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Is Amygdalin Outcomes Weighing Detriments of Sorafenib Treatment In Female Mice With Kidney Injury Induced By Ehrlich Ascites Carcinoma Model? Preliminary study

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ABSTRACT

Nowadays, attention is intensifying in using natural sources of real antitumor agents that breed fewer side effects than unadventurous chemotherapeutic drugs. The Ehrlich ascites carcinoma (EAC) model is a spontaneous adenocarcinoma that can use to induce cancer in the targeted organ. This preliminary study is implemented to weigh the best choice for cancer treatment with fewer side effects and rule out the concomitant use of targeted drugs against kidney injury in female mice induced by EAC. The mice were apportioned into seven groups. The most affected parameters in the EAC mice model as Kidney functions and hematological parameters were measured. Furthermore, to exact unearth the values of two drugs, histopathological studies were further evaluated. The results showed that the mice with EAC exhibited kidney functions with alterations of hematological parameters. Co-administration of vitamin B17 and sorafenib restored the kidney functions in serum. Ample histopathological variations were detected in kidney sections in EAC as marked damage and degenerated glomerular atrophy and necrosis of renal corpuscle and tubules. A moderate improvement in kidney sections noted in sorafenib (SOR) group as a mild degeneration in both renal corpuscles and renal tubules in addition to congestion of renal blood vessels were improved contrary to B17 group that improved better than SOR. It could be concluded that B17 has a potential defensive role against EAC cells-induced

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kidney toxicity rather than the undesirable effect of SOR in the tested parameters.

1. Introduction

Cancer is caused by an imbalance in the rate of proliferation and apoptosis or cell death and the positive effect of anticancer therapy is taken into consideration when its ability to initiate apoptosis in cancer cells [1]. Ehrlich ascites carcinoma (EAC) is one of the experimental breast tumor-derived from spontaneous mouse adenocarcinoma. EAC is referred to as an undifferentiated carcinoma, and is originally hyperdiploid, has the high transplantable capability, no-regression, rapid proliferation, shorter life span, 100% malignancy [2]. The Ehrlich ascitic tumor implantation induces per se a local inflammatory reaction, with increased vascular permeability which results in an intense edema formation, cellular migration, a progressive ascitic fluid formation which is essential for tumor growth since it constitutes a direct nutritional source for tumor cells [3].

In up-to-date medicine, 3 methods are largely designed for cancer treatment; chemotherapy, radiotherapy, and surgery. In our time, chemotherapy has been alleged to be the best actual therapy [4]. The main principle of chemotherapy, which serves as a drug treatment in cancer, is to avert the progress and metastasis of malignant cells. In concern, the aim is to deliver a disastrous effect of the used drugs to tumor progression. Furthermore, this effect is aimed to be specific for only malign cells [5]. However, all the used drugs for cancer therapy are not specific to cancer cells, in that they do not only affect the proliferated cells, but also the normal cells for instance sorafenib drug [5]. Therefore, all cancer therapeutics are toxic and their dosage-response curves are upright.

In this context, the (Scientists' studies about cancer therapy have engrossed in the idyllic drug being ineffective or minimally effective for normal cells. At this instant, the usage of natural sources is thought to have a great value for cancer control and programs' destruction [6].

Nature gives a great deal of effective anti-cancer agents such as amygdalin (Vit. B17) [7]. Several studies were reported to stimulate the immune system in different pathways. Into the bargain, B17 increased specific cellular and humoral immune responses [8]. Moreover, there is a growing trend for herbal drugs because of low toxicity and high medical effectiveness against cancer for example kidney cancer [9].

Accordingly, this study aims to evaluate the therapeutic nature of sorafenib versus amygdalin (B17) as a first-line treatment in female mice with kidney injury induced by the EAC model.

2. Materials and Methods

2.1 Reagents and chemicals:

Vitamin B17 was procured from Sigma-Aldrich, USA. Sorafenib was obtained from BAYER Company. All other chemicals and reagents used in the study were of analytical grade and were bought from local suppliers (Biomed, Egypt).

