

## Histopathological Effects of 5-Fluorouracil on the Liver of Male Rabbits ( with and without Folic Acid administration )

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### الخلاصة

**الاهداف:** تهدف الدراسة الى تقييم آثار الفلورويوراسيل على نسيج الكبد عند الأرناب ودراسة تأثير حامض الفوليك على تلك الآثار .  
**المواد وطرائق العمل:** استخدمت في العمل الحالي (9) ذكور أرناب ، أعمارهم 3 أشهر تقريبا ، تم تقسيمهم الى ثلاث مجموعات رئيسية (3 أرناب لكل مجموعة) : المجموعة الأولى : ضابطه، اعطيت محلول ملحي متعادل ( 1 مليلتر) . المجموعة الثانية : أعطيت حقنة 10 ملغم /كغم من الفلورويوراسيل داخل الصفاق مرتين أسبوعيا ولمدة 8 أسابيع . المجموعة الثالثة : أعطيت حقنة 10 ملغم /كغم من الفلورويوراسيل داخل الصفاق مرتين أسبوعيا ولمدة 8 أسابيع مع جرعة (5) ملغم أعطيت عن طريق الفم مرتين أسبوعيا ولمدة 8 أسابيع بواسطة (needle Cavage) **النتائج:** جميع المقاطع المأخوذة من الكبد للمجموعة الثانية أظهرت نتائج متشابهة ، كان هناك تغييرات في شكل الفصيص الكبدي السداسي ، زيادة في حجم خلايا الكبد ، نشوء فجوات كبيرة في غالبية خلايا الكبد . أظهرت بعض المقاطع وجود تدمير وموت للخلايا الكبدية . كان هناك احتقان كبير في الأوعية الدموية بين الفصيصية وحول الوريد المركزي مع احتقان وتوسع في اشباه الجيوب الدموية . تسلل الخلايا الالتهابية وحيدة النوى بشكل كبير في المنطقة البوابية وحول الوريد المركزي . اما بالنسبة للمقاطع المأخوذة من المجموعة الثالثة فقد أظهرت تغييرات بسيطة في شكل الفصيص الكبدي ، كذلك لم يتغير حجم خلايا الكبد ، وقليل من الخلايا الكبدية أظهرت وجود فجوات داخلها . لم تظهر المقاطع وجود خلايا ميتة . كما أظهرت المقاطع تسلل الخلايا الالتهابية وحيدة النوى في المنطقة البوابية. **الاستنتاجات:** التأثير السام لعقار الفلورويوراسيل قد لوحظ على أنسجة الكبد في الأرناب ، ويجب إعطاء بعض الادوية الفعالة مع الادوية المضادة للسرطان لغرض تحسين عمل الكبد.

### ABSTRACT

**Aims :** The objective of this study is to evaluate the effects of 5-fluorouracil (5-FU) on the liver tissue of rabbits, as well as the effect of folic acid on the effectiveness of 5-FU on the rabbit's liver. **Materials and Methods:** In this study nine adult male rabbits ,aged about 3 months were used and divided into three main groups .Group 1 : served as a control group and received normal saline only.Group 2 : treated by 5-fluorouracil , 10 mg / kg intraperitoneally twice weekly for 8 weeks. Group 3 : treated by 5-fluorouracil , 10 mg / kg intraperitoneally twice weekly for 8 weeks with 5mg of folic acid orally twice weekly for 8 weeks .The animals were sacrificed and livers were excised and processed for histological study. **Results:** The liver of the treated group 2 shows disturbance in the architecture of hepatic lobule , swelling and vacuolation of the hepatocytes ,with ballooning degeneration and areas of necrosis. Congestion in the portal blood vessels and congestion in the central vein , together with congestion and dilatation of the sinusoids. Infiltration of mononuclear inflammatory cells in the portal area and around the central vein .While liver sections of group 3 show slight disturbance in hepatic cords architecture , hepatocytes showed no swelling and few vacuolation in their cytoplasm ,mononuclear cells infiltration mainly in the portal area and in lesser degree than that found in group 2 ,necrosis were not present. **Conclusion:**Toxic effect of 5-FU was observed on the liver tissue of rabbits , and an effective drugs must be applied with anticancer agents to improve liver function .

