.

Bee products have long been used in traditional medicine. The raw materials, crude extracts and purified active compounds from them have been found to exhibit interesting bioactivities, such as antimicrobial, anti-inflammatory and antioxidant activities. In addition, they have been widely used in the treatment of many immune-related diseases as well as in recent times in the treatment of tumors (Premratanachai and Chanchao, 2014).

Bee venom, a complex mixture of substances is used to defend the bee colony against a broad diversity of predators from other arthropods to vertebrates. Bee venom, produced in the venom gland located in the abdominal cavity, contains several biologically active peptides, including melittin, apamin, adolapin, mast cell degranulating peptide and many enzymes, plus also non-peptide components, such as histamine, dopamine, phospholipase A2 (PLA2) and norephnephrine (Raghuraman and Chattopadhyay, 2007). Bee venom has traditionally been used as a non-steroidal anti-inflammatory drug for the relief of pain and the treatment of chronic inflammatory diseases, such as rheumatoid arthritis and multiple sclerosis as well as in the treatment of tumors (Son *et al*., 2007).

This study was carried out to investigate the bee venom (First Carniolan Hybrid) *Apis mellifera* L. as anticancer agent in lab animals.

MATERIALS AND METHODS

Materials

Bee Venom (BV): Bee venom was collected using the bee Venom Collector Device (VCD) according to Rybak *et al*. (1995).

Experimental animals: One hundred and twenty female swiss albino rats were purchased from National Research Center, Giza, Egypt weighting 25-30 g. The animals were kept at constant environmental and nutritional condition throughout the experimental period and kept at room temperature (22±2°C) with a 12 h on/off light schedule. Female rats were used in this study because they are susceptible to ehrlich ascites carcinoma cells more than male (Abu-Zeid *et al*., 2000).

Cancer cell lines: HeLa cell line (cervical carcinoma), HEPG-2 cell line (liver cancer), PC3 cell line (prostate cancer), A549 cell line (lung cancer), RDA cell line (Rhabdomyosarcoma), MCF 7 cell line (breast cancer), HCT116 cell line (colon cancer) and HEP-2C cell line (larynx cancer) were purchased from Vacsera, Cairo, Egypt and maintained in RPMI 1640 media with 10% fetal calf serum and antibiotics.

Anticancer experiment (*in vitro*)

SRB cell survival assay: Cytotoxicity assay determined using a forementioned cancer cell lines. Cell survival determined using Sulpho Rhodamine-B (SRB) method as previously described by Skehan *et al.* (1990).

Anticancer experiment (*in vivo*): One hundred and twenty female swiss albino rats (25-30 g) were fed for one week before starting the experiment on basal diet (15% casein, 10% corn oil, 5% cellulose, 4% salts mixture, 1% vitamins mixture and 65% starch) and then, randomly divided into six groups each of twenty. The twenty rats in each group were housed in single cage and fed on basal diet overall experimental period. Ehrlich Ascites Carcinoma (EAC) cells were injected intraperitoneally in swiss albino rats. The tumor cell line was maintained in rats through serial intraperitoneal transplantation of 1×10^6 viable tumor cells in 0.2 mL of saline. The six groups and their treatments are summarized in Table 1.

Total experimental period was 14 days and after administering the last dose, 6 animals in each group were fasted overnight, anesthetized and sacrificed to study of antitumor activity and biochemical parameters. The remaining animals were left to calculate the mean survival time.

Collection of blood samples: At the end of the experiment blood samples were collected from the experimental groups by cardiac puncture in accordance with the method of Frankenberg (1979). Each blood sample was placed in a dry clean centrifuge tube; the blood was centrifuged for

Table 1: Experimental groups and their treatments

Groups	Treatments
1 (Negative control)	Normal rats injected intraperitoneally with 0.2 mL of the physiological saline (0.9 g dL^{-1}) solution daily for 14 days. Normal rats saline treated group)
2	Normal rats injected intraperitoneally daily with 0.28 mg kg^{-1} day^{-1} of BV daily for 14 days. Normal rats 1/10 LD_{50} bee venom treated group)
3 (Positive control)	Normal rats injected intraperitoneally with 1×10^6 tumor cells/mouse. After 24 h of tumor inoculation, the rats were treated with 0.2 mL of the physiological saline daily for 14 days (EAC-bearing rats saline treated group)
4	Normal rats injected intraperitoneally daily with 20 mg kg^{-1} day^{-1} of 5-fluorouracil daily for 14 days (EAC-bearing rats 5-Fluorouracil treated group)
5	Normal rats injected intraperitoneally with 1×10^6 tumor cells/mouse. After 24 h of tumor inoculation, the rats were treated with 0.28 mg kg^{-1} day^{-1} of BV daily for 14 days (EAC-bearing rats 1/10 LD_{50} bee venom treated group)
6	Normal rats injected intraperitoneally with 1×10^6 tumor cells/mouse. After 24 h of tumor inoculation, the rats were treated with 0.56 mg kg^{-1} day^{-1} of BV daily for 14 days (EAC-bearing rats 1/5 LD_{50} bee venom treated group)

15 min at 4000 rpm to separate the serum from the cells. The supernatant was frozen kept in eppendorf tubes until used for biochemical analysis.