2.2 Experimental animals:

The experiment was carried out on 42 female swiss albino mice weighing 16– 21 g. Mice were kept inconsistent environments. The Mice

were set aside at a precise temperature. The animals were provided with a normal diet and water *ad libtium* during the period of the experiment.

2.3 Experimental design:

Forty-two mice were injected with EAC-cells (a million cells of EAC/mouse) and then separated into 6 groups (Gp, n=6) as follow:

Gp1: (Negative control group): Mice were injected with normal saline (0.9 % w/v, 300 µl/mouse intraperitoneal (i.p.) for 14 days.

Gp2: (EAC- bearing positive control group): mice inoculated with tumor cells alone, Mice were injected with EAC cells (300 µl of $2.0x10^6$ cells /mouse (i.p.) on the day "0".

Gp3 :(EAC-bearing group treated with B17): mice were inoculated with EAC and then treated with amygdalin (300 mg/ kg mouse) daily for 14 days.

Gp4: (EAC-bearing group treated with sorafenib IP): mice were inoculated with EAC, as in Gp.2, and then treated with sorafenib (30 mg/ kg mouse) (Intraperitoneal) daily for 14 days.

Gp5: (EAC-bearing group treated with B17 and sorafenib IP): mice were inoculated with EAC and then co-treated with amygdalin and sorafenib (Intraperitoneal) daily for 14 days.

Gp6 :(EAC-bearing group treated with sorafenib oral): mice were inoculated with EAC and then co-treated with amygdalin and sorafenib (oral) daily for 14 days.

Gp7 :(EAC-bearing group treated with B17 and sorafenib oral): mice were inoculated with EAC and then were treated with sorafenib (30 mg/ kg mouse) (Oral) daily for 14 days.

2.4 EAC Cell model:

EAC was attained from the Cancer Biology Unit (CBU), Cairo, upheld and propagated by serial transplantation (i.p) in an aseptic environment. $2x10^6$ viable EAC cells were injected i.p into each mouse in an aseptic condition and the day of tumor inoculation was considered as day 0 [10]. After 24 hours of inoculations, mice were treated with appropriate doses of amygdalin and sorafenib or both for 14 consecutive days. By day 14 mice were euthanized to investigate biochemical, hematological, and histopathological findings.

2.5 Blood samples collection:

Blood samples were collected from mice into the heparinized tubes and mixed well to avoid clot formation, blood specimens were stored refrigerated (at 4C) until used. The rest of the blood was collected in glass tubes for coagulation and serum formation, blood was permitted to sit for 30 minutes at 4C to clot and then centrifuged for 10 minutes at 3000 rpm at room temperature.

2.6 Determination of kidney functions

The level of B. urea was analyzed by using a commercial kit that was purchased from diamond, Egypt. The method used to determine urea was Patton et al. [11]. S. Creatinine concentration was evaluated according to the method of Larsen and Knud [12].

2.7 Hematological assays

The Sysmex® automated hematology analyzer XK-21N (Sysmex Corporation, Kobe 651-0073, and Japan) was used for the assay of hemoglobin (Hb)

concentration, count of Red blood cells, and platelets.

2.8 Tissue samples

Mice were euthanized, kidneys tissues were directly obtained after separation, washed in ice-cold saline, and dried on filter paper then weighted and partly placed in 10 % buffered formalin for histopathology and the rest was homogenized in phosphate buffer (PH 7.4) and frozen at –20^{o}C until assayed.

2.9 Histopathological investigation of kidney tissues

Instantaneously afterward dissection, tissues were detached and fixed in 10% neutral buffered formalin. Freshly isolated tumor from different groups was stained using routine hematoxylin and eosin counterstain method [13].

2.10 Statistical analysis

Statistical Analyses were evaluated using GraphPad Prism 5.0 Software. The experimental results were expressed as the mean ± the standard error means (SEM). Data were calculated by one-way analysis of variance (ANOVA) followed by the Tukey test for multiple comparisons test. Values for which P < 0.05 were considered statistically significant.