**Key words:** 5-fluorouracil , liver, rabbit.

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### INTRODUCTION

Chemotherapy is the treatment with drugs that kill cancer cells or make them less active , it destroys cancer cells anywhere in the body , although chemotherapy

is an effective treatment for many types of cancer, chemotherapy like other cancer treatments often causes side effects , it can also harm healthy cells and affect other

body organs. 5-Fluorouracil, is the most commonly used chemotherapy drug in the treatment of various cancers, including gastrointestinal cancer , breast cancer ,head and neck cancers. It belongs to the family of drugs called antimetabolites. <sup>(1)</sup>

Despite extensive investigations about their hepatotoxicity ,but there is very limited information about their effects on the structure and ultrastructure of liver cells in vivo. <sup>(2)</sup>

It is well known that 5-FU has the potential effect of hepatotoxicity and nephrotoxicity. <sup>(3)</sup> Thus folic acid which is the reduced form of folic acid is widely used to prevent the toxic effects of

5-FU and to strengthen the antineoplastic effect of 5-FU. <sup>(4)</sup> In this study we demonstrate the effect of 5-FU on the liver of the rabbit using light microscopy , together with the effect of folic acid on the rabbit's liver treated with 5-FU.

### MATERIALS AND METHODS

Nine adult male rabbits were used in the present work , aged about 3 months weighing 0.950-1.640 kg , kept in a standardized animal house condition with room temperature 25±2 Co and freely fed ,the animals divided into 3 groups ,3 animals for each group .

Group1 : served as a control group ,were injected intraperitoneally with 1 ml of normal saline twice weekly for 8 weeks .

Group 2 : rabbits of this group were treated with 10mg/kg of 5-FU twice weekly for 8 weeks intraperitoneally .

Group 3 : rabbits of this group were treated both with 10mg/kg of 5-FU twice weekly intraperitoneally , and 5mg of folic acid orally , for 8 weeks .

Body weight for all the animals were measured at the beginning of the experiment and at the end .Then after 8 weeks all the animals were sacrificed by ether inhalation anesthesia , dissection of the abdomen was done , livers were excised and small pieces of hepatic parenchyma were taken and fixed in 10% formalin fixative for 24 hours, then dehydrated in ascending grades of alcohol using 50%,70%,90% and 2 changes of absolute alcohol respectively with a period of one hour for each .Then cleared by 2 changes of xylene with a period of 1hour for each ,then embedded in 3 changes of 60Co melting point paraffin for 2 hours each , sections were obtained and stained with hematoxyline and eosin (H&E) for light microscopic examinations .<sup>(5)</sup>

### RESULTS

#### Physical and clinical observations :

The results demonstrate that there were increase in the body weight of the animals in group 1 and 3, and there were decrease in the body weight of the animals in group 2 (Table 1).

Table (1): Body weight (gm) of rabbits before and after treatments

Animal No.	Before treatments	After Treatments	Note
1	950	1235	Control Group I
2	1320	1405	
3	1400	1525	
4	1595	1580	Twice dose weekly 5-FU Group II
5	1640	1535	
6	1470	1425	
7	1465	1500	Twice dose weekly 5-FU & FA Group III
8	1460	1525	
9	1270	1380	

One of the animals in this group show marked hair loss , and localized alopecia.  
Histological findings :

**Group 1:**

All sections of liver were taken from rabbits in this group appeared to have

The normal histological structure .The hepatic lobule is roughly hexagonal in shape with centrally located terminal hepatic venule (central vein) Figure (1).

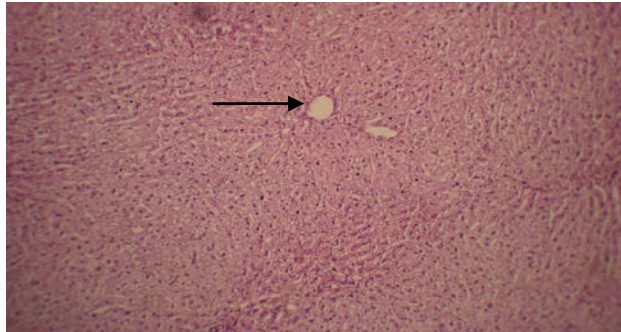


Figure (1) light micrograph of rabbit's liver of group 1 showing the hepatic lobule with it's central vein (arrow).H&E.( X -90).

The plates of liver cells or hepatocytes and hepatic sinusoids appear to radiate from the

central vein to the periphery of hepatic lobule , Figure (2).

