Asian Oncology Research Journal

3(4): 20-25, 2020; Article no.AORJ.56165

Therapeutic Impact of Etoposide against Ehrlich Solid Tumor Induced Cardiac Toxicity in Female Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IETES and ET designed the study, performed the statistical analysis, wrote the protocol and N author AAR wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Ramesh Gurunathan, Sunway Medical Center, Malaysia.
Reviewers:
(1) Christopher Larbie, Kwame Nkrumah University of Science and Technology, Ghana.
(2) Dr. Paras Nath Yadav, Tribhuvan University, Nepal.
(3) Dr John Ogedengbe, University of Abuja, Nigeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/56165

Original Research Article

Received 27 May 2020
Accepted 03 August 2020
Published 08 August 2020

ABSTRACT

Background and Objective: Breast cancer is one of the most common cause of cancer deaths among women worldwide. Etoposide is an important chemotherapeutic agent that is used to treat a wide spectrum of human cancers. It has been in clinical use for more than two decades and remains one of the most highly prescribed anticancer drugs in the world. The current study was conducted to compare the effect of Etoposide on cardiac function on none bearing tumor mice and bearing tumor mice.
Materials and Methods: A total of 40 albino females' mice were separated into four groups (1st, control group; 2nd, Etoposide group; 3rd, EST group; 4th, EST treated with Etoposide group).
Results: EST induced changes in cardiac function and Electrolytes under study compared with control group. We found that LDH (lactate dehydrogenase), CK-MB (creatinine kinase) and CK (creatinine kinase), potassium ions (k+), chloride ions (Cl^-), alkaline phosphatase (ALP) and aspartate aminotransferase (*AST*) were significantly increase in EST group compared with control

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group. In contrast, serum sodium ions had a significant decrease in EST group compared with control group. On the other hand, treatment with Etoposide for 14 days improved these changes in cardiac functions.

Conclusion: The present study confirmed the cardiac potential Etoposide. Further studies are warranted to explore its mode of action and safety for medicinal use in cancer therapy.

Keywords: Ehrlich solid tumor; Etoposide; mice; cardiac functions; electrolytes.

1. INTRODUCTION

Cancer is the largest single cause of death in humans and is a cellular malignancy that results in the loss of normal cell-cycle control, such as unregulated growth and the lack of differentiation, can develop in any tissue of any organ, and at any time [1]. Cancer is initiated due to abnormalities in DNA of the affected cells leading to an extra mass of tissue termed a tumor. Many cancer therapies indirectly activate apoptosis by chemical or physical damage of DNA [1-3].

Breast cancer is the most common type of cancer among women and the second most frequent kind of tumor worldwide [4]. Ehrlich solid tumor is used as experimental models of breast cancer [3,5,6]. Ehrlich carcinoma is an undifferentiated carcinoma characterized by rapid proliferation, no-regression, high translatable capability, 100% malignancy, does not have tumor specific transplantation antigen and short life span [6-8]. Ehrlich solid tumor (EST) is often used as a transplantable tumor model for the examination of breast cancer because it imitates the antineoplastic activity of various chemical compounds in breast cancer [9-11].

Many of research investigated the effect of chemotherapy drugs on normal animal cells and tissues [12-21]. Etoposide is chemotherapeutic drugs that originated from the plant *Podophyllum pelltatum* and inhibits topoisomerase II activity and it has been used for treatment of human cancer [22,23]. This study aims to find the protective effect of etoposide against EST induced cardiac toxicity in female mice.

2. MATERIALS AND METHODS

2.1 Chemical

Etoposide (also commonly known as VP-16) is a semisynthetic derivative of podophyllotoxin used in the treatment of certain neoplastic diseases. It is 4'-demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-β-D-glucopyranoside]. It is very soluble in methanol and chloroform, slightly soluble in ethanol and sparingly soluble in water and ether. It is made more miscible with water by means of organic solvents. It has a molecular weight of 588.58 and a molecular formula of C 29H 32O 13.

2.2 Experimental Animals

Experiments were performed on 40 female Swiss albino mice weighing 20–25 g obtained from animal house colony Egypt Vaccine Company, Giza, Egypt. The animals were randomized and housed under ambient room- temperature at 22 - 25^0C and relative humidity conditions a 12 h light/ 12 h dark cycle, a commercial diet and water were provided ad libitum for two weeks.

2.3 Induction of Ehrlich Solid Tumor (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, A fixed number of viable cells (usually 2.5-3x10^6 cells/20 g body weight) were implanted into subcutaneous in the left thigh of each recipient mouse [3].

2.4 Experimental Design

After one week of acclimation, a total of 40 female mice were randomly and equally assigned into four groups, 10 mice each.

First group: Mice were not injected with anything kept as a control.

Second group: Mice were injected with Etoposide (50 mg/kg body weight/ twice a week) intraperitoneal for two weeks [22].

Third group: Mice were injected subcutaneously with 2.5-3 million cells of EAC per mouse diluted

in physiological saline to initiate tumor EST for 2 weeks [3].

Fourth group: Mice were injected subcutaneously with 2.5-3 million cells of EAC per mouse diluted in physiological saline to initiate tumor and left for 2 weeks till the development of solid tumor then treated with Etoposide for another 2 weeks.

2.5 Blood Sampling

By the end of the experiment, mice were sacrificed with intraperitoneal injection of sodium pentobarbital and subjected to a complete necropsy. Blood samples were individually collected from the inferior vena cava of each mice into non-heparinized glass tubes for estimation of cardiac function biomarkers and some electrolytes.

