Ehrlich solid tumor induced injury and toxicity in liver and kidney in female mice

ABSTRACT

Background and Objective: Cancer can develop in any organ at any time; it is a cellular malignancy that causes a breakdown in normal cell-cycle regulation, leading to unchecked proliferation and a lack of differentiation. It is the main factor in human mortality. The therapeutic efficacy of currently available drugs is minimal, and they do not prevent the growth of cancer. Current work aimed to study the effect of Ehrlich solid tumor (EST) on liver and kidney in female mice.

Materials and Methods: Twenty mice were randomly and equally divided into two groups and each group consists of 10 mice; 1st group is control group in which mice did not receive any treatment and 2nd group is EST group, including mice, each injected subcutaneously with 2.5-3×10^6 EAC cells.

Results: Current results revealed that; EST induced significant increase in the levels of serum urea, creatinine, potassium, chloride, aspartate transaminase (AST) and alanine transaminase (ALT) activities and alkaline phosphatase (ALP), kidney and liver injuries and cconversely, a significant decrease in sodium, calcium as associated to control.

Conclusion: Current results indicated that EST induced damage and toxicity in liver and kidney.

Key words: Ehrlich solid tumor; mice; kidney functions; liver functions.

1. INTRODUCTION

Cancer is an abnormal cell growth brought on by aberrant cell proliferation. It can spread through the blood stream to other bodily organs. Both internal and external variables, including immunological disorders and inherited genetic abnormalities, can contribute to the development of cancer [1]. Any of a broad range of illnesses known as cancer are defined by the growth of aberrant cells that proliferate rapidly and have the capacity to invade and destroy healthy bodily tissue [2&3].

Cancer is a condition where cells accumulate abnormally as a result of an imbalance between proliferation and planned cell death [4]. The general word for all malignant tumors is cancer. Both benign and malignant tumors exhibit unchecked growth, but the latter are characterized by their propensity to differentiate, be invasive, and spread to other parts of the body [5&6]. Multiple genetic alterations are required for the emergence of cancer, which result in the loss of control over a variety of processes. Thus, cancer rates rise with age as starting mutations accumulate over a longer period of time and the impact of molecular repair mechanisms and the immune system is diminished. In addition to point mutations, which can result from insufficient DNA replication or repair during any cell division event, a variety of environmental variables, or so-called mutagens, can alter an organism's genetic makeup.

Breast cancer is the most public cancer amongst women world-wide (1.38m new cases/year, 23% of all cancers). Graded as fifth cause of death (the first in women) from cancer overall (45800 deaths). In Egypt, it represents almost 37% of cancer in women (18% overall).

The Ehrlich tumour, which was created from mouse breast cancer, is only one example of the

numerous in-vivo experimental models that are based on experimental animals [7-10]. Depending on how it is inoculated—subcutaneously or intraperitoneally—this aggressive and swiftly developing cancer may manifest in either the solid (EST) or ascetic form (EAC) [11-15].

2. Material and methods

2.1 Induction of Ehrlich Solid Tumour (EST)

The Egyptian National Cancer Institute (NCI; Cairo University, Egypt) provided the mice that carried Ehrlich ascites carcinoma (EAC). To maintain the tumor line and evaluate EST, viable cells (2.5-3×106 cells/mouse) were implanted subcutaneously into the left thigh of each recipient mouse according to Elgharabawy et al., [7].

2.2 Experimental Animals

Twenty mice were randomly and equally divided into two groups; group 1: (Control) and group 2: (EST). Throughout the study period, mice were raised and treated in line with the Faculty of Science, Tanta University guide for animals, which was approved by the Institutional Animal Care and Use Committee (IACUC-SCI-TU-0223).

2.3 Experimental Design and mice groups

Group 1: Control group in which mice did not receive any treatment.

Group 2: EST group, including mice, each injected subcutaneously with 2.5-3×10^6 EAC cells.

