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Original Article

Investigating the anticancer effect of a new drug originating from plant and animal: In vitro and in vivo study

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ABSTRACT

In this study, a new combination drug (BH) from herbal and animal materials was designed and its anticancer activity was examined in experimental and animal models. The animal and herbal sections of drug were originated from Bee, date syrup as well as grape syrup, respectively. After drug preparation, anticancer (Human Colon Cancer (CaCO2) Cell Line), anticoagulant, and hemolysis activity were evaluated by MTT, coagulometer, and RDA methods, respectively. Two different doses of the drug were tested on rats with colon tumor in the 18-day period by determination of the size and weight of the tumor. The mice were killed after 18 days and their three important tissues (heart, kidney, and liver) were histologically evaluated. The drug has shown an effective anticancer effect on cancer cells, so that this drug completely prevents the growth of cancer cells at a low concentration within 72 hours and the percentage of live cells approximately reached zero after 72 hours. This drug has a more limited effect on normal cells with proper selectivity (Toxicity on normal cells was 32%). The study of hemolysis activity on red blood cells showed that the drug had no hemolytic activity on RBCs. This drug did not effect on the coagulation system (PT and PTT pathways). The extent of the drug's ability to differentiate between cancerous and normal cells was almost constant at a time interval of 24, 48, and 72 hours. In treatment group, the weight and size of the tumor significantly decreased compared with the control group. However, there was no significant difference in comparison with the chemotherapy drug, but even in this group, the tumor size and weight reduction was higher in the new drug group. Histological data showed that both the new drug and the 5FU drug resulted in necrosis of the tumor cells. Necrosis was 75% for 5FU and 90% for the new drug. The new drug has no symptoms on the normal tissues of the body. In general, this drug can distinguish between cancerous and normal cells with a specific mechanism, and its formula is patterned from traditional medicine during extensive research. This drug can introduce as new complementary drug for cancer treatment.

Keywords: Anticancer, Toxicity, Drug, Bee, Tumor

Introduction

Cancer is one of the main causes of death in the world. Annually, many people die because of cancer around the world [1]. There are many publications for development of new strategies for cancer treatment. Different therapies such as surgery, chemotherapy, radiotherapy, and hormone therapy are used to treat a variety of cancers [2-4]. All strategies have different side effects on normal cells [5]. So researchers are looking for new approaches for cancer treatment. In the meantime, natural compounds can be very promising for enhancement of treatment efficacy and reduction of side effects. Plants are the potential source of drug combinations with positive effects and low side effects. Today, the use of plant extracts and pure chemicals extracted from the plant are used after laboratory tests in the treatment of diseases [6-8]. Date and Grape syrup are products acquired from dates and Grape in Iranian industrial foods [9-11]. Hemolymph is a fluid, analogous to

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Abbas Mokarramat-Yazdi, Hossein Soltaninejad, Hadi Zare-Zardini, Fatemeh Shishehbor, Ashraf Alemi, Farzaneh Fesahat, Fatemeh Sadeghian. Investigating the anticancer effect of a new drug originating from plant and animal: In vitro and in vivo study. J Adv Pharm Edu Res 2020;10(S2):72-78.
Source of Support: Nil, Conflict of Interest: None declared.

the blood in vertebrates that circulates in the interior of the arthropod body remaining in direct contact with the animal's tissues [12, 13]. Many insect have toxic and valuable compounds in their hemolymph [14, 15]. These agents have potent biological activities such as anticoagulant, antibacterial, antiviral, antifungal, and etc. Hemolymph from adult bees has been analyzed in immunological, nutritional, mycological, proteomic, epigenetic, and biochemical research [16, 17]. In Iranian traditional medicine Bee, Date, and Grape have a special place in traditional medicine. Based on this data, in this study, we extracted and fractionated all compounds of bee hemolymph. This extraction was mixed by suitable concentration of Date and Grape syrup. Then, anticancer activity was examined in experimental and animal conditions.