2.6 Cardiac Functions and Enzymes Biomarker

Serum lactate dehydrogenase (LDH) activity was measured by a kinetic method using kits (Vitro Scient, Cairo, Egypt) according to a method described by Tousson et al. [24]. The level of creatine kinase (CK) in serum was determined by akinetic method using kits (Vitro Scient) according to the method described by Zilva and Pannall [25]. Creatine kinase myoglobin (CK-MB) activity in serum was determined using an assay kit (Bioassay Systems, Hayward, CA, USA) based on the method of Bishop et al. [26]. Activities of serum AST and ALP were assessed in the sera as per Al-Rasheed et al. [19].

2.7 Electrolytes Biomarkers

Determination of the levels of serum electrolytes (K+, Na+, Ca++ and Cl-) using commercial kits (Sensa core electrolyte, India)in the sera as per Abd Eldaim et al. [27].

2.8 Statistical Analysis

Data were expressed as mean values±SD and statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) tests to assess significant differences among treatment groups. The criterion for statistical significance was set at $p<0.05$. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® Inc., USA).

3. RESULTS

3.1 Changes in Cardiac Function

Table 1 shows the changes in cardiac functions in different groups under study. It was revealed that, CPK, LDH, and CK-Mb significantly increased in EST group compared with control group. ETO group reveal a moderate increase in CPK and LDH while CK-Mb shows a moderate decreased compared with Control group. EST+Eto group shows a significant decreased in CPK, LDH, and CK-Mb compared with EST group.

3.2 Changes in Serum Enzymes

Table 2 shows the changes in serum enzymes in different groups under study. It divulged that serum AST and ALP have a noticeable increase in EST group compared with control group. Eto group divulge significant increase in AST and ALP levels compared with control group. EST+Eto group showed decrease in serum AST and ALP compared with EST group.

3.3 Changes in Electrolytes

Table 3 shows the changes Electrolytes in different groups under study. Divulge that; serum k^+ and Cl^- have a noticeable increase in EST group compared with control group. On the other hand, Na^+ has a noticeable decreased in EST group compared with control group. EST+Eto group showed decreased in k^+ and Cl^- compared with EST group on the other hand; serum Na^+ divulge a slight increased compared with EST group.

Table 1. Changes in cardiac functions in different groups under study

	CPK (U/l)	LDH*103 (U/l)	CK-Mb (ug/ml)
Control	$295.8^{\#}$± 6.24	$1.435^{\#}$± 0.024	$0.268^{\#}$± 0.008
Eto	$396.2^{\#*}$± 10.67	$2.854^{\#*}$± 0.16	$0.254^{\#}$± 0.01
EST	892^{*}± 5.15	10.05*± 0.27	0.364^{*}± 0.006
EST+Eto	$495.8^{*\#}$± 12.9	5.61*#± 0.21	$0.294^{*\#}$± 0.0031

*Data are expressed as mean ± S.E.M. Where, ETO, Etoposide group; EST, Ehrlich solid tumor group; Est+Eto, treated Ehrlich solid tumor with Etoposide group. #significat for control, * significant for EST*

Table 2. Changes in serum enzymes in different groups under study

	AST(U/l)	ALP(U/l)
Control	100.4#±3.586	122#± 2.168
Eto	128.6#*± 5.6	154*#± 3.05
EST	225.4*±5.446	198.8*±3.707
EST+Eto	192.2*#±5.113	174.6*#±2.75

*Data are expressed as mean ± S.E.M. Where, ETO, Etoposide group; EST, Ehrlich solid tumor group; Est+Eto, treated Ehrlich solid tumor with Etoposide group. #significat for control, * significant for EST*

Table 3. Changes in electrolytes in different groups under study

	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)
Control	$136.3^{\#}$±0.552	$4.704^{\#}$±0.1087	$101.1^{\#}$±0.6367
Eto	$144^{\#}$± 2.115	$5.022^{\#*}$ ±0.1345	$110.8^{*\#}$±0.9356
EST	96.8^{*}± 4.168	6.612^{*}± 0.0735	120.1^{*}± 1.111
EST+Eto	$127.1^{\#*}$± 2.44	$5.956^{*\#}$±0.07325	$113.1^{*\#}$± 2.033

*Data are expressed as mean ± S.E.M. Where, ETO, Etoposide group; EST, Ehrlich solid tumor group; Est+Eto, treated Ehrlich solid tumor with Etoposide group. #significat for control, * significant for EST*

4. DISCUSSION AND CONCLUSION

Breast cancer is the most widespread cancer in females, and the major public health problem worldwide with increasing incidence and mortality in the world. Among the available treatment options for cancer, chemotherapy is the therapy for treating a diversity of cancer patients [9]. This work aimed to investigate the protective effect of etoposide against EST induced cardiac toxicity in female. In the current study; a significant increase in CPK, LDH, and CK-Mb indicated the cardiac toxicity were detected after the treatments of mice with etoposide as compared with control. This result is in harmony Qusti et al. [28], Noureldeen et al. [29] Our data revealed that induction of EST altered cardiac function, which is indicated by the increased serum levels of potassium and chloride ions and decreased serum level of sodium ions that might be due to EST induced heart tissue injury. These findings were in line with that of Abd Eldaim et al. [27]. The current study revealed that, treatment of EST+ Eto group shows a significant decrease in cardiac function such as CPK, LDH, and CK-Mb compared with EST group on the other hand Na^+ adivulge a slight increase compared with EST group. The present study confirmed anticarcinogenic potential of Etoposide. Further studies are warranted to explore its mode of action and safety for medicinal use in cancer therapy.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Rearing and treatment of mice all over the experimental period were conducted in accordance with the Faculty of Science, Tanta University guide for animal. The study protocol was approved by Institutional Animal Care and Use Committee (approval number: IACUC-SCI-TU-0047).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/56165