2.4 Blood Sampling:

Mice were sacrificed at the end of the study by administering sodium pentobarbital intraperitoneally, and they received a full necropsy. For the purpose of determining the activities of the liver, individual blood samples were taken from the inferior vena cava of each mouse and placed in non-heparinized glass tubes.

2.5 Liver enzymes and functions

Serum aspartate transaminase (AST) and alanine transaminase (ALT) activities and alkaline phosphatase (ALP) levels were assessed by Tousson et al, [16&17]. Serum albumin was determined according to the approach proposed by Moustafa et al. [18] while total protein concentration was determined according to the approach proposed by El Moghazy et al. [19].

2.6 Kidney functions and Electrolyte estimation

Creatinine and urea were assessed after Tousson et al. [20] and Patton and Crouch [21] respectively. The method planned by El-Masry et al. [13] was surveyed to measure the levels of Potassium, calcium, sodium, and chloride ions using marketable kits of Indian Sensa-core electrolyte.

2.7 Histological preparation

After necropsy the kidney and liver were immediately removed and fixed by immersion in 10% neutral buffered formalin solution for 24-48 h. The specimens were then dehydrated, cleared, and embedded in paraffin. Serial sections (5 µm thick) were sliced using a rotary microtome (Litz, Wetzlar; Germany) and stained with haematoxylin and eosin according to Tousson [22].

2.8 Statistical Analysis

Data were expressed as the significance of difference was analyzed by one – way ANOVA. Values are expressed as means ± SE. *and# significant difference from control and from EST group respectively at $p< 0.05$.

3.0 Results

3.1. EST morphology

Examination of untreated tumor-bearing mice showed that EAC cells infiltrated and mostly replaced the subcutaneous tissue with necrosis of the remaining skeletal muscles. Mice that inoculated with EAC cells intramuscularly in the right thigh of the hind limb developed a palpable solid tumor in 14 days following inoculation. Numerous newly formed blood capillaries (neovascularization) were seen

in the surrounding tissue with mild or no inflammatory response. Such tumor showed tissue architectural disarray, as well as marked degree of cellular anaplasia, pleomorphism, and anisocytosis, with nuclear vascularity, a typicality, hyperchromasia, and mitoses. Some tumor cells were differentiated into gland-like structures surrounding a lumen containing eosinophilic material. Also; liver sections in EST revealed marked degeneration and necrosis (Figure 1).

3.2. EST induced liver toxicity:

A significant ($P<0.05$) increase in the activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase while a significant ($P<0.05$) decrease in albumin and total protein levels in EST group as compared with control mice (Table 1).

3.3. EST induced kidney toxicity:

A significant elevation in the levels of kidney functions (urea and creatinine), and electrolytes (potassium and chloride) while a significant depletion in electrolytes (calcium and sodium) in EST group as compared with control mice (Table 2).

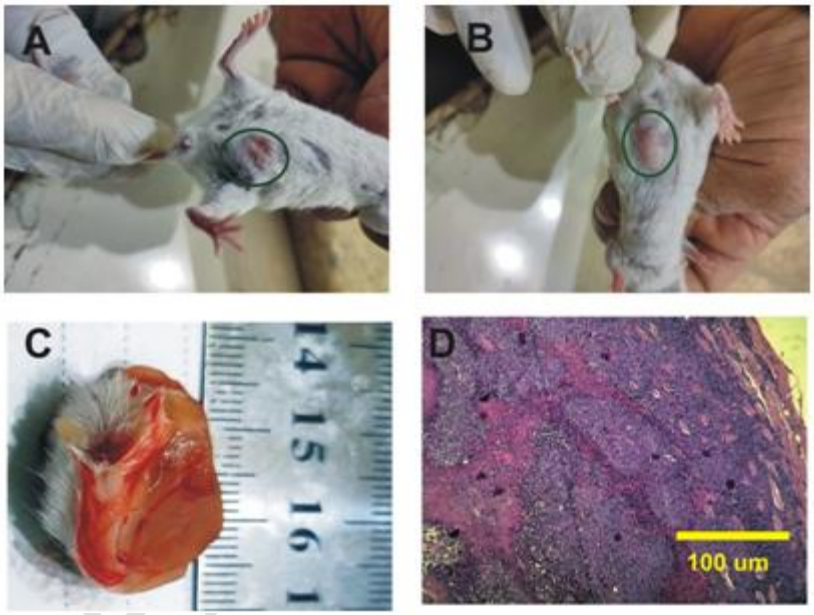

Figure 1: A&B: Ehrlish solid tumor (EST) in female mouse. **C:** EST after isolated from mouse. **D:** Marked degree of cellular anaplasia, pleomorphism, and anisocytosis, with nuclear vascularity in EST.