Collection of Ehrlich Ascites Carcinoma (EAC) cells: Ascetic fluid was individually withdrawn from ether anaesthetized rats of tumor-bearing none treated and tumor bearing treated groups using an 18 gauge needle. Volume of individual ascetic fluid was measured.

Collection of organs: Samples of peritoneum, lung, liver and kidney were immediately removed and kept in formalin 10% then stored at room temperature until used for histological exam.

Antitumor activity: Antitumor effect of BV was assessed by observing the changes with respect to body weight (%), ascites tumor volume, Packed Cell Volume (PCV), viable and non-viable tumor cell, tumor cell count and Mean Survival Time (MST). The MST of each group containing 14 rats was monitored by recording the mortality daily:

$$MST = \frac{\text{Day of first death+Day of last death}}{2}$$

Tumor cell count: Each primary square of the WBC hemocytometer, with cover-slip in place, represents a total volume of 0.1 mm^3 or 10^{-4} cm^3. Since, 1 cm^3 is equivalent to approximately 1 mL and the total number of cells will be determined using the following calculations:

$$\text{Cells per mL} = \text{Average count per square}\times\text{Dilution factor}\times10^4$$

Viability test: Viability assays measure the percentage of a cell suspension that is viable. This is generally accomplished by a dye exclusion stain, where cells with an intact membrane are able to exclude the dye while cells without an intact membrane take up the coloring agent:

$$\text{Viability (\%)} = \frac{\text{No. of viable cells}}{\text{No. of viable and dead cells}}\times100$$

Biochemical analysis

Determination of serum aminotransferease: Aspartate aminotransferease (AST) and alanine aminotransferease (ALT) were measured in serum using kits according to the method described by Reitman and Frankel (1957).

Determination of serum alkaline phosphatase (ALP): The activity of alkaline phosphatase in serum was determined using kits according to the method of Kind and King (1954).

Determination of total protein: It is determined using kits by measuring the increase in absorbance at 530-570 nm, according to Doumas *et al*. (1971) and Grant *et al*. (1987).

Determination of albumin: It is determine dusing kits by measuring the increase in absorbance at 580-630 nm, according to Young (1990).

Determination of globulin concentration: Serum globulin was determined as the difference between serum total protein and albumin.

Determination of serum cholesterol: It is determined by measuring the increase in absorbance at 500-550 nm, according to Ellefson and Caraway (1976).

Determination of serum urea: It is determined by measuring the increase in absorbance at 578-630 nm, according to Rock *et al*. (1987) and Young (1990).

Histopathological analysis: Tissue samples from peritoneum, liver, kidney and lung were fixed in 10% neutral buffered formalin. Paraffin sections of 5 µ thickness were prepared from all specimens and were stained by haematoxylin and eosin (H and E) according to Bancroft and Stevens (1996). The sections were examined by A Carl Zeiss microscope and the histopathological results of the different groups were photographed.

Statistical analysis: Data obtained were statistically analysed by using factorial analysis and F-test. Means were compared according to Duncan. Differences among means were determined by Duncan's multiple range test. Simple and multiple correlations for the relations between studied factors were calculated by SAS (1996).

RESULTS AND DISCUSSION

Anticancer effects of bee venom

***In vitro* cytotoxicity assay:** The growth inhibitory effect of bee venom administered in various concentrations (0, 0.01, 0.1, 1, 10 and 100 µg mL^{-1}) to HeLa cell line (cervical carcinoma), HEPG-2 cell line (liver cancer), PC3 cell line (prostate cancer), A549 cell line (lung cancer), RDA cell line (rhabdomyosarcoma), MCF 7 cell line (breast cancer), HCT116 cell line (colon cancer) and HEP-2C cell line (larynx cancer is presented in Fig. 1(a-h). Bee venom decreased the percentage of viable cells inHeLa cell line (cervical carcinoma), HEPG-2 cell line (liver cancer), PC3 cell line (prostate cancer), HEP-2C cell line (larynx cancer), A549 cell line (lung cancer), RDA cell line (rhabdomyosarcoma), MCF 7 cell line (breast cancer) and HCT116 cell line (colon cancer) by about 92.49-59.89, 85.38-8.42, 93.18-14.86, 94.31-14.36, 80.74-10.52, 75.48-16.07, 78.82-18.78 and 87.19-62.1% at treatment 0.1-10 µg mL^{-1}, respectively. The IC_{50} value of bee venom for HeLa cell line, HEPG-2 cell line, PC3 cell line, HEP-2C cell line, A549 cell line, RDA cell line, MCF 7 cell line and HCT116 cell line were 11.07, 1.32, 2.48, 2.63, 1.63, 0.304, 1.56 and 8.20 µg mL^{-1} in Fig.1(a-h), respectively.

The results indicated that the growth inhibition by bee venom was dose dependent in all cell lines. The highest death cell was observed in HEPG2, A549 and HEP-2 cell lines treated with bee venom, while the lowest was recorded with HCT116 cell line. These results are in agreement with previous studies which showed the inhibitory effects of bee venom on certain types of cancer including lung (Jang *et al*., 2003), liver (Liu *et al*., 2008), breast (Ip *et al*., 2008), cervical (Orsolic *et al*., 2009), prostate (Park *et al*., 2010), ovarian cancer (Alizadehnohi *et al*., 2012), colon (Slaninova *et al*., 2012), renal (Putz *et al*., 2006) cancer cells as well as leukemia cells (Slaninova *et al*., 2012).