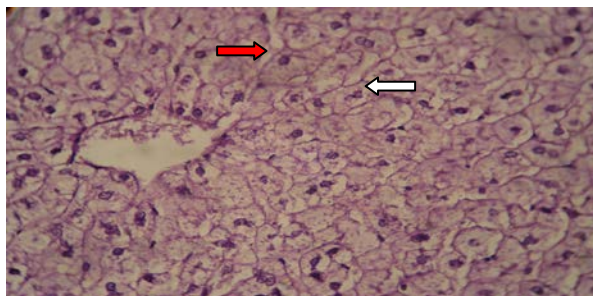


Figure (2) light micrograph of rabbit's liver of group I showing central vein (C.v) , hepatocytes (white arrows) ,sinusoid (red arrow) .H&E.(X-350)

The portal areas which is located at the corners of the lobules houses the terminal

branches of hepatic artery ,portal vein and bile duct .

**Group 2:**

All sections of liver were taken from this group showed similar changes characterized by disturbance in the architecture of hepatic cords, Figure (3). The hepatocytes become swollen and vacuolated Figure (4) giving the appearance of ballooning degeneration with focal areas of necrosis. Congestion in the portal blood vessels, central vein, and sinusoids, Figure (5). Infiltration of mononuclear inflammatory cells noticed in the portal area, and around the central vein figure (6, 7 and 8).

**Group 3:**

Liver sections were taken from this group showed slight disturbance in hepatic cords architecture according to that of group 2. Congestion of blood vessels and sinusoids were not present, but there is dilatation of the sinusoids. Hepatocytes showed neither swelling nor ballooning degeneration, and the cytoplasmic vacuoles very few Figure (9). Infiltration of mononuclear inflammatory cells especially in the portal area but in lesser degree than that found in group 2, necrosis were not present Figure (10).

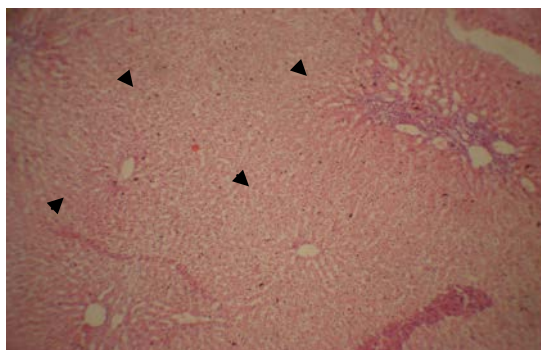


Figure (3): Light micrograph of rabbit's liver of group 2 showing disturbance in the architecture of hepatic cords (arrows).H&E.(X-75)

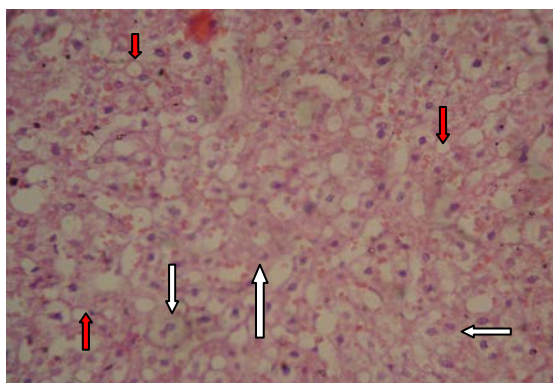


Figure (4): High magnification of figure (3) showing the swelling of hepatocytes (white arrows) and the presence of spherical vacuoles in their cytoplasm (red arrows). H&E. (X-350).

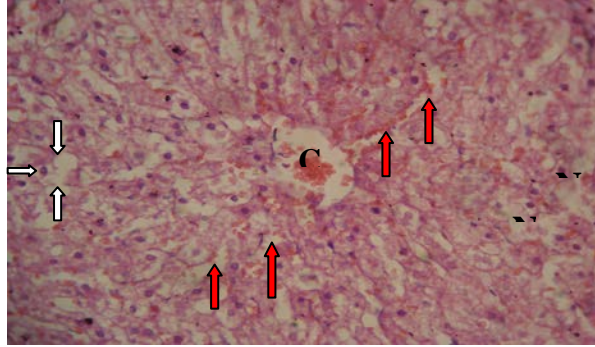


Figure (5): light micrograph of rabbit's liver of group 2 showing congestion of central vein(C.V),ballooning degeneration of hepatocytes (white arrows) , sinusoidal dilatation and congestion (red arrows) , areas of necrosis (N). H&E.(X-350)