Materials and Methods

New agent named BH was purified and identified from Bee hemolymph by gel filtration and Q-sepharose chromatography. Anticancer and toxicity of these new combined drug was determined by MTT [3-(4, 5-dimethyl-2-thiazolyl) -2, 5-diphenyl-2H- tetrazolium bromide] assay on Human Colon Cancer (CaCO2) and human mesenchymal stem cells (MSCs) cell lines, respectively. For the assessment of hemolytic activity, 5 ml of the human blood from healthy volunteers was collected in a heparinized tube. The hemolysis assay of this drug was performed by two methods: Radial Diffusion Assay (RDA) and spectrophotometric assay. Effect of new drug was evaluated on blood coagulation system by determination of Partial Thrombin Time (PTT) and Prothrombin Time (PT). This experiment was done on *a* blood sample was taken from healthy volunteers with normal physical activity. For evaluation of PTT, 10 µl from serial dilution of drug was added to 90 µl of human plasma and all samples were incubated for 5 min at 37 °C. Then, 100 µL of calcium chloride solution (CCl4) was added and the sample was placed into the STAGO instrument for clotting time determination. Control was plasma treated by Na2SO4/0.2M HAc/NaAc solution. The experiment was done triplicate. For evaluation of PT, 10 µl from serial dilution of nanostructures was also added to 90 µl of human plasma and all samples were incubated for 5 min at 37 °C. After this time, 200 µL of clotting factors were added to tubes and the sample was placed into the STAGO instrument for clotting time determination. Control was plasma treated by Na2SO4/0.2M HAc/NaAc solution. The experiment was done triplicate. We also examined this drug in animal model. Two different doses of the drug were tested on rats with colon tumor in the 18-day period. The drug was given to mice twice a day in the morning and in the evening. To compare the effect of the drug, one group received no drug as a control and one group was treated with the equivalent dose of 5-Fluorouracil (5FU). Then, the size and weight of the tumor was evaluated. The mice were killed after 18 days and their different tissues (Heart, kidney and liver) were prepared for histological examination.

Results and Discussion

The result of anticancer activity of new designed drug (BH) was summarized in table 1. As shown in this table, new drug has potent anticancer activity on cancerous cell line. This drug had low toxicity on normal cell lines. The cell lines were treated with three different concentrations of new drug for 48 and 72h. The mean percentage of live cells was presented in Table 1. Overall, CaCO2cells were more vulnerable to all concentrations of drug than MSCs cell lines. According to the results, 72 hours treatment with highest concentration had the highest anticancer activity among all times and concentrations. There is similar data for bee venom on proliferation of different cancer cells such as breast, gastric cells [18-20]. The results of determining the size and weight of the tumor showed that both the weight and size of the tumor in the treated group with the new drug decreased significantly compared with the control group (Figure 1 and 2). The reduction of size and weight of the tumor was also higher than 5FU group; however, there was no significant difference. The study of hemolysis activity on red blood cells also showed that the drug does not have hemolysis activity on the blood cells (Figure 3). This drug did not affect the coagulation system (PT and PTT pathways) (Figure 4). There are many researches about drug induced hemolysis as one important frequent complications associated with chemotherapy drug. Direct interaction of these drugs with erythrocyte membrane leads to hemolysis [21, 22]. Unlike chemotherapy drugs, our new drug had no hemolysis activity. This is one of the strengths of the new drug. Increase in thromboembolic phenomena after chemotherapy has been described in cancer patients. Increases in fibrinopeptide A levels, changes in plasmatic coagulation and fibrinolysis, decrease in fibrinolytic activity, and increase in functional plasminogen activator inhibitor were described in various studies as the reasons for the effect of chemotherapy on the coagulation system [23, 24]. Our new drug had no effects on intrinsic and extrinsic pathway. Histopathological data ewer summarized in figure 5. For kidney, results showed that glomerular atrophy, dilatation of Bowman's capsule, and mild hyaline degeneration of tubules was observed in 5FU group. In high dose of our drug, only mild hyaline degeneration was observed. Portal inflammation including lymphocytes and neutrophils and hydropic degeneration of liver cells was observed in 5FU group. For high dose of our drug, congestion of portal vein and central vein was observed. For cardiac tissue, disorganization and degeneration in myocardial fibrosis with separation of myofibril was observed in 5FU group. In high dose of our drug, only mild edema in interstitium was observed. Based on these data, the toxicity of our drug on normal tissues was lower than 5FU. Different studies showed that 5FU has different severe side effects such as increased risk of getting an infection, breathlessness and looking pale, bruising, bleeding gums or nosebleeds, tiredness and weakness (fatigue) during and after treatment, diarrhea, feeling or being sick, kidney damage, heart problems, mouth sores and ulcers, soreness, redness and peeling on palms and

