Histopathological study of the Influence of Estradiol on Liver Regeneration in Male Rabbits

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Accepted on:29/5/2013

Summary

This study was investigated to recognize the generative capacity of liver that influenced by administration of estradiole benzoate. Twenty four healthy local breed male rabbits weighed 1.5-2 kg were used .They were classified into two equal groups control and treated groups. Animals anaesthetized by intramuscular injection of Ketamine (35mg /kg B.W) and Xylazine (5mg\kg B.W.) In control group partial hepatectomy of liver was done, while in treated animals were subject to a same operation then received an intramuscular injection 0.1ml of estradiol benzoate 0.2% at 3rd and 5th days post operation. At 3rd days microscopic finding showed biopsy were taken at 3rd, 7th and 14 day, post operation to study the histopathological changes in liver regeneration .At 3rd day it showed early proliferation of hepatocyte character by irregular hyperchromatic multinucleated cell .While in control group it showed necrotic area of liver parenchyma surrounded by thick fibrous connective tissue infiltrated with inflammatory cells. At 7th day in treated animals still progress in proliferation of hepatocyte while, liver proliferation phase showed in control group .At 14th day in treated animals showed large cells of hepatocyte with double nuclei aggregation without central vein , while in control group showed small hyperchromatic hepatocyte with present of necrotic area. In conclusion the results of this study indicate that administration of estrogen after partial resection in male rabbits has a role in liver regeneration by early initiate or facilitate heypatocyte proliferation.

Keywords: Estradiol, Liver regeneration, Male rabbits.

Introduction

Liver is a vital organ, it has a wide range of function which include synthesis, metabolism, storage and redistribution of amino acids, carbohydrates, fats and vitamins (1). It also important immunological function in filtrating the portal blood via the reticuloendothelial system, removing bacteria and endotoxin translocate from the gastrointestinal tract (gut) to the portal circulation by its production of bile (2). Liver resection was first described centuries ago, but until the latter half of the 20th century, the majority of such resection were performed for management of either injuries or infection. These procedures are performed not only for treatment of acute emergencies (e. traumatic injuries or abscesses) but also as potential curative therapy for a variety of benign and malignant hepatic lesions (3 and 4). Liver regeneration is a complex and well-orchestrated process, during which hepatic cells are activated to produce large single molecules in response to liver injury or mass reduction (1) , actually regeneration is a compensatory hyperplasia, which is mediated by the proliferation of surviving hepatocytes, during liver regeneration hepatocytes are primed to proliferate, maintain metabolic function , secrete interleukin(IL-6), protease and protease inhibitor, and hepatocyte growth factor (HGF) (5 and 6). Kupffer cells are liver resident macrophage, during the priming phase of liver regeneration Kupffer cells are Activated and secret pro-inflammatory cytokines, most prominently tumor necrosis factor (TNF)-α, IL6 and 1B, which can initiate the acute phase response in hepatocytes (7). Hepatic stellate cells (HSC) also known as fat storing cells (2). Liver fibroblasts, following liver damage (HSC) can secret collagens and proteoglycans, growth factors such as hepatic growth factor, fibroblast growth factor transforming growth factor and cytokines such as IL-6, (6).

Liver Dendritic cells isolated from the liver at 6 hours after partial hepatectomy exhibit enhanced estrogen receptor expression, concomitant with increased serum estradiol level receptor. There has been considerable experimental evidence to suggest that estrogen may modulate liver regeneration after

hepatectomy (8). After partial hepatectomy increase estrogen receptors in hepatocytes also estrogen induces DNA synthesis, mitosis of hepatocytes and enhanced stimulation of plasma rennin substrate synthesis ,in the same time there is a mechanism by which estridiol protect and limit the liver from complication after surgery it may be possible to exploit the estrogen –mediated protective pathway (9 and 10).

Also (11) refer that sex steroid and pituitary hormone influence on cytoplasmic estrogen receptors in fully differentiated liver cells, and refer that estrogen and androgen receptor are present in mammalian liver. Because estrogen administration in male mice and rat after partial resection attenuated hepatocellular injury that lead to increase their survival rate and promote liver regeneration, this study investigated to recognize the generative capacity of liver influenced by administrating of estradiole benzoate**.**

Materials and Methods

Twenty four healthy, local breed male rabbits, weighing 1.5-2 kg were used. The rabbits were divided into two equal groups, control and treated groups .Animals anaesthetized by intramuscular injection of Xylazine 5mg\kg B.W and Ketamine 35mg\kg B.W. Abdominal region from xyphoid to umbilical region prepared aseptically .In control group, left paracostal lapratomy incision was made and left lateral lobe of liver traction outside abdominal cavity then part of lobe was resected after suturing with horizontal interrupted mattress suture by absorbable suture material (2.0, polyglactine 910) then the abdominal wall was closed routinely.

