Asian Oncology Research Journal

3(2): 42-48, 2020; Article no.AORJ.55393

Therapeutic Impact of Etoposide against Ehrlich Solid Tumor Induced Improvement in Electrolytes, Liver and Kidney Toxicity in Female Mice

Ibrahim El Tantawy El Sayed[1], Ehab Tousson[2] and Manar Atia Sakr[1*]

[1]Department of Chemistry, Faculty of Science, Menoufia University, Egypt.
[2]Department of Zoology, Faculty of Science, Tanta University, Egypt.

Authors' contributions

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

Article Information

Editor(s):
(1) Dr. Bing Yan, Hainan Branch of PLA General Hospital, China.
(2) Dr. Dinithi C. Peiris, University of Sri Jayewardenepura, Sri Lanka.
Reviewers:
(1) Sachiko Ezoe, Osaka University, Japan.
(2) Waseem Hassan, COMSATS University Islamabad, Pakistan.
Complete Peer review History: http://www.sdiarticle4.com/review-history/55393

Original Research Article

Received 08 February 2020
Accepted 15 April 2020
Published 22 April 2020

ABSTRACT

Background and Objective: Breast cancer is the most common type of cancer among women and the second most frequent kind of tumor worldwide. This study aims to find the renal and hepatic protective effect of etoposide against Ehrlich solid tumor induced liver and kidney toxicity in female.
Materials and Methods: A total of 60 albino females' mice were separated into four groups (1st, control group; 2nd, Etoposide group; 3rd, EST group; 4th, EST treated with Etoposide group). EST group divulge changes in liver, kidney function and Electrolytes under study compared with control group.
Results: Our results revealed that; serum urea, creatinine, potassium ions (K+), chloride ions(Cl^{-}), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) have a significant increase in EST group compared with control group. In contrast, serum sodium ions (Na^{+}), calcium ions (Ca^{++}), total protein and albumen have a significant decrease in EST group compared with control group. On the other hand, treatment with Etoposide for 14 days improved these changes in liver and kidney functions.

**Corresponding author: E-mail: manarsakr93@gmail.com;*

Conclusion: The present study confirmed that Etoposide has therapeutic role against liver and kidney toxicity induced by Ehrlich solid tumor. Further studies are warranted to explore its mode of action and safety for medicinal use in liver and kidney therapy during cancer treatments.

Keywords: Ehrlich solid tumor; etoposide; mice; liver and kidney functions.

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 [2-4].

Breast cancer is the most common type of cancer among women and the second most frequent kind of tumor worldwide [5]. Ehrlich solid tumor is used as experimental models of breast cancer [4,6]. 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 [7-9]. 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 [10-12].

Many of research investigated the effect of chemotherapy drugs on normal animal cells and tissues [13-18]. 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 [19,20]. Vepesid or Etopophos and toposar or etoposide phosphate are other names for Etoposide. In some cases, health care professionals may use the trade name VP-16 or other names Vepesid or etopophos or toposar or etoposide phosphate when referring to the generic drug name etoposide [20]. Vepesidis commonly used alone or with another anticancer agent for the treatment of Hodgkin's lymphoma and AID's and sexual organ cancers as testicular, ovarian, uterine, bladder and prostate or for the treatment of other organs as lung and stomach cancer [19]. This study aims to find the renal and hepatic protective effect of etoposide against EST induced liver and kidney toxicity in female.

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

Sixty adult female Swiss albino mice weighing 22-25g were purchased from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Cairo, Egypt). Mice were fed commercial standard pellet diet and supplied tap water *ad libitum*. Rearing and treatment of mice all over the experimental period were conducted in accordance with the Faculty of Science, Tanta University guide for animal, which approved by Institutional Animal Care and Use Committee (IACUC-SCI-TU-0041).

2.3 Induction of Ehrlich Solid Tumor (EST)

Mice with Ehrlich ascites carcinoma (EAC) were purchased from Egyptian National Cancer Institute (NCI; Cairo University, Egypt) and used as source of EAC cells. To initiate solid tumor in the experimental mice, 0.2 ml of acitic fluid were aspirated from EAC bearing mice, diluted with physiological saline and EAC cells were counted. About 2.5 to 3 million of EAC cells were injected subcutaneously in the left thigh of the lower limb of each mouse.