soles of feet, allergic reaction, changes to your hearing, periods stopping, loss of appetite, numbness or tingling in fingers and toes, loss of taste or a metallic taste in your mouth, hair thinning, brittle, chipped and ridged nails, skin sensitivity to sunlight, watery or sore eyes, liver changes, darkened skin, confusion or unsteadiness, eyes moving quickly from side to side, low blood pressure, Headaches, and etc. [25-33]. Our result was also showed the severe side effects of 5FU on heart, kidney, and liver tissues. In comparison of 5FU, our new drug has no these side effects on normal cells and tissues. Histologic results also indicated that both the new drug and the 5FU resulted in necrosis of the tumor cells. Necrosis was 75% for 5FU and 90% for the new drug. The percentage of necrosis in new drug was higher than 5FU (Figure 6).

Conclusion

Based on the study, new reported drug can distinguish between cancerous and normal cells with a specific mechanism, and its formula is patterned from traditional medicine during extensive research. This drug can introduce as new complementary drug for cancer treatment.

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Table 1. The effect of drug in three different concentrations on cancerous and normal cells after 48 and 72 hours

Mean percentage of live cells (48h)		
Compounds	**CaCO2**	**Normal cell lines**
1U	18.2	51.7
0.5U	26.5	75.4
0.25	28	83
Mean percentage of live cells (72h)		
Compounds	**CaCO2**	**Normal cell lines**
1U	08.30	53.5
0.5U	16.50	68.15
0.25	20.30	70.30

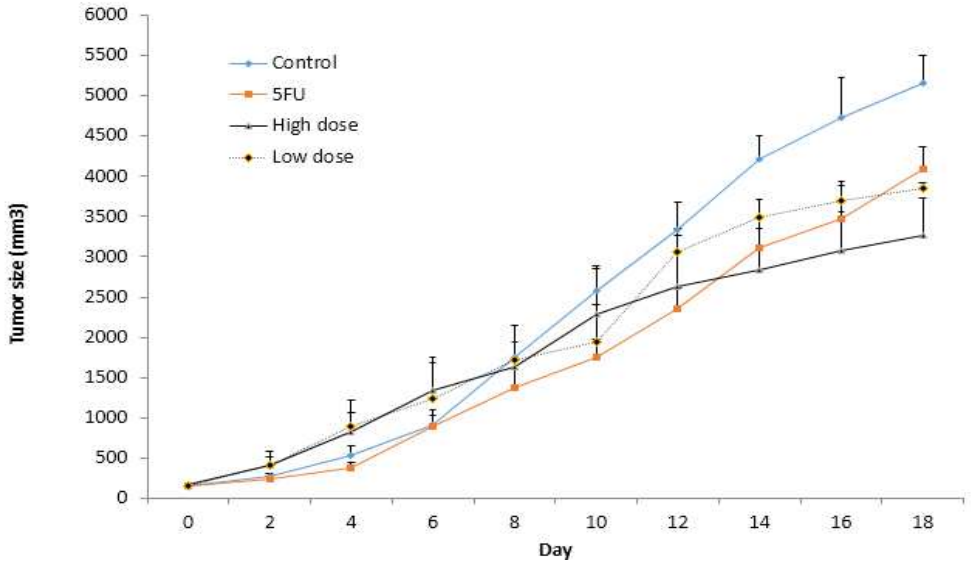

Figure 1. Comparison of the size of the tumor from the beginning of the treatment to the eighteenth day.