In treated group animals subject to similar procedure and administered intramuscular injection of 0.1 ml Estradiol benzoate (Estradiol 2mg\ml, Chongqing Fangtong Animal pharmaceutical Co.) at 3rd and 5th days post operation. Penicillin (10000IU\kg BW) and streptomycin (10mg\kg BW) intramuscularly were injected for 5days post operation. Biopsies of liver were harvested after animal's scarified at 3, 7 and14 day post operation in both groups.

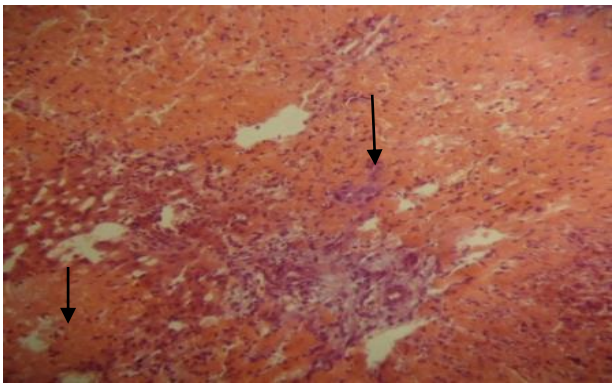
Results and Discussion

Clinical findings revealed that animals was tolerate the surgical operation, and the time of anesthesia was sufficient to perform this operation. Macroscopic finding showed adhesion of incision site with stomach, diaphragm and omentum that need blunt dissection to separate the liver from around.

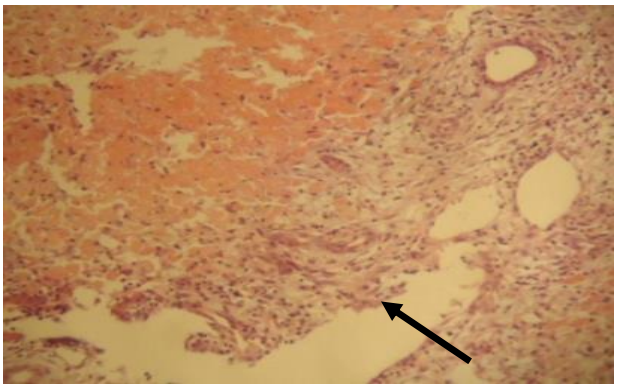
Histopathological examination in the liver at 3rd day postoperation in control group refered multiple necrotic area in the liver parenchyma (Fig. 1) and this necrotic area surrounded by thick fibrous connective tissue capsule infiltrated with neutrophils (Fig. 2) ,in other section showed necrostic area surrounded by thick fibrous connective tissue infiltrated with inflammatory cells mainly macrophages ,lymphocytes and noutrophils with congestion of blood vessels in the liver parenchyma (Fig. 3). While in treatment group 3 days postoperation reveals presence of necrotic area infiltrated with notrophils with congestion and dilatation sinusoids and proliferation of hepatocytes which characterized by irregular hyperchromatic cells with vacuolation in their cytoplasm which extend in incision part (Fig. 4).

In control group at 7 day post operation revealed newly form of hepatocyte character by hyperchromatic, pleomorphic, multinucleated, cells surrounded by mature fibrous connective tissue capsule (Fig. 5). Also showed necrotic area surrounded by thick fibrous connective tissue capsule congestive blood vessels and infiltrated with mononuclear cell (Fig. 6). While in treated group at 7 day showed congestion of sinusoids and progress in proliferation of hepatocytes which characterized by hyper chromatic multinucleated hepatocytes together with fibrin network and inflammatory cells in the liver incision (Fig. 7). At 14 day post operation in control animals in the liver still presence of large necrotic area surround by fibrous connective tissue capsule infiltrated with mononuclear cell and contain blood vessel (Fig. 8). Other section showed hepatocytes characterized by hyper chromatic small cell extend to thick fibrous connective tissue (Fig. 9). At the same time the treated group showed main lesion characterized by hyperchromatic