2.4 Experimental Design

After one week of acclimation, a total of 60 female mice were randomly and equally assigned into eight groups, 15 mice each.

2.4.1 First group

Mice were not injected with anything kept as a control.

2.4.2 Second group

Mice were injected with Etoposide (50 mg/kg body weight/twice a week) intraperitoneal for two weeks [19].

2.4.3 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 [4].

2.4.4 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 Etoposidefor another 2 weeks.

2.5 Blood Sampling

At the end of the experimental period, mice were euthanized with intraperitoneal injection of sodium pentobarbital and subjected to a complete necropsy. Blood samples were individually collected from the inferior vena cava of each mouse in non-heparinized glass tubes for estimation of liver and kidney functions biomarkers.

2.6 Liver Function Biomarker

Serum aspartate transaminase (AST) and alanine transaminase (ALT) were estimated in the rat sera according to Moustafa et al. [21] and Bolkiny et al. [22] respectively while alkaline phosphatase (ALP) was estimated in the rat serum according to El-Moghazy et al. [23]. Serum albumin was estimated according to Saggu et al. [24] while serum total proteins level was estimated according to Tousson et al. [25].

2.7 Electrolytes and Kidney Functions Biomarker

Serum urea and creatinine respectively were determined in the rat sera according to Oyouni et al. [26] and Abd-Eldaim et al. [27] respectively. Estimation of the levels of serum electrolytes (Potassium, sodium, calcium and chloride ion) by using commercial kits (Sensa core electrolyte, India) according to El-Masry et al. [28].

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 Liver Function

Table 1 showed the changes in liver functions in different groups under study. Reveal that ALT, AST, and Alp a significantly increase in EST group compared with control group. While Alb and total protein a significantly decrease in EST group compared with control group. Eto group reveal a moderate increase in ALT, AST and Alp while ALB and total protein shows a moderate decrease compared with Control group. EST+Eto group shows a significant decrease in ALT, AST and Alp compared with EST group while Alb and total protein shows a slight increase compared with EST group.

3.2 Changes in Kidney Function

Table 2 showed the changes in kidney function in different groups under study. Divulge that serum urea and creatinine have a noticeable increase in EST group compared with control group. Eto group divulge significant increase in urea and creatinine levels compared with Control group. EST+Eto group shows decrease in serum urea, creatinine compared with EST group on the other hand Na^+ and Ca^{++} divulge a slight increase compared with EST group.

3.3 Changes in Electrolytes

Table 3 showed 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^+ and Ca^{++} have a noticeable decrease in EST group compared with control group. EST group shows that; serum k^+, Na^+ and Cl^- have a moderate increase on the other hand, Ca^{++} have a moderate decrease in EST group compared with control group. EST+Eto group shows decrease in k^+ and Cl^- compared with EST group on the other hand; serum Na^+ and Ca^{++} divulge a slight increase compared with EST group.

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

	ALT (U/l)	AST (U/l)	ALB (gm/dl)	ALP (U/l)	Total protein (gm/dl)
Control	47.2$^{\#}$±2.745	142.8$^{\#}$±2.437	4.404$^{\#}$±0.0432	128.4$^{\#}$±3.614	6.07$^{\#}$±0.044
Eto	72.56$^{*\#}$±2.53	239.8$^{\#*}$ ±9.074	3.864$^{\#*}$ ±0.059	161.6$^{\#*}$ ±4.02	5.69$^{\#}$±0.099
EST	91.0*± 3.51	305.6*±9.277	3.474*±0.147	193*±5.128	5.056*±0.095
EST+Eto	78.1$^{*\#}$± 1.15	143.8$^{\#}$±2.672	3.538*±0.051	137.6$^{\#*}$ ±4.13	5.304$^{*\#}$±0.060

*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 kidney function in different groups under study

	Urea (mg/dl)	Creatinine (mg/dl)
Control	27$^{\#}$±1.332	0.432$^{\#}$±0.037
Eto	53.3$^{*\#}$±2.468	0.936$^{*\#}$±0.032
EST	51.1*±1.952	1.05* ± 0.023
EST+Eto	47.8$^{*\#}$±1.617	0.764$^{\#*}$±0.024