Table 1: Changes in the liver functions in the two groups.

	control	EST
AST (U/l)	$40.5^{\#}\pm1.98$	$82.3^{*}\pm1.92$
ALT(U/l)	$49.2^{\#}\pm2.36$	$86.9^{*}\pm2.03$
ALP (U/l)	$45.2^{\#}\pm2.83$	$61.2^{*}\pm1.97$
Albumin (mg/dl)	$3.02^{\#}\pm0.14$	$2.54^{*}\pm0.25$
Total protein (g/dl)	$6.7^{\#}\pm0.22$	$6.1^{*}\pm0.31$

Values are expressed as means ± SE. *and# significant difference from control and from EST group respectively at p< 0.05.

Table 2: Changes in the kidney functions and electrolytes levels in the two groups.

	control	EST
Creatinine (gm/dl)	$0.91^{\#}$± 0.8	1.72*± 0.07
Urea (gm/dl)	$30.0^{\#}$±1.12	45.8*±1.2
Na^{+} (mmol/l)	$137.2^{\#}$±0.7	125*± 0.87
K^{+} (mmol/l)	$5.1^{\#}$±0.1	8.24*±0.21
Cl^{-} (mmol/l)	$106^{\#}$±0.59	111.7*±1.36
Ca^{++2} (mmol/l)	$1.01^{\#}$±0.05	0.74*±0.02

Values are expressed as means ± SE. *and# significant difference from control and from EST group respectively at p< 0.05.

3.4 Liver Histopathology

The histopathological changes in the liver sections in the two groups were showed in Figures 2(A-C). Regarding the histopathological examination of the liver sections in the normal control group revealed normal histological pattern with normal central vein is surrounded by radiating cords of hepatocytes that are arranged in the form of anastomosing cords (strands) forming a network that extend from a central vein to the periphery of the hepatic lobules at which the portal tracts appeared (Figure 2A). Liver sections in EST revealed marked cellular damage, degeneration in hepatic cords in addition to karyomegally and pyknotic nuclei indicating apoptosis, moderate fibrosis, and marked diffuse necrosis of hepatic tissue, marked inflammatory cells and congested blood sinusoids (Figure 2A&C).

3.5 Kidney Histopathology

The histopathological changes in the kidney sections in the two groups were showed in Figures 3(A-C). Regarding the histopathological examination of the kidney sections in the normal control group revealed normal histological structure (Figure 3A). The control kidney is composed of two main parts: the renal cortex and the medulla which possess normal histological features. The renal cortex is enclosed by numerous renal corpuscles, each made up of a glomeruli and the Bowman's capsule (Figure 3A). There is a characteristically normal space between the glomeruli and Bowman's capsule to allow renal filtration. The renal corpuscles are surrounded by proximal and distal convoluted tubules.

Kidney sections in EST group revealed variable pathological changes in glomeruli and some parts of the urinary tubules as a marked damage and degenerated to the renal tissues, Glomerular atrophy, the Malpighian corpuscles that lost their characteristic configuration (Figure 3B&C).

4.0 Discussion

Every year, 10 million new cancer identifies are made; the trend in cancer diagnosis suggests that; by 2030, 20 million new cancer diagnoses will have been made [23]. Cancer refers to any one of large number of diseases characterized by the development of abnormal cells that divide uncontrollably and

have the ability to infiltrate and destroy normal body tissue [24]. Ehrlich carcinoma has a similarity with human tumors which are the most sensitive to chemotherapy due to the fact that it is undifferentiated and has a rapid growth rate [25]. Therefore, the current study aimed to studies the effect of Ehrlich solid tumor (EST) in liver and kidney structure and functions.