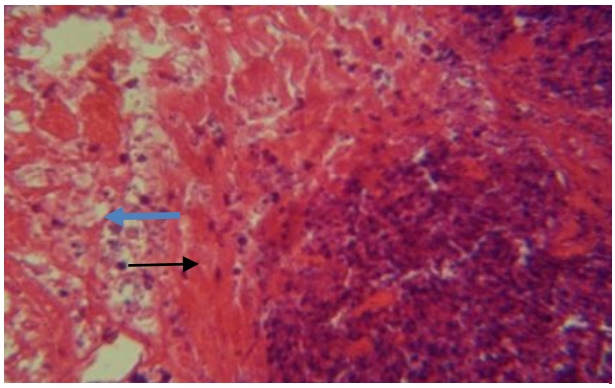
multinucleated hepatocytes with divided nuclei aggregation without central vein surrounded by mature fibrous connective tissue (Fig. 10), while (Fig. 11) show proliferation of hepatocyte lesion charectarized by large newly form hepatocyte hyperchromatic multinucleated hepatocyte with divided nuclei aggregation without central vein surrounded by mature fibrous connective tissue.



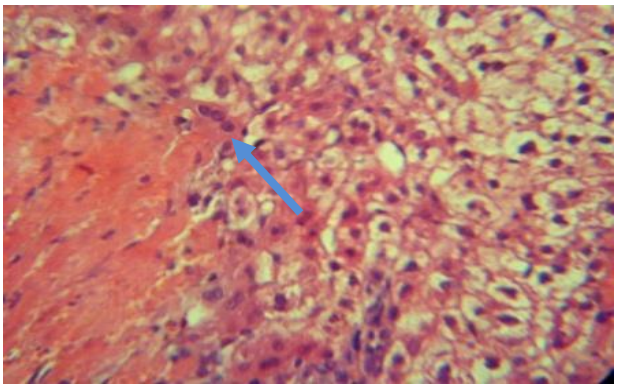
Figure, 1: The histopathological section in control group at 3rd day showed multiple necrotic area in the rabbit liver parenchyma (H&E400x).



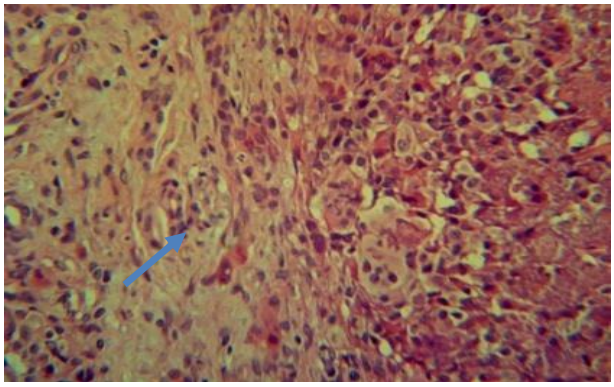
Figure, 2: In control group at 3rd day showed necrotic area surrounded by thick fibrous connective tissue capsule infiltrated with neutrophils (H&E400x).



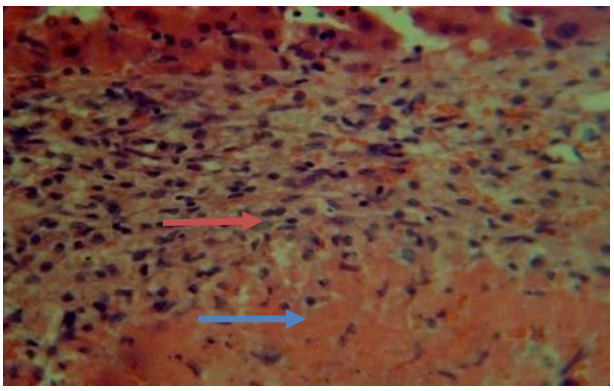
Figure,3: In control group at 3rd day showed necrosis area surrounded by severe fibrous connective tissue infiltrated with inflammatory cell manly macrophage ,lymphocyte and neutrophils (⟶) with congestion blood vessel (⟵) (H&E400X).