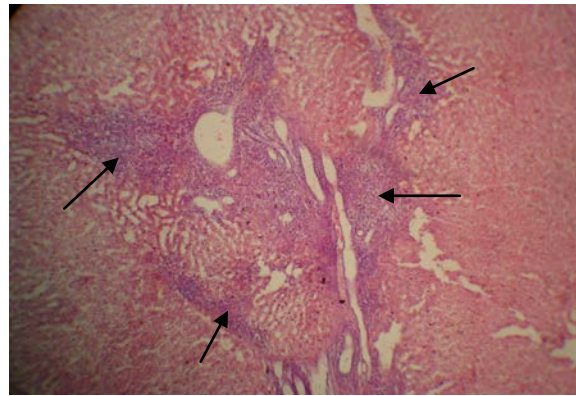


Figure (6): light micrograph of rabbit's liver of group 2 showing mononuclear cell infiltration in the portal area (arrows). H&E(X-75)

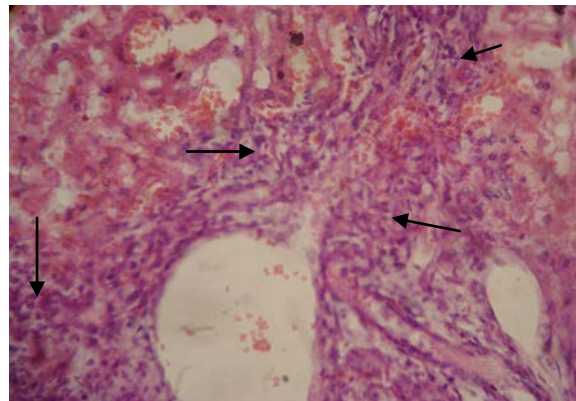


Figure (7): light micrograph of rabbit's liver of group 2 showing mononuclear cell infiltration in the portal area (arrows). H&E(X-350)



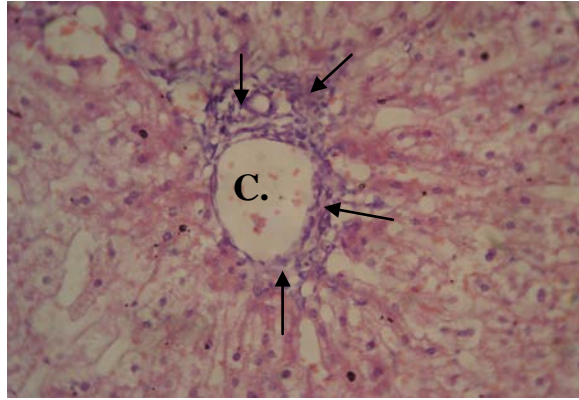


Figure (8): light micrograph of rabbit's liver of group 2 showing mononuclear cell infiltration (arrows) around the central vein(C.V). H&E(X-350)

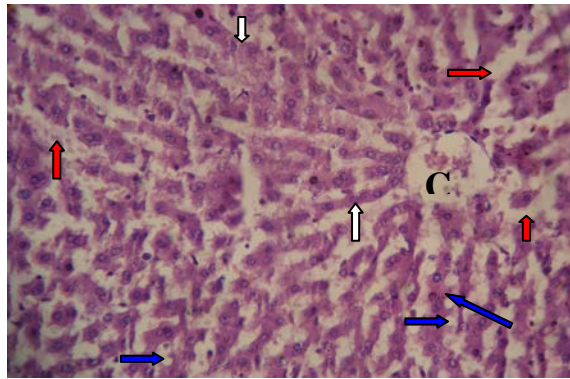


Figure (9): light micrograph of rabbit's liver of group 3 showing hepatic cords (white arrows),central vein (C.V), dilated sinusoids (red arrows) , cytoplasmic vacuoles in hepatocytes (blue arrows) . H&E (X-350)

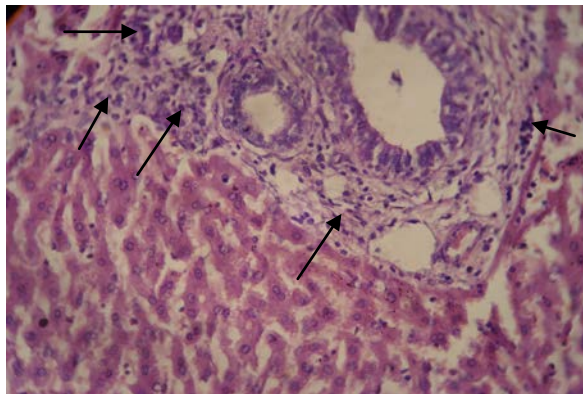


Figure (10): light micrograph of rabbit's liver of group 3 showing mononuclear cell infiltration in and around the portal area (arrows). H&E.(X-350).