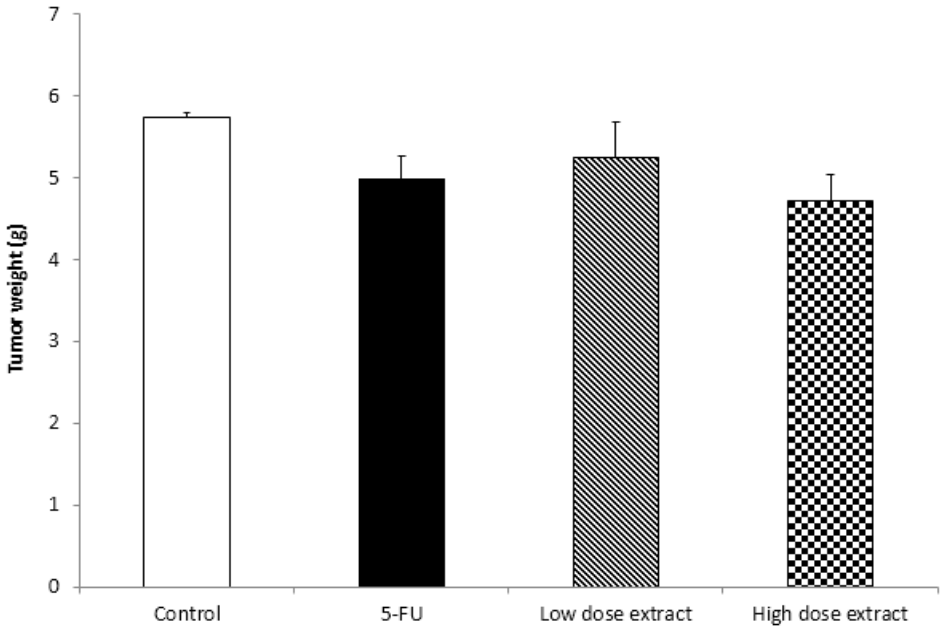

Figure 2. Comparison of the weight of the tumor on the eighteenth day after treatment.

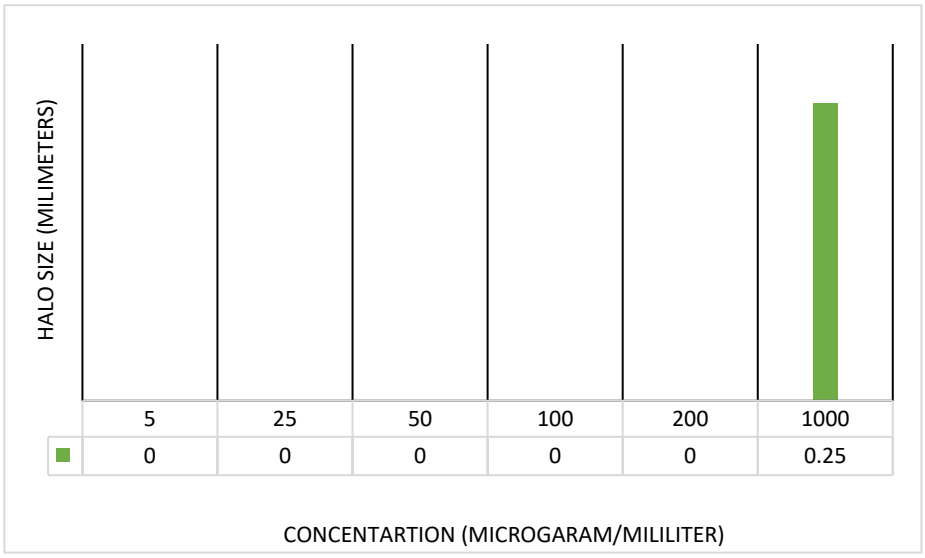

Figure 3. The hemolytic activity of new drug on human blood cells based on the diameter of the halo around the well in different concentrations