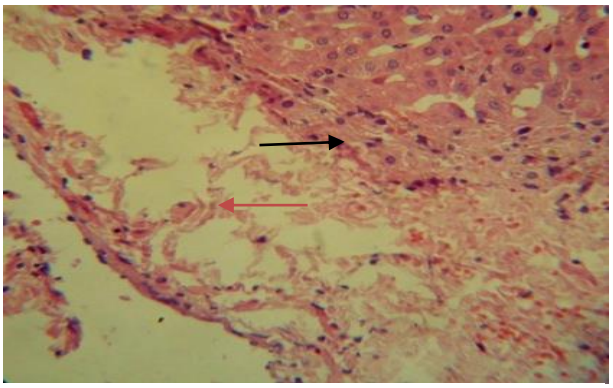
Figure, 4: In treatment group at 3rd day showed proliferation of hepatocytes which characterized by irregular hyperchromatic cells with vaculation in their cytoplasm which extend in incision part (H&E 400X).



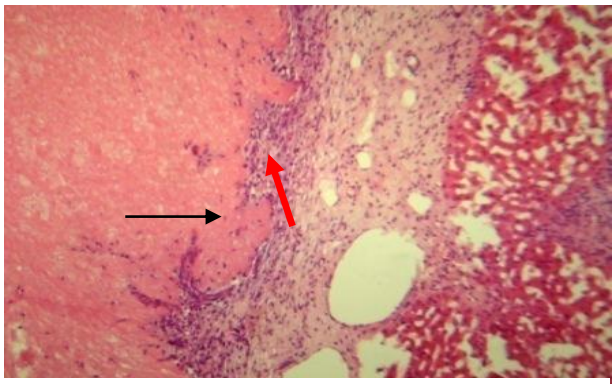
Figure, 5: In control group at7 day showed newly form of hepatocyte character by hyper chromatic plearomorphic multinucleated cell surrounded by mature fibrous connective tissue capsule (H&E 400X).



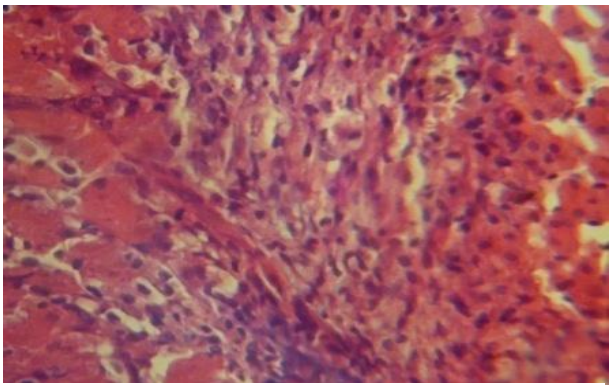
Figure, 6: In control group at7 day showed necrosis area surrounded by thick fibrous connective tissue capsule (⟶) contain congestive blood and infiltrated withmononuclear cell (⟶) (H&E 400X).



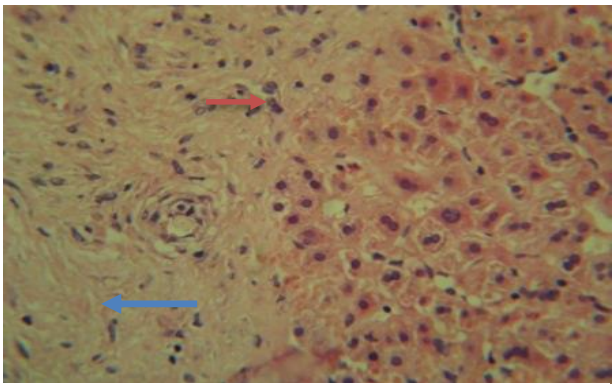
Figure, 7: In treatment group at 7day refer congested of sinusoid and proliferated of hepatocyte (→)which characterized by hyper chromatic multinucleated hepatocyte together with fibrin network (→) and inflammatory cells in the liver incision (H&E 400X).



Figure, 8: In control group at 14 day showed large necrotic area (→) surround by thick fibrous connective tissue capsule infiltrated with mononuclear cell (→) and contain blood vessel (H&E 40X) .



Figure, 9: In control group at 14 day showed hepatocyte character by hyper chromatic small cell extend to thick fibrous connective tissue (H&E40X).



Figure, 10 :In treatment group at 14 day refer large newly form hepatocyte (→) characterized by hypochromatic multinucleated plearomorphic hepatocyte with divided nuclei aggregation without central vein surrounded by mature fibrous connective tissue (→)(H&E 40X).