The present study demonstrates direct cytotoxic effect of bee venom on tumor cells. The cell cytotoxic effect through the activation of antiapoptotic gene products (PLA2) by melittin has been suggested to be the critical mechanism for the anti-cancer activity of bee venom (Orsolic, 2012).

Anticancer effects of bee venom (*In vivo*)

Effect of bee venomon some antitumor parameters of EAC tumor bearing rats: Data of the effect of bee venom on survival of tumor bearing rats are shown in Fig. 2. The mean of survival days for the untreated EAC control (G3) was 15.5 days. The intraperitoneal administration with 0.28 and 0.56 mg kg^{-1} b.wt., of bee venom to EAC bearing rats (G5 and G6) increased the survival days to 25.5 and 26.5 days, respectively.

Figure 3 and 4 show the bee venom effects on body weight and volume of ascitic fluid in tumor bearing rats, respectively. The untreated EAC control (G3) revealed a high significant ($p \leq 0.05$) increase in body weight reached to 34.41 g compared with normal control (G1) (27.166 g). There was no significant effect in animals body weight in normal rats group treated with bee venom (0.28 mg kg^{-1} b.wt.) (G2). The results indicated that the beevenom at the studied concentrations or 5-fluoruracil caused high significant ($p \leq 0.05$) reduction in body weights of EAC bearing rats which reached approximately to normal rats body weight. The EAC bearing rats treated with different dose of bee venom and 5-fluoruracil showed significant reduction in the tumor volume (Fig. 4) compared with untreated EAC control (G3) (6.8 mL). The tumor volumes of the EAC bearing rats administrated with 0.28 and 0.56 mg kg^{-1} of bee venom (G5 and G6) were 2.87 and 2 mL, whereas the group of EAC bearing rats treated with 5-fluoruracil (20 mg kg^{-1}) (G4) showed a high significant ($p<0.05$) decrease in tumor volume reached to 0.575 mL.

Figure 5, 6 and 7 illustrated the total number of EAC cells, Packed Cell Volume (PCV) and viable tumor cell percent in EAC bearing rats treated with bee venom, respectively and 5-Flurouracil compared to untreated EAC bearing rats. The EAC bearing rats treated with different doses of bee venom and 5-fluoruracil showed high significant ($p<0.05$) reduction in the total number of EAC cells (Fig. 5) compared with untreated EAC control (G3) (68.58×10^6). The total number of EAC cells of the rats administrated with 0.28 and 0.56 mg kg^{-1}