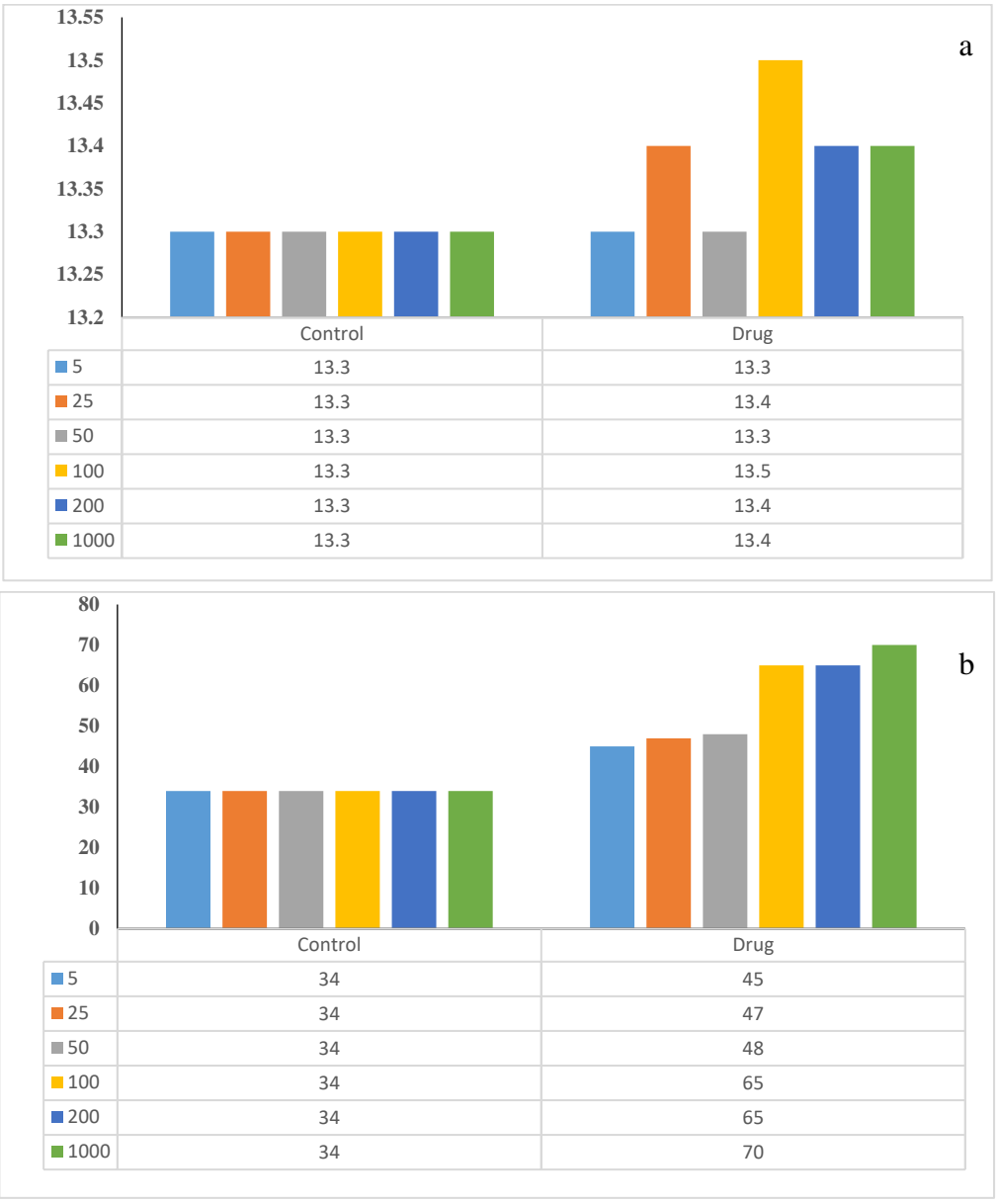

Figure 4. The effect of drug on Prothrombin time (PT) (a) and partial thromboplastin time (PTT) (b)