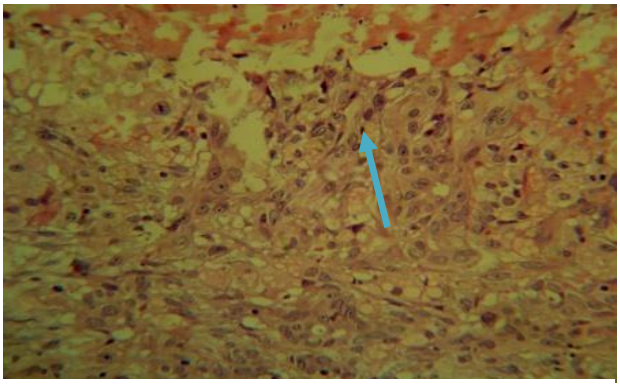


Figure ,11 :In treatment group at 14day show proliferation of hepatocyte(H&E40X).

Liver has a remarkable capacity for proliferation after a partial hepatectomy and can precisely regulate its growth and mass to adjust its size. The exact mechanisms of stimulation and regulation of hepatic regeneration remain unclear. It is well known that various cytokines and growth factors and perhaps cell populations, other than hepatocytes are involved (11). Many different substances have been reported to stimulate liver cell growth in vivo and invitro, including a number of known hormones, serum factors and some small nutrient molecules (10and11). In this work estradiol benzoate was administrated at 3rd and 5th days post operation. This phenomena was coincided with (12) which refer partial hepatectomy results in a substantial increase in serum estradiol concentrations, as well as mammalian liver cell of both sexes contains estrogen receptors (13), it has been suggested that serum estrogen concentration may initiate or facilitate liver regeneration. As well as (14) refer that early little exposure to 17α ethanol estradiol after heptectomy can stimulate liver growth in rat, while prolonged exposure inhibits the proliferation of hepatocytes, also the plasma concentration of estrogen is increase in the first hours following hepatectomy also (15) has been suggested that this may be play an important role in facilitate initial liver regeneration for that used in early period post operation only. As well as it is observed macroscopically amass formed at 3rd day post operation in treated group more than in control group but it little differ in both group at 7th day , this disagree with gain of mass was similar for both group after 36 hour and higher in the experimental group at 7 th days (16).

Male rabbits were used in this research because female already has estrogen hormone and (17) refer that specific reductions in the hepatic content of the male estrogen-binding (MEB) protein and estrogen 2-hydroxylase activity have been demonstrated in adult male rat liver following a major hepatic resection rather than female.

In liver necropsy shown adhesion of regeneration site with stomach, diaphragm and omentum this observation agreed with (18) this adhesion may be result from tissue trauma , an inflammatory process ,the presence of foreign body or insufficient of local fibrinolysis , reduce peritoneal fibrinolytic capacity always provide favorable environment for fibroblast and extracellular matrix deposition ,resulting in adhesion formation .Also (19) reported after liver resection in sheep show intra abdominal adhesion in all animals in different degree of evaluation and occurred among liver, different organ and abdominal wall . Histopathological results at 3rd days in control group (Fig. 1,2 and 3) indicated a natural response of liver resection that include inflammatory reaction due to tissue injury that include accumulation of inflammatory cells with necrosis area of liver parenchyma and dead of cell at incision line with release of lysozomal enzyme from neutrophile, this agreed with (20) whom reported that many cytokines are produce during acute liver injury, including TNF-α, IL-1, IL-6, hepatocyte growth factor, TGF-α, macrophage inflammatory protein-2 (MIP-2), stem cell factor (SCF), and many others ,while many of these molecules contribute to hepatic inflammation via direct effects on the vascular endothelium, and/or neutrophil recruitment and activation, they have also been shown to be involved in hepatic repair and regeneration . .While in treated group at 3rd day (Fig. 4),all that's mean earlier in proliferation of hepatocyte in treated group than in control group may be due to effect of estrogen administration immediately after operation effect on happen of regeneration , this agree with (21) that referred estrogen induces increase activity of DNA synthesis , endogenous aromatize activity was induced by IL-6 which is a key factor for living regeneration and increase serum estradiole level ,therefore it is very likely that estradiole and IL-6 synergize initiate stimulation of hypatocyte proliferation during liver regeneration ,as well as (1) records liver dendritic cells isolated from liver 6 hour after 75% partial hepatectomy exhibit enhanced estrogen receptor expression ,concomitant with increased serum 17-β –estradiol levels these result indicated that the increase numbers of estrogen –exposed dendritic cells may play key role in local immune suppression and

promote progression of liver regeneration (22), and agreed with macroscopic finding at this period which include are increase in regenerated mass of liver in treated group more than in control group.