Current study revealed that; EST induced elevations in the levels of ALT, AST, ALP and depletion in albumin and total proteins indicate liver injury. These results concurred with those of Said et al. [26], Aldubayan et al. [11], AbdEldaim et al. [27] who reported that; EST induced liver toxicity in mice. In addition to EST induced liver tissue injury. Also; Tousson et al. [28] and Abd Eldaim et al., [9] who reported that Ehrlich ascites carcinoma (EAC) induced changes in liver functions and induced liver damage. Current study revealed that; EST induced elevation in the levels of urea, creatinine, potassium, chloride ions and depletion in calcium and sodium ions indicate kidney toxicity.

These results concurred with those of Abd Eldaim et al., [8] who reported that; EST induced elevation in kidney functions and damage in renal tissue structure. Ehrlich ascites carcinoma (EAC) induced changes in kidney functions and damage in kidney tissues. Also our results agree with Mutar et al. [14] and Abd Eldaim et al., [27] who reported that; EAC induced renal damage and toxicity. Our results conclude that; EST induced toxicity and damage in liver and kidney.

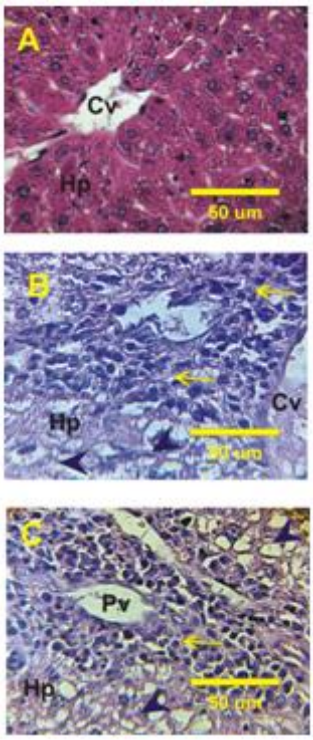

Figure 2: Photomicrograph of mice liver sections in the different experimental groups stained with Haematoxylin & Eosin. A: Liver sections in control group revealed normal histological pattern with normal central vein (CV) is surrounded by radiating cords of hepatocytes (Hp). B&**C:** Liver sections in EST revealed marked cellular damage, degenerated hepatocytes (arrow heads), cellular infiltrations (arrows), atrophy, and moderate congestion in central and portal veins.

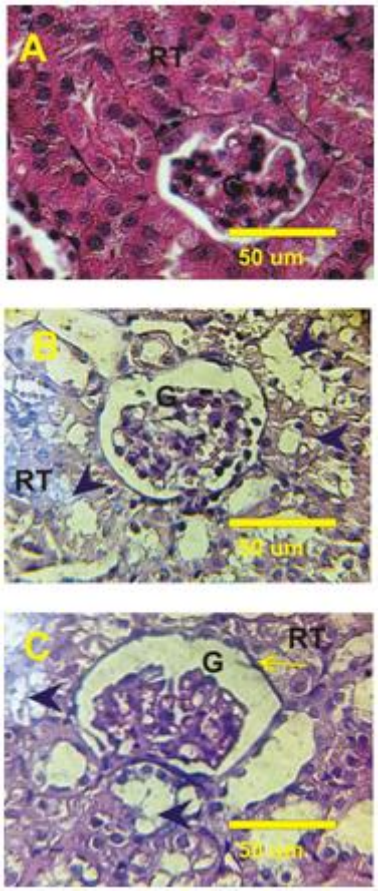

Figure 3: Photomicrograph of mice kidney sections in the different experimental groups stained with Haematoxylin & Eosin. A: Kidney section in control group revealed normal histological structure. **B&C:** Kidney sections in EST revealed marked degenerated to the renal tissues (arrow heads) and Glomerular atrophy (arrows).

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