## DISCUSSION

Although a number of studies have indicated some side effects of 5-FU as a chemotherapeutic drug of various cancers, very few studies and limited information about the effect of 5-FU on the structure and ultra structure of liver cells. Various types of chemotherapy-associated hepatopathy have been reported. The effect of Cisplatin, Doxorubicin and 5-FU was demonstrated for the first time on rat liver toxicity using both light and electron microscopy in 2009.<sup>(2)</sup> Our study confirms the original finding on the adverse side effects of 5-FU as a chemotherapeutic agent on the rabbit liver. The first histopathological effect seen in group 2 liver is the disturbance in the architecture of hepatic cords and this was mentioned in other studies.<sup>(6)</sup>

The swelling of hepatocytes and the presence of numerous spherical vacuoles in their cytoplasm is mainly consequence of considerable disturbance in lipid droplets and fat metabolism occurring during pathological changes.<sup>(7)</sup> Necrosis was an obvious change seen in the current study is due to that 5-FU interferes with the mechanism of action of RNA, resulting from the formation of "fraudulent" RNA by the incorporation of F-UTP (5-fluorouridine-5'-triphosphate), in the ribonucleotides. Incorporation into RNA has been associated with toxicity and has major effects on both the processing and functions of RNA, by interference with the formation of DNA and RNA, 5-FU provokes unbalanced growth and death of cells,<sup>(8)</sup> and this result is in accordance with other studies which showed many histopathological and ultrastructural abnormalities in the liver after intraperitoneal administration of 5-FU including apoptotic cell death, appearance of numerous areas of inflammatory cells, and the cytoplasmic organelles were markedly affected with collapse. The histopathological results of liver sections taken from group 3 revealed that the administration of folic acid has ameliorative effect on the histopathological changes induced by 5-FU, therefore the toxicity of 5-FU was significantly greater in folate-deficient rats than in folate supplemented

genous fibrils in a number of necrotic cells.<sup>(2)</sup>

Congestion in the portal blood vessels and central vein together with congestion and dilatation of sinusoids are mostly due to inflammation, after a transient vasoconstriction, vasodilatation occurs resulting in locally increased blood flow and engorgement of the down-stream capillary bed. The microvasculature becomes more permeable resulting in the movement of protein-rich fluid into the extravascular tissue and emigration of leukocytes, this causes RBCs to be more concentrated, thereby increasing blood viscosity and slowing the circulation. These changes are reflected microscopically by numerous dilated small vessels packed with erythrocytes.<sup>(9)</sup> and this effect is in agreements with other studies which demonstrate that non-neoplastic livers exposed to cytotoxic agents showed significantly more frequent sinusoidal dilatation, centrilobular venular fibrosis, hepatocyte plate disruption, compared to the control group.<sup>(10)</sup> However it has been suggested that the initial toxic injury to the sinusoidal endothelial cells by the chemotherapeutic drug may cause sinusoidal wall disruption, activation of hepatic stellate cells and sinusoidal matrix deposition<sup>(11)</sup> the sinusoids become markedly dilated and red blood cells extravasate into the Disse's spaces through the discontinuities in the endothelial lining. Infiltration of mononuclear cells (mostly lymphocytes) is a prominent response of body tissues against any injurious agent.<sup>(8)</sup>

The decreased in the weight of the animals in group 2 is mostly due to the decreased food intake observed during the period of experiment which is mostly due to the loss of appetite caused by the anticancer therapy. Hepatotoxicity might be contributed to this loss of weight.<sup>(12)</sup> rats.<sup>(13)</sup> It has been reported that folic acid has both proliferative and regenerative effects on the liver cells,<sup>(14)</sup> thus folic acid which is the reduced form of folic acid is often used to potentialize the antineoplastic effect of 5-FU as a chemical modulator,<sup>(4)</sup> and there is improvement in liver functions

and increased bile secretions the day following a chemotherapy protocol including 5-FU and folic acid in patients with advanced gastric carcinoma .<sup>(15)</sup>

### CONCLUSIONS

Our results confirm that 5-FU cause direct hepatic toxicity, an appropriate protective measures must be applied with anti-cancer treatment for improving liver function , as a result of our finding we believe that folic acid has more protective effect by preventing the liver hepatotoxicity.

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