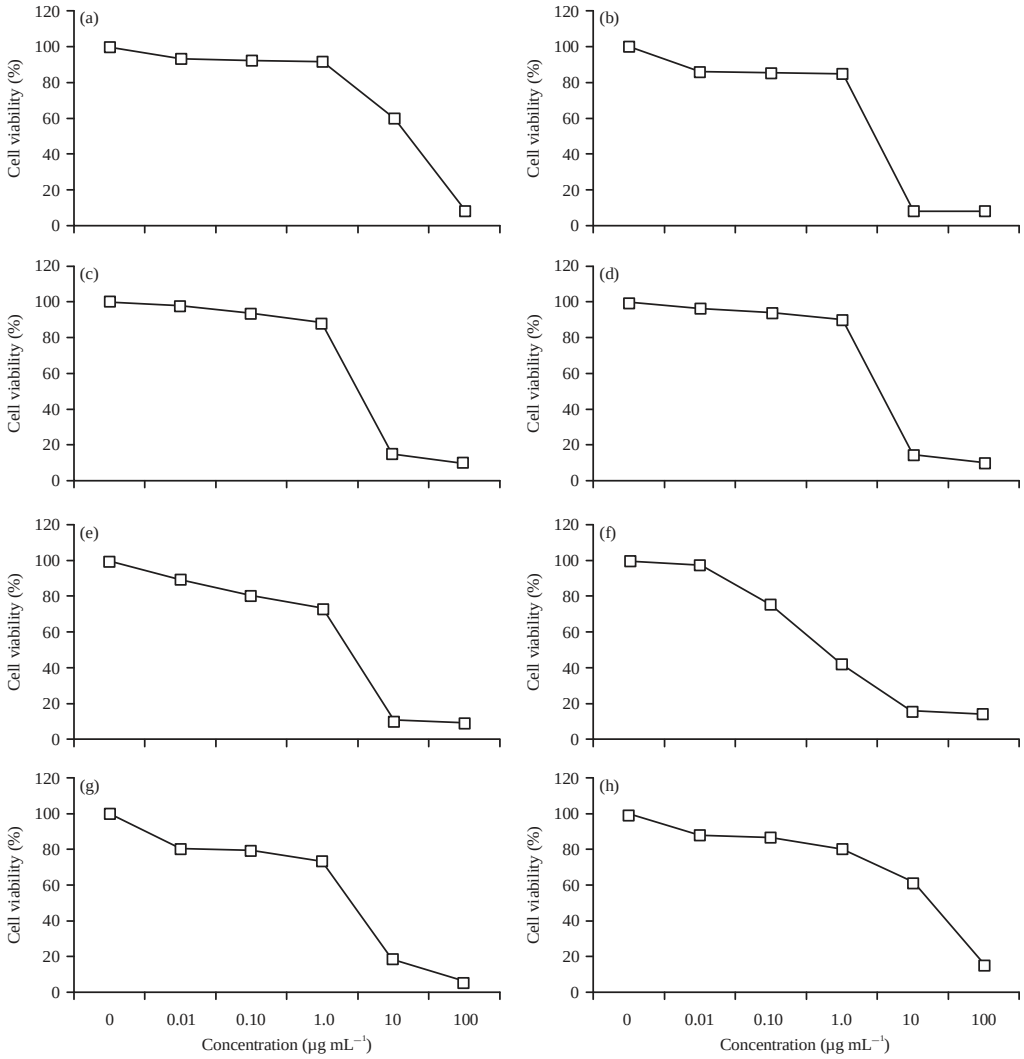

Fig. 1(a-h): Effect of bee venom at different concentrations on viability of different cancer cells for 72 h, (a) HeLa cell line (cervical carcinoma), (b) HEPG-2 cell line (liver cancer), (c) PC3 cell line (prostate cancer), (d) HEP-2C cell line (larynx cancer), (e) A549 cell line (lung cancer), (f) RDA cell line (rhabdomyosarcoma), (g) MCF7 cell line (breast cancer) and (h) HCT116 cell line (colon cancer)

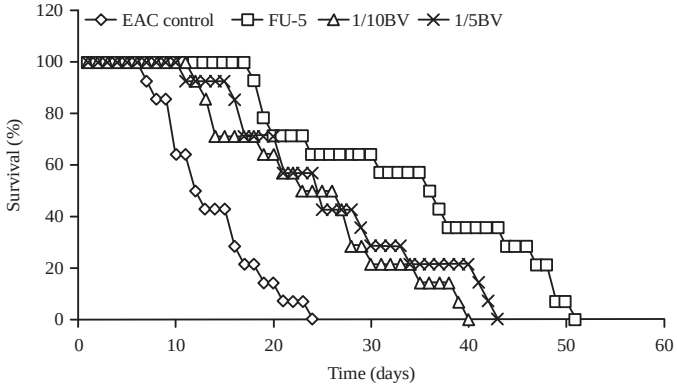

Fig. 2: Effect of bee venom on the percentage of survival (%) of EAC bearing rats