3. Results

3.1 Effect of B17 and/or sorafenib treatment on kidney functions in different mice groups.

Creatinine and urea in serum were significantly increased in Gp2 (P<0.0001) as Gp1. In the case of Gp4 (P < 0.01), and EAC/B17/SOR Oral (P<0.01) indicated a significant improvement in creatinine concentration in serum when compared to EAC bearing positive control group. EAC/SOR Oral (P<0.0001) and (EAC/B17/SOR IP) (P<0.0001) showed better significant improvement (decrease) in serum creatinine concentration when compared to EAC bearing positive control group and other groups as shown in **Table (1)**. The results revealed that the concomitant administration of SOR and B17 could improve the kidney functions rather than the administration of B17 alone i.e.: *B17 alone could not neutralize the effect of EAC on kidney injury.*

3.2 Effect of B17 and/or sorafenib treatment on hematological profile in different mice group.

3.2.1 Effect on Hb and RBCs

Cancer-bearing animals showed a significant reduction in red blood corpuscles (RBCs) count (7.37±0.26 X I0$^{\wedge 6}$ cells/µl) and the level of hemoglobin (Hb) (10.05±0.36 g/dl) with significant P-value (0.001) and (0.0001) respectively. The Group EAC/SOR oral animals showed a remarkable decrease in RBC count (5.52±0.39 X 10$^{\wedge 6}$ cells/mm^3) and Hb levels (8.30±0.22 g %) (P< 0.0001) as compared with EAC bearing mice as shown in **Figure (1)** and **(2)**. From these results, we subtract that the negative effect of SOR was raised contrary to B17 which improves the results near to the negative control group.

3.2.2 Effect on Platelets

The effects of SOF and B17 on platelet counts were recorded in **Figure (3)**. The EAC-bearing mice showed a marked increase in platelet count (669.0±10.06) with P<0.0001 if compared with the normal mice group. Also, a marked decrease was revealed after treatment in the groups with B17 and SOR either oral or IP relative to EAC bearing positive control group. However, the

marked decrease was in favor of EAC/B17/SOR Oral.

3.3 Effect of B17 and/or sorafenib treatment on kidney histochemistry

In the normal control group, the histopathological examination showed normal renal corpuscles, proximal and distal convoluted tubules as shown in **Figure 4 (A)**. EAC-bearing mice showed large aggregation of pleomorphic, darkly basophilic cells surrounding congested blood vessels and renal corpuscles as shown in **Figure 4 (B)**. In EAC/B17 group, the sections showed small aggregations of pleomorphic, darkly basophilic cells surrounding the blood vessels and renal corpuscles, mild degeneration in both renal corpuscles and renal tubules as shown in **Figure 4 (C)**. In kidney of EAC/ sorafenib (IP) showing small aggregations of diffuse pleomorphic, darkly basophilic cells inside the renal parenchyma (tailed arrow), mild degeneration in both renal corpuscles (arrowhead) and renal tubules as shown in **Figure 4 (D)**. According to EAC/B17/sorafenib IP group showed a small focus of diffuse pleomorphic, darkly basophilic cells inside the renal parenchyma, and mild degeneration in both renal corpuscles and renal tubules in addition to congestion of renal blood vessels as shown in **Figure 4 (E)**. kidney of EAC/sorafenib oral showing small foci of diffuse pleomorphic, darkly basophilic cells inside the renal parenchyma, degenerative changes in renal corpuscles and renal tubules as shown in **Figure 4 (F)**. In EAC/B17/sorafenib oral showing presence of diffuse pleomorphic, darkly basophilic cells inside the renal parenchyma, necrosis in renal tubules, and shrinkage of renal glomeruli as shown in **Figure 4 (G)**.

4. Discussion

Cancer is a genetic msease of cells characterized by a shift in the control mechanism that rules cell proliferation and differentiation [14]. Cancer chemotherapy is the most promising of current methods of treatment. The antitumor chemotherapeutic drugs (sorafenib), while producing their beneficial effects unfortunately produce undesired adverse side effects. Hence, the current trend to overcome these undesirable effects was raised globally [15].