*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)	Ca^{++} (mmol/l)	Cl^{-} (mmol/l)
Control	135.7$^{\#}$±0.611	4.506$^{\#}$±0.079	1.075$^{\#}$±0.039	101.8$^{\#}$±0.723
Eto	138.1$^{\#}$±0.768	5.284$^{\#*}$±0.051	0.919$^{\#*}$±0.007	116* ± 2.28
EST	121.9* ± 1.84	6.566*±0.032	0.859*±0.022	116.8*±1.997
EST+Eto	134. $^{\#}$2±0.772	6.06$^{*\#}$ ± 0.096	0.896$^{*\#}$±0.010	115.7* ± 2.29

*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

Cancer is a class of diseases characterized by out-of-control cell growth. Breast cancer is the commonest females' cancer and the leading cause of cancer death worldwide (1.38 m new cases/year, 23% of all cancers) ranked as fifth cause of death (the first in women) from cancer overall (45800 deaths). Breast cancer is the most widespread cancer in females, and the chief cause of cancer-related mortality in the world. Ehrlich carcinoma is an undifferentiated carcinoma that is originally hyperdiploid, has high transplantable capability, no-regression, rapid proliferation, short life span, 100% malignancy and also does not have tumor-specific transplantation antigen. There are a number of in vivo experimental models based on laboratory animals including the Ehrlich solid tumor (EST), is an undifferentiated solid tumor that derived from the mouse breast adenocarcinoma which is an aggressive and fast growing carcinoma able to develop both in the ascetic or in the solid form depending whether inoculated intraperitoneally or subcutaneously, respectively [6,10]. Among the available treatment options for cancer, chemotherapy is the therapy for treating a diversity of cancer patients [10].

This work aimed to investigate the protective effect of etoposide against EST induced liver and kidney toxicity in female. In the current study; a significant increase in serum ALT, AST, ALP and a significant decrease albumen and total proteins indicated the liver toxicity were detected after the treatments of mice with etoposide as compared with control. This result is in harmony with Gupta et al. [29], and Sakr et al [30]. The recorded increase in the levels of AST, ALT and ALP in serum may be interpreted as a result of liver damage or as changes in membrane permeability indicating the severity of hepatocellular damage by Ehrlich solid tumor. the significant decrease in Serum albumin and total proteins level observed in Ehrlich solid tumor bearing mice correlates with liver damage which reflected in the decreased biosynthetic capabilities of the liver. Our data revealed that induction of EST altered kidneys function, which is indicated by the increased serum levels of urea, creatinine, potassium and chloride ions and decreased serum level of sodium ions that might be due to EST induced renal tissue injury. These findings were in line with that of Eldaim et al. [4] and Aldubayan et al. [10]. Also; our results agree with Aldubayan et al. [10] who reported that;

elevation in kidney functions in the serum of Ehrlich solid tumour (EST). Such increase in blood urea concentration was attributed to the tumor's catabolic effect and the increase in urea production. Serum urea and creatinine level elevation in clinical experiments means renal dysfunction. The current study revealed that, treatment of EST+Eto group shows a significant decrease in liver function such as ALT, AST and ALP compared with EST group while Alb and total protein shows a slight increase compared with EST group.

Whereas in kidney function we found that EST+Eto group shows decrease in urea, creatinine, k^+ and Cl^- compared with EST group on the other hand Na^+ and Ca^{++} divulge 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.