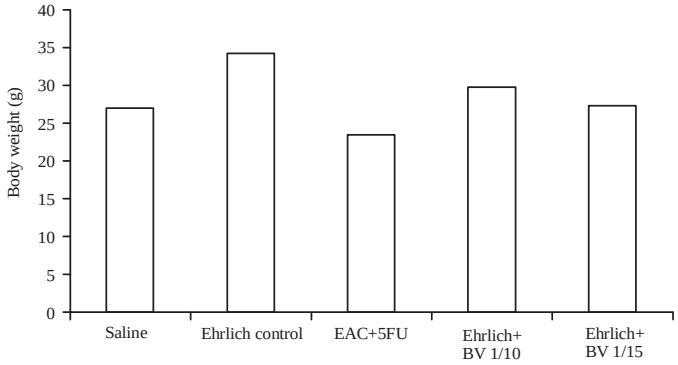

Fig. 3: Changes in body weight (g) in different groups of rats

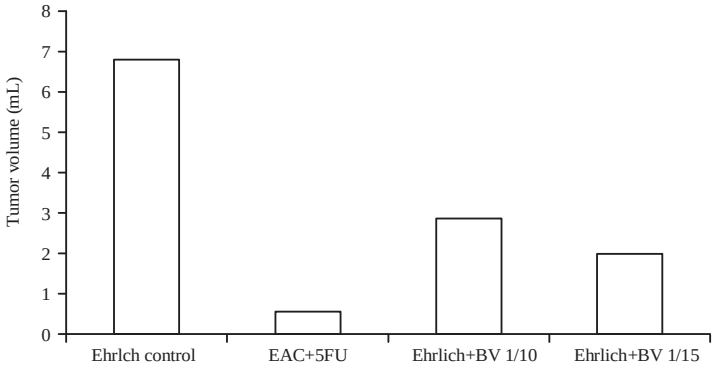

Fig. 4: Changes in ascites tumor volume in different groups of rats

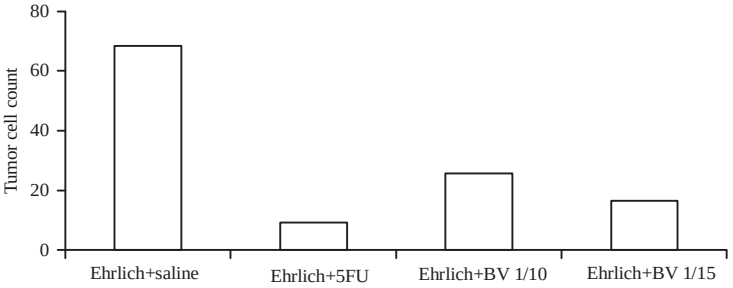

Fig. 5: Changes in total EAC cells ($N \times 10^6$) in different groups of rats

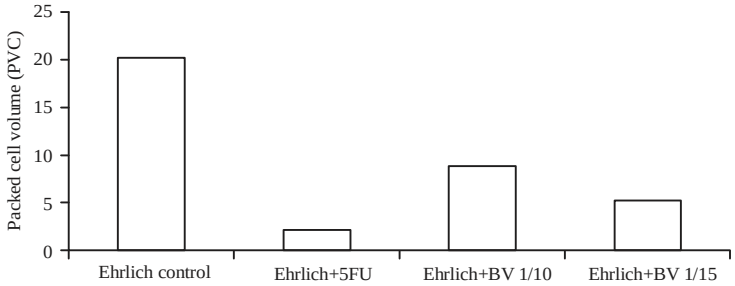

Fig. 6: Changes in tumor PCV in different groups of rats

b.wt., of bee venom (G5 and G6) were 25.66×10^6 and 16.5×10^6, respectively, whereas, the EAC bearing rats treated with the standard drug 5-fluoruracil (20 mg kg^{-1}) (G4) decreased the total number of EAC cells to 9.2×10^6.

The PCV% (Fig. 6) of untreated EAC control (G3) was 20.16%, while the different dose of bee venom and 5-fluoruracil showed high significant ($p<0.05$) reduction in PCV% of EAC cells compared with untreated EAC control group. The PCV% of EAC cells of the rats administrated with 0.28 and 0.56 mg kg^{-1} b.wt., of bee venom (G5 and G6) were 8.83 and 5.33% respectively, whereas the group of EAC bearing rats treated with 5-fluoruracil (G4) decreased the PCV% of EAC cells to 2.16%.

Dead tumor cells percentage (Fig. 7) in untreated EAC control (G1) was 4.16%, while the different doses of bee venom and 5-fluoruracil groups showed high significant ($p\leq0.05$) increase in dead cells percentage compared with untreated EAC control group. The dead cells percentage in the rats administrated with 0.28 and 0.56 mg kg^{-1} of bee venom (G5 and G6) were 58.33 and 66.5%, respectively, whereas the group of EAC bearing rats treated with 5-fluoruracil (G4) increased the dead cells percentage to 73.83%.

The aforementioned results indicated that the groups treated with 0.28 and 0.56 mg kg^{-1} of bee venom (G5 and G6) and 5-fluorouracil revealed increasing of MST, dead cells% with a reduced in body weight, volume of the ascitic fluid, total number of EAC cells, PCV% and life cells% this could be due to interfere with the gross of EAC cells directly during early phase of treatment leading to a considerable elimination of these cells. This results are in agreement with those of Merit (2006) who suggested that intraperitoneal administration with bee venom to tumor bearing rats increased the survival time and (Orsolic *et al*., 2009) who reported that bee venom injection decreased tumor size and induced some sort of tumor shrinkage and delay of tumor growth.

Effect of bee venom injection on some serum parameters (Biochemical analysis) of EAC tumor bearing rats: Data in Fig. 8, showed that aspartate aminotransferease (AST) and alanine aminotransferease (ALT) and alkaline phosphatase (ALP) activities were significantly ($p<0.05$) increased in untreated EAC bearing rats (G3) which reach to 151, 59.5 and 99.166 IU L^{-1}, respectively compared with normal rats control group (G1) (113.66, 37.166 and 65.66 IU L^{-1}, respectively). AST, ALT and ALP activities were significantly ($p<0.05$) decreased in EAC bearing rats treated groups with 0.28 and 0.56 mg kg^{-1} of bee venom (G5, G6) and 5-fluoruracil (G4), while the normal rats treated with 0.28 mg kg^{-1} b.wt., of bee venom (G2) showed non-significant change compared to normal rats control group (G1).

Figure 9 presented the effect of bee venom injection on serum protein, albumin and globulin levels. The results showed that protein and albumin levels were significantly ($P\leq0.05$) decreased in untreated EAC bearing rats (G3) to reach 6 and 1.98 g dL^{-1}, respectively compared with normal rats control group (G1) (7.6 and 3.25 g dL^{-1}, respectively). Total protein and albumin levels were significantly ($p\leq0.05$) increased in EAC bearing rats treated with 0.28 and 0.56 mg kg^{-1} of bee venom (G5, G6) and 5-fluoruracil (G4). Also, the total protein and albumin levels of normal rats treated with 0.28 mg kg^{-1} of bee venom (G2) significantly ($p<0.05$) increased compared to normal rats control group (G1). No significant changes for globulin level between the experimental groups were observed.