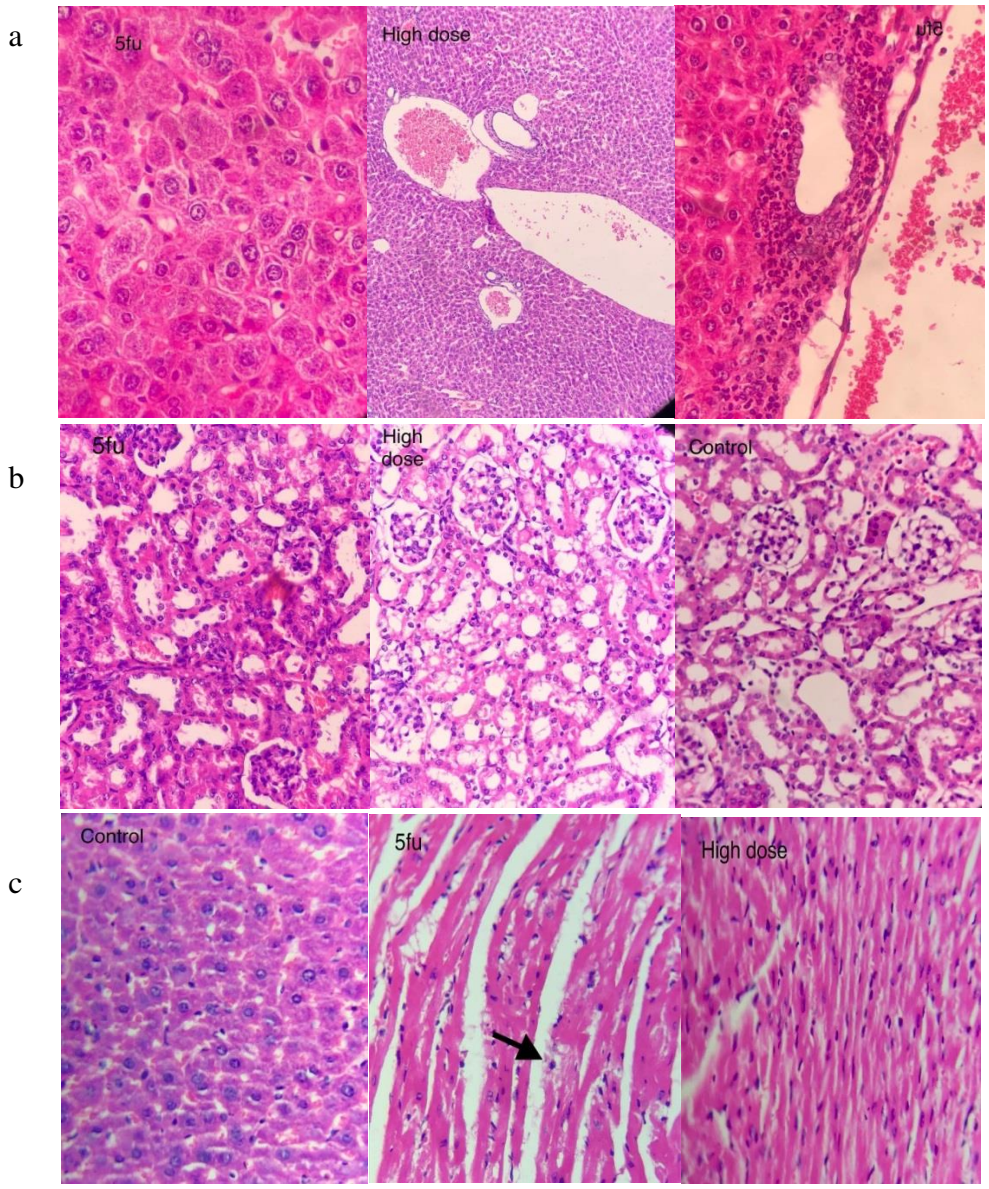

Figure 5. Comparison of liver (a), kidney (b), and heart (c), and histology between the three groups (high and low dose of new drug and 5FU).

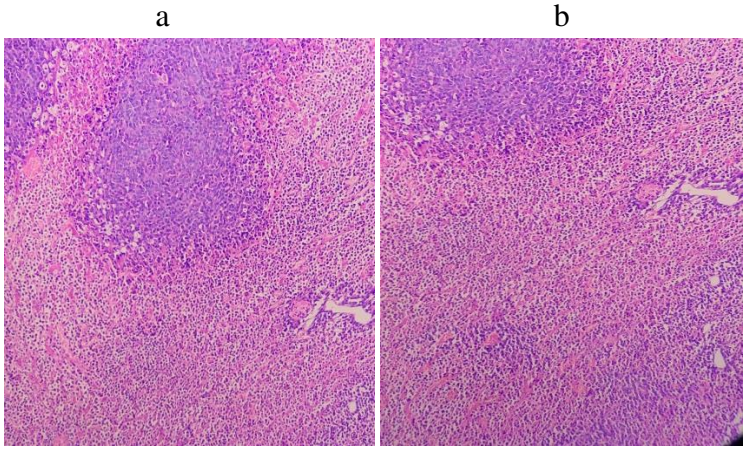

Figure 6. Comparison of tumor histology between the 5FU (a) and new drug (b)