On one hand, amygdalin (B17) is an effective anticancer chemotherapeutic agent, which is used against a variety of malignant tumors [16]. On another hand, EAC is a rapidly growing carcinoma with very aggressive behavior and can grow in almost all strains of mice [17].

In the current preliminary study, our data publicized that S. creatinine and B. urea concentration in serum (mg/dl) was significantly increased in EAC bearing positive control group ($P < 0.001$) as compared to a normal control group. However, insignificant results were found in the groups treated with B17, SOR IP, or combination with B17 administration as compared with the control group. This is concordant with Abd Eldaim *et al*., (2019) who reported that EAC has led to kidney injury and significant fluctuations in the renal function in mice through an increase in the levels of B. urea, S. creatinine, potassium, and chloride ions and decreased sodium ions [18]. On the other hand, the presence of B17 in treated groups leads to ameliorative levels of B. urea and S. creatinine,

our results correspond with Chang *et al*., (2020) who reported that amygdalin inhibits renal fibrosis and ameliorate the biochemical parameters in chronic kidney disease [19]. Moreover, Shi *et al*., (2019) found that B17 inhibits the development of kidney cell carcinoma cells *in vitro* [20].

In the case of sorafenib treatment I.P, the preliminary study found that an improvement in kidney functions was noticed. The improvements came higher in SOR I.P that give a good impression to shed the light on how the SOR I.P mechanism is and may change the future of SOR administration to decrease the mortality of cancer. To enhance these improvements, the coadministration of B17/SOR I.P was applied and the outcomes were noted as the S. urea and S. creatinine were clinically improved. In this context, indeed there is no difference between sorafenib and B17 to determine which first-line treatment is the safest.

According to the studies on hematological profiles, and in cancer chemotherapy, the chief complications encountered; are myelosuppression and anemia [21]. So, our results revealed that the EAC group disclosed a reduction in hemoglobin and RBC count. This may be due to excessive hemolysis and iron deficiency. Both B17 and SOR I.P, oral administration enriched the hemoglobin and RBCs count of tumor-bearing animals. One explanation for that B17 might have facilitated the synthesis of hemoglobin or prevented hemolysis rather than sorafenib. The results thus suggest that the B17 is capable of protecting the hemopoietin system [22]. At this point, the B17 is considered the first-line treatment in targeting the decrease of undesirable effects on the hemopoietin system contrary to sorafenib treatment.

The histopathological changes in the kidney structure in the study groups revealed that the normal structure of the glomeruli and renal tubules was observed in the control group and treated mice with B17. Kidney sections in inoculated mice with EAC revealed variable pathological changes in glomeruli and renal tubules as marked damage and degenerated to the renal tissues, glomerular atrophy. The cotreated EAC/B17 group showed an enhancement and arrangement of the kidney structure when compared to the EAC group. On contrary, the treated group by SOR either I.P or Oral showed the same results as a mild improvement to the renal tissue. Our histopathological findings are in partial agreement with a study held by Stavniichuk *et al.*, (2020) who use a sorafenib induced model of kidney injury who revealed that rats administered sorafenib with a dose (20 mg/kg per day) in combination with a high sodium diet develop marked 4-fold higher glomerular injury characterized by glomerular sclerosis, mesangiolysis, and glomerular capillary injury [23]. Furthermore, our earlier study published to explore the effect of B17/SOR on liver pathology showed the same results in histopathology of kidneys according to SOR treatment [24].

Conclusion

At this point, we can assume that sorafenib in large dose (p.o.) can be used as a model to induce kidney injury as a result of the marked degeneration in renal corpuscles and renal tubules in addition to small aggregations of diffuse pleomorphic, darkly basophilic cells inside the renal parenchyma and this

considered a significant clue to weigh the merits of B17 treatment and it could be used as a first-line for kidney treatment according to a preliminary study. According to the good outcomes of SOR I.P administration, it can change the future of SOR by modifying the formulation and its administration route of SOR. As a result, it could be concluded that B17 has a potential defensive role against EAC cells-induced kidney toxicity rather than the undesirable effect of SOR in the tested parameters. Again, this is a way for kidneys injury induction model, so another study will be underway for in-depth investigations as per B17/SOR outcomes in case of kidney injury.