5. CONCLUSION

Although the many side effects of chemotherapy during the cancer treatments; Etoposide improved the changes in liver and kidney functions after Ehrlich solid tumor. Our study revealed that Etoposide has therapeutic role against liver and kidney toxicity induced by Ehrlich solid tumor. Further studies are warranted to explore its mode of action and safety for medicinal use in liver and kidney therapy during cancer treatments.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre, Giza, Egypt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. DeSantis C, Ma J, Bryan L and Jemal A. Breast cancer statistics, CA: A Cancer Journal for Clinicians. 2014;64(1):52-62.
2. Knijnenburg TA, Wang L, Zimmermann MT, Chambwe N, Gao GF, Cherniack AD, Fan H, Shen H, Way GP, Greene CS, Liu Y. Genomic and molecular landscape of DNA damage repair deficiency across The Cancer Genome Atlas. Cell Reports. 2018; 23(1):239-54.
3. El-Atrsh A, Tousson E, Elnahas EE, Massoud A, Al-Zubaidi M. Ameliorative effects of spirulina and chamomile aqueous extract against mice bearing ehrlich solid tumor induced apoptosis. Asian Oncology Research Journal. 2019;2(1):1-17.
4. Eldaim MA, Tousson E, El Sayed IE, El AE, Elsharkawy HN. Grape seeds proanthocyanidin extract ameliorates Ehrlich solid tumor induced renal tissue and DNA damage in mice. Biomedicine & Pharmacotherapy. 2019; 115:108908.
5. Siegel R, DeSantis C, Jemal A. Colorectal cancer statistics. CA: A Cancer Journal for Clinicians. 2014;64(2):104-17.
6. El-Keey MM, El Ghonamy MA, Ali TM, Ibrahim WM, Tousson E. Effect of sulforaphane and methotrexate combined treatment on histone deacetylase activity in solid ehrlich carcinoma. Journal of Bioscience and Applied Research. 2017;3 (3):62-69.
7. Mishra S, Tamta AK, Sarikhani M, Desingu PA, Kizkekra SM, Pandit AS, Kumar S, Khan D, Raghavan SC, Sundaresan NR. Subcutaneous Ehrlich ascites carcinoma mice model for studying cancer-induced cardiomyopathy. Scientific Reports. 2018;8 (1):1-1.
8. Mutar TF, Tousson E, Hafez E, Abo Gazia M, Salem SB. Ameliorative effects of vitamin B17 on the kidney against Ehrlich ascites carcinoma induced renal toxicity in mice. Environmental Toxicology. 2019;1-10.
9. Tousson E, Hafez E, Gazia MM, Salem SB, Mutar TF. Hepatic ameliorative role of vitamin B17 against Ehrlich ascites carcinoma–induced liver toxicity. Environmental Science and Pollution Research. 2020;27:9236–9246
10. Aldubayan MA, Elgharabawy RM, Ahmed AS, Tousson E. Antineoplastic activity and curative role of avenanthramides against the growth of ehrlich solid tumors in mice. Oxidative Medicine and Cellular Longevity. 2019;12.
[ID: 5162687]
Available:https://doi.org/10.1155/2019/5162687