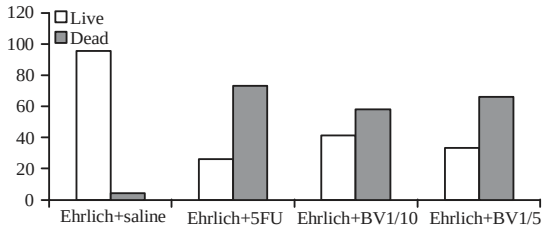

Fig. 7: Changes in viability (%) in different groups of rats

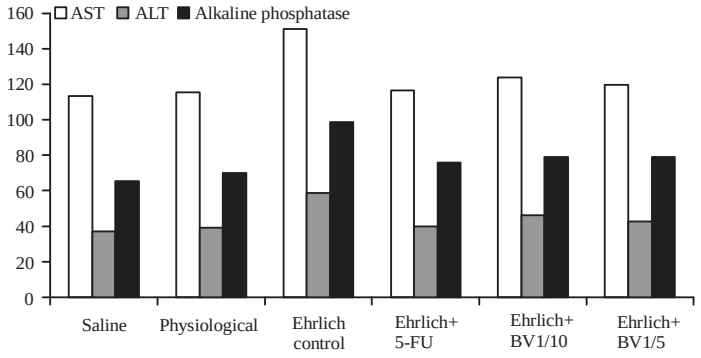

Fig. 8: Changes in AST, ALT and ALP activities in different rats groups

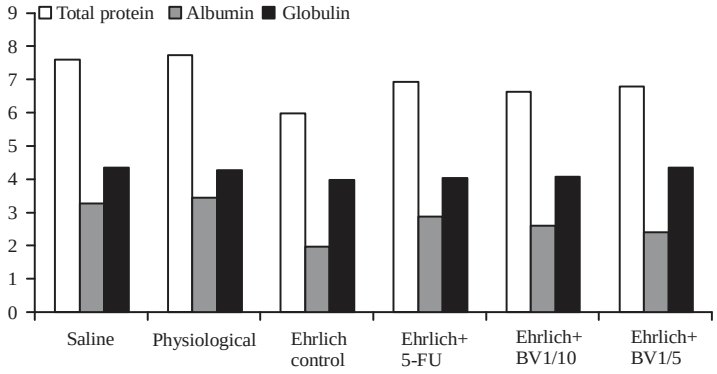

Fig. 9: Changes in serum total protein, albumin and globulin levels in different rats groups

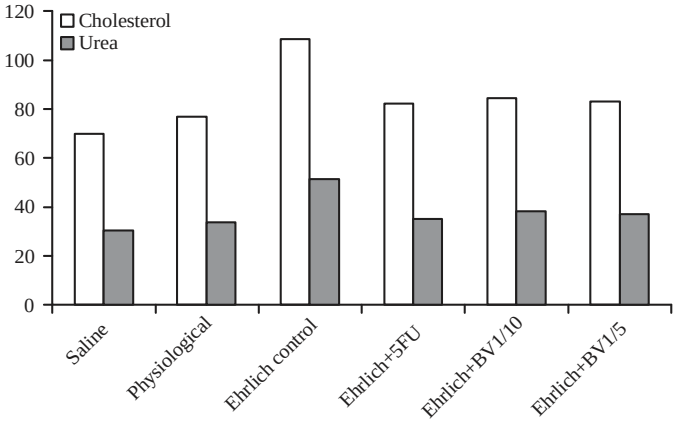

Fig. 10: Changes in serum cholesterol and urea concentration in different rats groups

Data in Fig. 10 showed that urea and cholesterol levels were significantly ($p \leq 0.05$) increased in untreated EAC bearing rats (G3) to reach 51.83 and 108.83 mg dL^{-1}, respectively compared with normal rats control group (G1) (30.5 and 70 mg dL^{-1}). Urea and cholesterol levels were significantly ($p \leq 0.05$) decreased in EAC bearing rats treated with 0.28 or 0.56 mg kg^{-1} of bee venom (G5, G6) and 5-fluoruracil (G4). Also the normal rats treated with 0.28 mg kg^{-1} of bee venom (G2) showed significantly ($p \leq 0.05$) increased compared to normal rats control group (G1).

Biochemical analysis of serum revealed hypoproteinemia and hypoalbuminemia in the untreated EAC bearing rats (G3), this may be attributed to increase mitotic division of tumor cells with high bloody fluid withdrawal and the capillary permeability, which permit the escape of plasma proteins into the peritoneal cavity (Garrison *et al*., 1987). Also hypoalbuminemia in domestic animals may be due to excessive nephritis and liver diseases (Coles, 1986), which confirmed with the result of increased ALT and AST activities in the untreated EAC bearing rats (G2) which describe presence of hepatic and renal damages as a result of cancer cells invasions.

While in the EAC bearing rats treated with bee venom (G5 and G6) there is an improvement of parameters towards the normal control (G1) as a cause of bee venom protective effect against organ dysfunction and cellular injury of liver and kidney, this result was in good agreement with Merit (2006) who reported that bee venom injection in different concentration reduced the effects on some serum parameters; ALT, AST, alkaline phosphatase, albumin, globulin and urea levels in serum of carcinogenated rats.