When compared control group at 7th day (Fig. 5 and 6) with treated group at same time (Fig. 7), found that newly hepatocyte proliferation which accurse in control group while advance in proliferation phase that happen earlier at 3rddays in treated group, this agreed with (23) who found Promoting effect of estrogen on regeneration of the liver transplanted to an ectopic site in mice and estrogen raised the mitotic activity of the regenerating hepatocytes markedly and for a long period.

In control group at 14th day post operation (Fig. 8 and 9) showed presence of large necrotic area surround by fibrous connective tissue infiltrated by mononuclear and small hepatocyte character by hyperchromatic extend to fibrous connective tissue . while in treated group at the same period (Fig. 10 and 11) show large newly form hepatocyte, character by hyperchromatic with divided nuclei(double nuclei) aggregation without central vein surrounded by mature fibrous connective tissue , this findings agreed with (5) who described that cells with double nuclei can be seen in liver tissue under normal condition but cells with double nuclei are also the main regenerating liver tissue at a higher rate than normal ,and agree with (24**)** that reported after surgical removal of two-thirds of the liver, remaining hepatocytes replicate and restore hepatic mass within 2 weeks. In conclusion estrogen has a role in liver regeneration by early initiate or facilitate hypatocyte proliferation that included early proliferation of hepatocyte at 3rd day character by irregular hyperchromatic multinucleated cell , at 7th day still progress in proliferation of hepatocyte while at this time appeared proliferation phase in control group, and at 14th day in treated group it was shown large cells of hepatocyte with double nuclei aggregation without central vein , at the same period in control group it showed small hyperchromatic hepatocyte and presence of necrotic area.

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دراسة نسجية مرضية لتأثير الاسترادايول على تجديد الكبد في ذكور الأرانب

أريج كامل مهدي

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الخلاصة

صممت هذه الدراسة لمعرفة مدى تأثر قابلية الكبد للتجدد بإعطاء الاسترادايول بنزويت . استعملت في هذه التجربة 24 أرنب محلي وبأوزان تتراوح 1.5-2 كغم , قسمت الى مجموعتين متساويتين , سيطرة ومعالجة . تم تخدير الحيوانات عن طريق الحقن العضلي بالكيتامين 35 ملغم \كغم والزيلازين 5ملغم \كغم من وزن الجسم في مجموعة السيطرة ثم ازالة جزء من الكبد , خضعت حيوانات مجموعة المعالجة لنفس العملية مع حقن الحيوانات عضليا 0.1 مل من عقار الاسترادايول بنزويت وبتركيز 0.2% في الايام 3و5 بعد العملية اظهر الفحص العياني لمكان القطع ان الكبد اكثر وضوحا عند اليوم الثالث بعد العملية لمجموعة المعالجة عند مقارنتها مه السيطرة . تم جمع عينات الكبد عند الايام 14,5,3 بعد العملية لدراسة التغيرات النسجية . لوحظ في اليوم الثالث تكاثر مبكر لخلايا الكبد تميز بعدم انتظام الكروماتين لخلايا متعددة الانوية . في نفس الفترة شوهد في مجموعة السيطرة مناطق متنخرة من النسيج الخلالي للكبد محاط بالنسيج الليفي الضام السميك المتخلل بالخلايا الالتهابية . اما عند اليوم السابع لمجموعة المعالجة استمر تكاثر خلايا الكبد بينما لوحظ في نفس الوقت في مجموعة السيطرة بداية لتكاثر خلايا الكبد . وشوهد عند اليوم الرابع عشر في مجموعة المعالجة وجود بعض التجمعات لخلايا كبدية كبيرة تحتوي نواتين متجمعة بدون وريد كبدي مركزي , بينما في مجموعة السيطرة في نفس الفترة شوهد خلايا كبدية صغيرة عالية الكروماتين و شوهد بقاء مناطق متنخرة من نسيج الكبد. نستنتج من هذا إن إعطاء الاستروجين بعد إزالة الكبد جزئيا في ذكور الأرانب له دور في إعادة تجدد نسيج الكبد من خلال البدء المبكر أو تسريع تكاثر الخلايا الكبدية .

الكلمات المفتاحية:الاسترادايول, تجدد الكبد,ذكور الارانب.