11. El-Masry TA, Al-Shaalan NH, Tousson E, Buabeid M, Alyousef AM. The therapeutic and antineoplastic effects of vitamin B17 against the growth of solid-form Ehrlich tumours and the associated changes in oxidative stress, DNA damage, apoptosis and proliferation in mice. Pak. J. Pharm. Sci. 2019;32(6):2801-10.
12. El-Masry T, Al-Shaalan N, Tousson E, Buabeid M, Al-Ghadeer A. Potential therapy of vitamin B17 against Ehrlich solid tumor induced changes in Interferon gamma, Nuclear factor kappa B, DNA fragmentation, Bcl2, survivin, VEGF and TNF-α Expressions in mice. Pak. J. Pharm. Sci. 2020;33(1):393-401.
13. Basuony M, Hafez E, Tousson E, Massoud A, Elsomkhraty S, Eldakamawy S. Beneficial role of *Panax ginseng* root aqueous extract against Cisplatin induced blood toxicity in rats. Am J Biol Chem. 2015;3(1):1-7.
14. Al-Rasheed NM, El-Masry TA, Tousson E, Hassan H, Al-Ghadeer A. Hepatic protective effect of grape seed proanthocyanidin extract against Gleevec-induced apoptosis, liver Injury and Ki67 alterations in rats. Braz J Pharm Sci. 2018; 54(2):e17391. DOI:org/10.1590/s2175-97902018000217391
15. Al-Rasheed NM, El-Masry TA, Tousson E, Hassan HM, Al-Ghadeer A. Protective potential of grape seed proanthocyandins extract against glivec (*Imatinib mesylate*) induced liver toxicity and oxidative Stress in male rats. Annual Research & Review in Biology. 2017;20(6):1-9.
16. Tousson E, Hafez E, Masoud A, Hassan AA. Abrogation by curcumin on testicular toxicity induced by cisplatin in rats. J Cancer Res Treat. 2014;2(3):64-8.
17. Tousson E, Hafez E, Zaki S, Gad A. P53, Bcl-2 and CD68 expression in response to amethopterin-induced lung injury and ameliorating role of l-carnitine. Biomedicine & Pharmacotherapy. 2014;68(5):631-639.
18. Tousson E, Hafez E, Zaki S, Gad A. The cardioprotective effects of L-carnitine on rat cardiac injury, apoptosis and oxidative stress caused by amethopterin. Environmental Science and Pollution Research. 2016;23(20):20600-20608.
19. Tousson E, Bayomy MF, Ahmed AA. Rosemary extract modulates fertility potential, DNA fragmentation, injury, KI67 and P53 alterations induced by etoposide in rat testes. Biomedicine & Pharmacotherapy. 2018 Feb 1;98:769-74.
20. 20 Almakhatreh M, Hafez E, Tousson E, Masoud A. Biochemical and Molecular Studies on the Role of Rosemary (Rosmarinus officinalis) Extract in Reducing Liver and Kidney Toxicity Due to Etoposide in Male Rats. *Asian Journal of Research in Medical and Pharmaceutical Sciences*, 2019; 7 (4): 1-11.
21. Moustafa AH, Ali EM, Moselhey SS, Tousson E, El-Said KS. Effect of coriander on thioacetamide-induced hepatotoxicity in rats. Toxicology and industrial health. 2014 Aug; 30(7):621-9.
22. Bolkiny Y, Tousson E, El-Atrsh A, Akela M, Farg E. (2019) Costus Root Extract Alleviates Blood Biochemical Derangements of Experimentally-Induced Hypo-and Hyperthyroidism in Mice. *Annual* Research & Review in Biology. 2019; 31(5):1-0.
23. El-Moghazy M, Zedan NS, El-Atrsh AM, El-Gogary M, Tousson E. The possible effect of diets containing fish oil (omega-3) on hematological, biochemical and histopathogical alterations of rabbit liver and kidney. Biomedicine & Preventive Nutrition. 2014;4(3):371-7.
24. Saggu S, Sakeran MI, Zidan N, Tousson E, Mohan A, Rehman H. Ameliorating effect of chicory (*Chichorium intybus* L.) fruit extract against 4-tert-octylphenol induced liver injury and oxidative stress in male rats. Food and Chemical Toxicology. 2014;72:138-146.
25. Tousson E, El-Moghazy M, Massoud A, El-Atrash A, Sweef O, Akel A. Physiological and biochemical changes after boldenone injection in adult rabbits. Toxicology and Industrial Health. 2016;32(1):177-82.
26. Oyouni AA, Saggu S, Tousson E, Rehman H. Immunosuppressant drug tacrolimus induced mitochondrial nephrotoxicity, modified PCNA and Bcl-2 expression attenuated by *Ocimum basilicum* L. in CD1 mice. Toxicology Reports. 2018;5:687-694.
27. Abd Eldaim MA, Tousson E, El Sayed IE, Awd WM. Ameliorative effects of *Saussurea lappa* root aqueous extract against Ethephon-induced reproductive toxicity in male rats. Environmental toxicology. 2019;34(2):150-159.
28. El-Masry TA, Al-Shaalan NH, Tousson E, El-Morshedy K, Al-Ghadeer A. P53 expression in response to equigan induced testicular injury and oxidative stress in

male rat and the possible prophylactic effect of star anise extracts. Annual Research & Review in Biology. 2017;14(1): 1-8.

29. Gupta M, Mazumder UK, Kumar RS, Sivakumar T, Vamis ML. Antitumor activity and antioxidant status of *Caesalpinia bunducella* against ehrlich ascites carcinoma in Swis albino mice. Journal of pharmacological Science. 2004;94:177–184.

30. Sakr SA, Badr OM, Abd-Eltawab HM. Ameliorative effect of saffron extract on mice bearing solid tumors. ISESCO Journal of Science and Technology. 2011; 12(22):60-70.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/55393