Histopathological analysis: Histopathological results of experimental rats groups are shown in Fig. 11-14. It can be noticed from the results that, the peritoneum of group 1 (Fig. 11a) showed normal histological structure. Mild hyalinization in the abdominal muscle (Fig. 11b), large tumor mass represented by clusters or sheets from large polymorphic cells with large vesicular hyperchromatic nuclei with mitotic activities and distinct cytoplasm replacing the omental fat (Fig. 11c), few number of neoplastic cells with necrosis in some cancer cells (Fig. 11d), neoplastic cells with

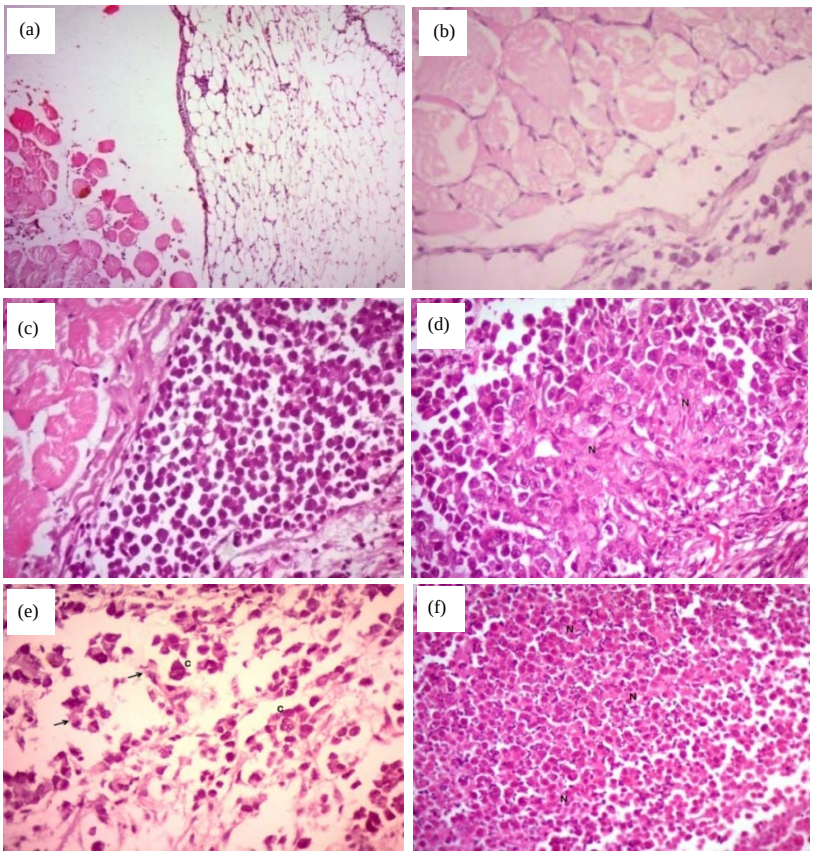

Fig. 11(a-f): Effect of bee venom injection on peritoneum of experimental rats groups

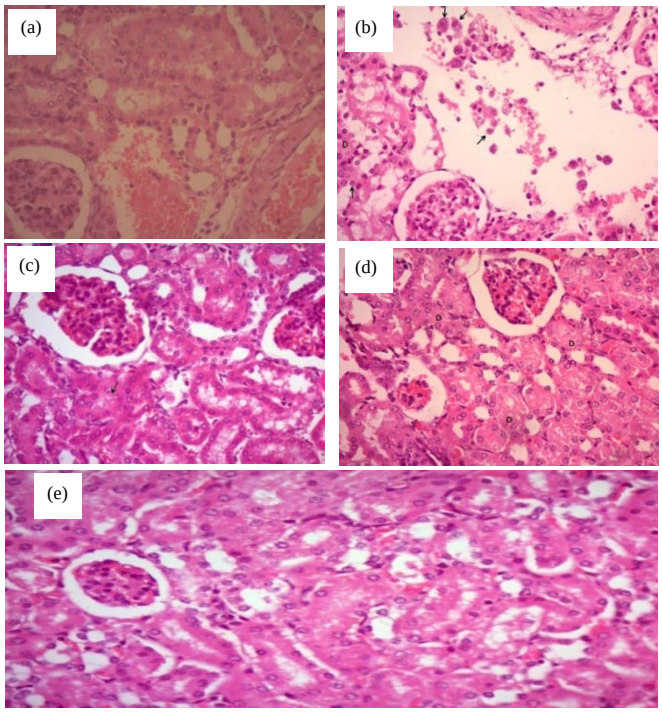

Fig. 12(a-e): Effect of bee venom injection on kidney of experimental rats groups