5. References

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Table1: Impact of B17 and sorafenib on kidney injury induced by EAC**.**

Groups	B. Urea (mg/dl) (Mean±SEM)	S. Creatinine (mg/dl) (Mean±SEM)
Control (Gp1)	25.75±1.05[a]	0.60±3.27[a]
EAC (Gp2)	81.50±4.51[a,b,c]	1.7±4.72[a,b,c]
EAC / B17 (Gp3)	79.50±2.11[a]	1.38±3.20[a]
EAC / SOR IP (Gp4)	49.00±0.58[a,b]	1.00±1.90[a,b]
EAC / SOR Oral (Gp5)	63.50±3.49[a,b,c]	0.89±2.31[a,b,c]
EAC/B17/SOR IP (Gp6)	39.00±1.28[a,b,c]	0.75±4.05[a,b,c]
EAC/B17/SOR Oral (Gp7)	48.25±1.38[a,b,c]	0.85±2.49[a,b,c]

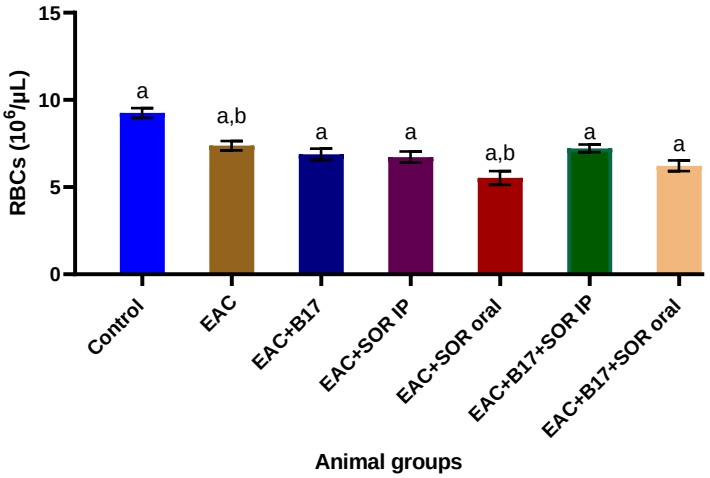

Fig. (1): RBCs count of control and different mice groups.

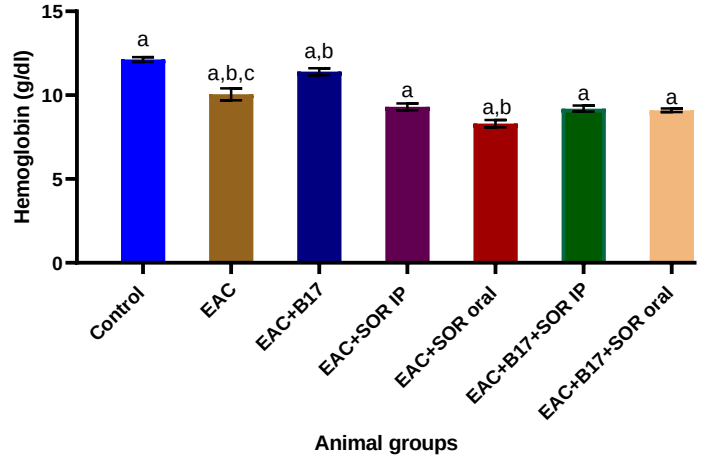

Fig. (2): Hb concentration of control and different mice groups.