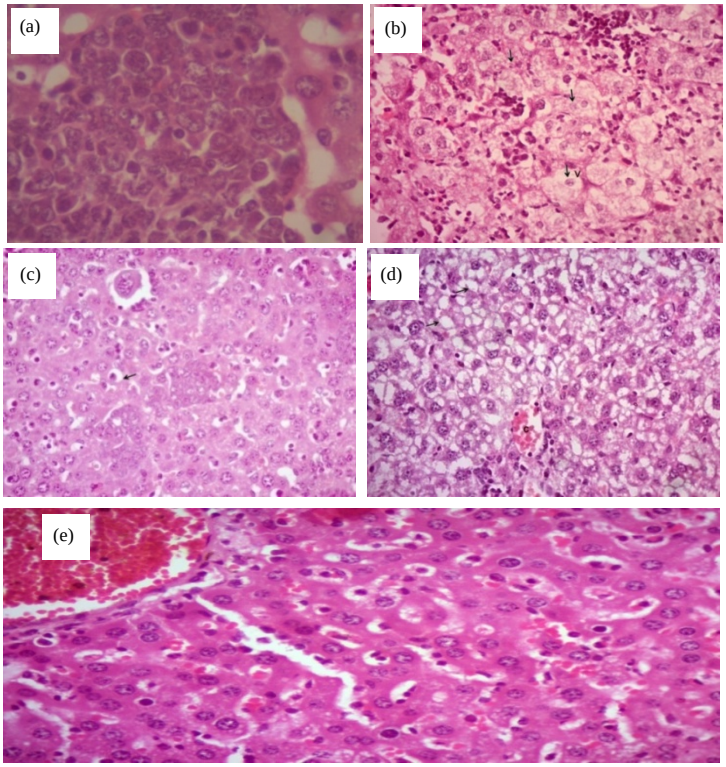

Fig. 13(a-e): Effect of bee venom injection on liver of experimental rats groups

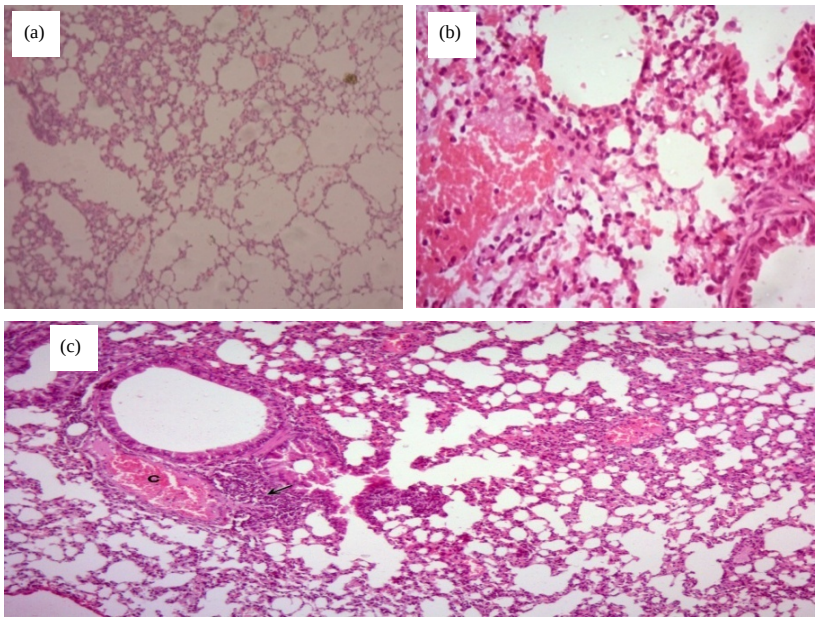

Fig. 14(a-c): Effect of bee venom injection on lungs of experimental rats groups

few necrotic cells (Fig. 11e) and neoplastic cells with sever necrosis (Fig. 11f) were observed for groups 2, 3, 4, 5 and 6, respectively.

Kidneys of groups 1 and 2 showed normal histological structure (Fig. 12a). Mild tubular degeneration was showed for groups 4 and 5 (Fig. 12c and d, respectively). Neoplastic invasions with pressure atrophy and necrosis of the surrounding tubules (Fig. 12b) and normal histological structure (Fig. 12e) were observed for kidneys of groups 3 and 4, respectively.

Liver of groups 1 and 2 showed normal histological structure (Fig. 13a). Focal coagulative necrosis of the hepatic parenchyma was common as well as vacuolation in their cytoplasm (Fig. 12b), mild vacuolation in their cytoplasm with a sever congestion (Fig. 12c), hydropic degeneration as well as congestion in the hepatic sinusoids (Fig. 12d) and mild degeneration (Fig. 12e) were observed for groups 3, 4, 5 and 6, respectively.

Lungs of groups 1, 2, 5 and 6 showed normal histological structure (Fig. 14a). Congestion in the interalveolar capillaries as well as focal hemorrhages (Fig. 14b) and congestion in the peribronchial blood vessels and infiltration of inflammatory cells (Fig. 14c).

Bee venom inhibits the proliferation of carcinoma cells and tumor growth *in vivo* due to the stimulation of the local cellular immune responses in lymph nodes. The mechanism of action of bee venom involves apoptosis, necrosis and lysis of the tumor cells (Gajski and Garaj-Vrhovac, 2013; Gajski *et al*., 2014). Bee venom has also been reported to induce apoptosis through caspase-3 activation in synovial fibroblasts (Hong *et al*., 2005).

CONCLUSION

The results of this study indicated that the bee venom has an inhibitory effect against cancer cell lines depending on bee venom concentration. Also, the results of *in vivo* experiment demonstrated that the bee venom (0.28 or 0.56 mg kg^{-1}) reduced the body weight, ascites tumor volume, Packed Cell Volume (PCV), viable tumor cell, tumor cell count number and increased Mean Survival Time (MST). Aspartate aminotransferease (AST) and alanine aminotransferease (ALT), alkaline phosphatase (ALP), total proteinand albumin levels significantly ($p \leq 0.05$) decreased in Ehrlich ascites carcinoma bearing rats treated groups bee venom.

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