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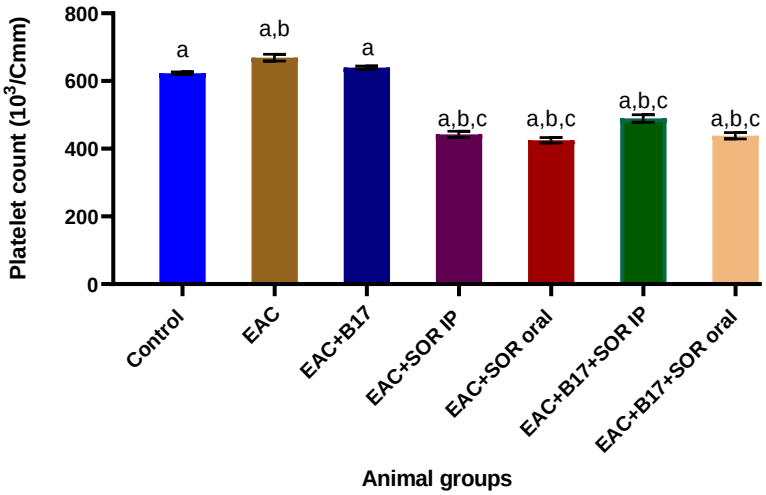

Fig. (3): Platelets count of control and different mice groups.

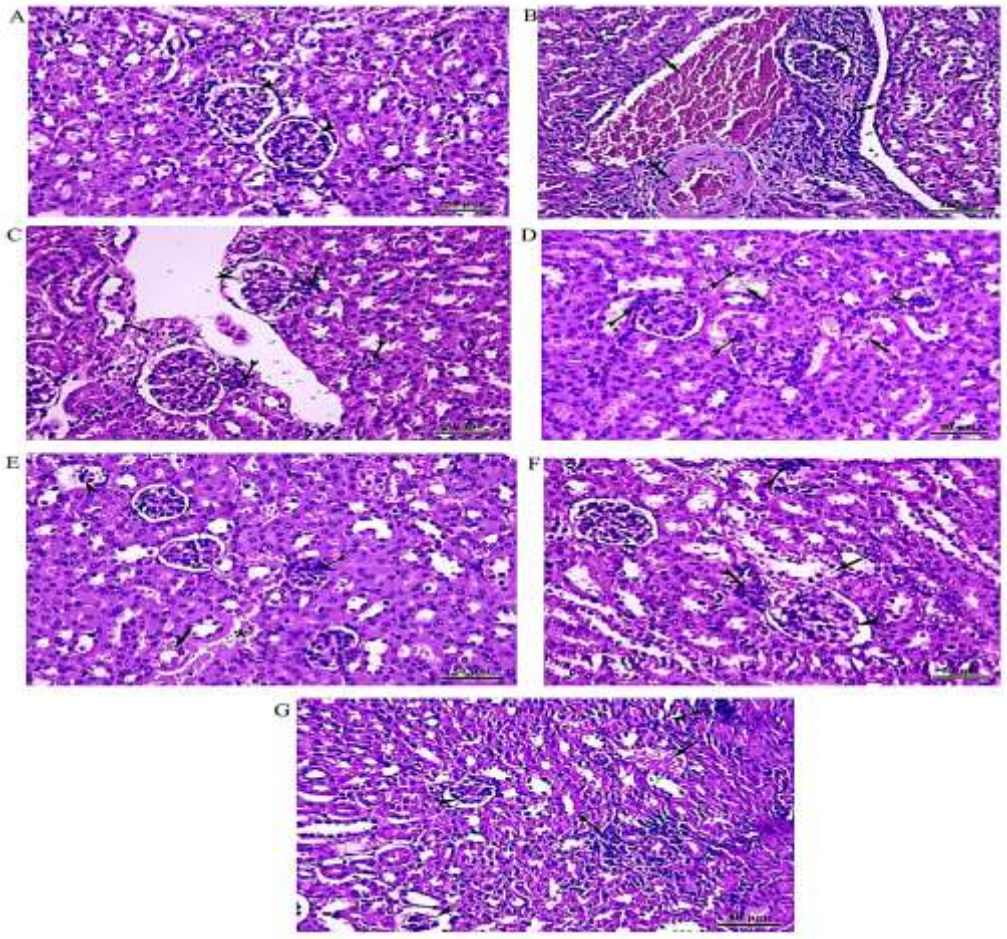

Fig. (4): Histopathological examination in different mice groups