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Liver Repair in Rabbits Using 532 nm Nd:YAG Laser; *In Vivo* Study

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Abstract: The main goal of this *in vivo* study was to evaluate the effect of 532nm Q-switched Nd: YAG Laser in combination with Human Serum Albumin 20% concentration (as a welding aid) on the liver tissue repair clinically, and histologically. The animals used in this study were 21 male rabbits divided into three main groups: control group (3 rabbits), conventionally treated group (9 rabbits) and Laser treated group (9 rabbits). Each two main groups (conventional and laser treated) consist of three sub-groups depending on the response evaluation at three different periods. The Laser group was treated using 532nm Q-switched Nd: YAG laser after adding human serum albumin immediately on the incised liver's tissue. The energy of was 460mJ, and 4Hz frequency and 60-90 second exposure time. Both groups were compared with the control group. The clinical findings emphasized an effective laser technique in treating the incised liver tissues. The histopathological studies showed a marked regenerative capacity followed by a peak of mitosis. From this work it was concluded that this laser soldering technique has great promise, and could potentially reduce morbidity and mortality associated with liver injury.

Introduction

The *liver* is the second-largest organ of the body after the skin (Jun Queira and Carneiro, 2003) and the largest vital gland in the body, weighing about 3.4% of the body weight (Jones, *et al.,* 1986). This organ has a soft parenchyma richly interspersed with vasculature and thinly protected by a delicate fibrous capsule with limited internal fibrous support. This makes them prone to injury and lacerated with blunt abdominal trauma (Yasmin, et al., 2000).

Hemorrhage is life-threatening in liver injury, as the liver is highly vascular organ, and injuries of it are frequently accompanied by hypothermia and iatrogenic hemodilution compromising native coagulation (Prahl, 2009). Laser tissue soldering has been shown to provide safe and effective tissue closure by creating an immediate leak-free anastomosis with minimal scar formation (Andrew, et al., 2001) and Lauto et al., 2006). Closure of surgical incisions and excisions using a rapid, simple, scarless technique remain a goal in surgery (Kamegaya, et al., 2005).

Laser repair of liver using albumin is a promising method for treating liver trauma (Scott, et al., 2001).

Laser tissue welding is the process of uniting or fusing two pieces of tissue with a laser. Upon cooling, a bond is established between tissue edges (Joie, et al., 1995).

Chromophores are light-absorbing substances within tissue. Absorption is a function of wavelength and chromophore. In the visible (blue, green and yellow), absorption is principally due to hemoglobin and melanin (ECFLDP., 1999-2001 and P.R., et al., 2009). The technique seems to enable faster procedures, less scarring, and possibly fewer infections, since it seals openings completely and leaves no gap through which bacteria can penetrate (Kochevar and Redmond, 2009) and (Meyer, and Haverkampf, 1982)

The purpose of this in *vivo* study was to evaluate the effect of 532nm Q-switched Nd: YAG Laser in combination with Human Serum Albumin 20% concentration (as a welding aid) on the liver tissue repair, clinically and histologically.

Materials and Methods

Twenty-one Local-breed *Oryctolagus cuniculus* male rabbits were used in this study, their weights were in between 1.5-1.8 kg and their ages were in between 9-11 months. All animals were weighed to calculate the dose of anesthetic (combelen 0.5mg/kg intramuscularly) with mixture of ketamine 50mg/kg and xylazins 20mg/kg) intramuscularly and antibiotic (a combination of streptomycin and penicillin 0.25ml/kg and Ivermectin0.25ml/kg) which would be given.

The laser device used in this experiment was Q-switched Nd-YAG (maximum energy: 800 mJ, frequency1-5Hz, pulse width; ≤ 10 ns, (diamond beauty) GUANG ZHOU HUA FEITONGDA CO., LTD, MADE IN CHINA)
The rabbits were divided into three groups:-

- Group A (control group); consists of three rabbits.
- Group B (conventional group); contains nine rabbits.
- Group C (lased group) (9 rabbits); Group C; also contains nine rabbits treated with 532 nm Q-switched Nd:YAG laser of output 460mJ, 4Hz. (3.68 J/ cm^2 energy density) with exposure time between 60-90s and spot size of 0.199cm and contact of hand piece with liver tissue.

The liver wound (incision and segmentectomy) was repaired using conventional (Surgicel) and laser soldering repair, both of the treated injuries were evaluated: clinically and histologically. Liver specimens were collected postoperatively, after four, eight, and twenty days for each group. The animals were observed and checked for normal activities, with daily medical follow-up.

Results

Clinical Observations

Laser Soldering Treated Groups

- All laser-soldering experiments yielded uniformly positive results, with no evidence of dehiscence and with minimal blood loss as shown in Figures 1 and 2.
- All animals recovered from the procedure without complications.
- There was no evidence of hepatic venous thrombosis or abscess formation.
- Early healing.

Conventional Treated Group

- In the early stages, signs like; arched back (due to pain); Pyrexia rising slightly at night, and anorexia were monitored due to inflammation. Figures 3 and 4.
- Loss of appetite.
- Weight loss.

Histopathological Tests

For control group

Slight congestion of central vein and sinusoids which is normally exists due to anesthesia as shown in Figure 5.

The laser repaired groups

The histological appearance of repaired liver, generally confirmed the acceleration of liver regeneration process by increasing the mitotic activity as shown in Figures 6 and 7. Quiescent hepatocytes become proliferative and restore liver functional capacity as shown in Figures 8 and 9. Thermally denatured albumin near the surface of the incised surface was clear in the early stages of treatment as shown in Figure 10. In the later stages, the repaired area of the liver is completely covered with early fibrous tissue and some residual denatured albumin is present in the outer surface that was being removed by phagocytes with infiltration of fibroblasts in the soldered site, finally the soldered liver surface had healed, covered by a layer of mature fibrous tissue and an outer capsule of dark elastic fibers walled off the entire region as shown in Figures 11, 12, and 13. The liver has a marked regenerative capacity; regeneration is mainly a result of hyperplasia and has a rapid onset. The onset of hepatocytes synthesis occurs 12-16 hours post-operatively, followed by a peak of mitosis at 32-34 hours; the mass of liver is restored within 8-20 days as shown in Figure 14.

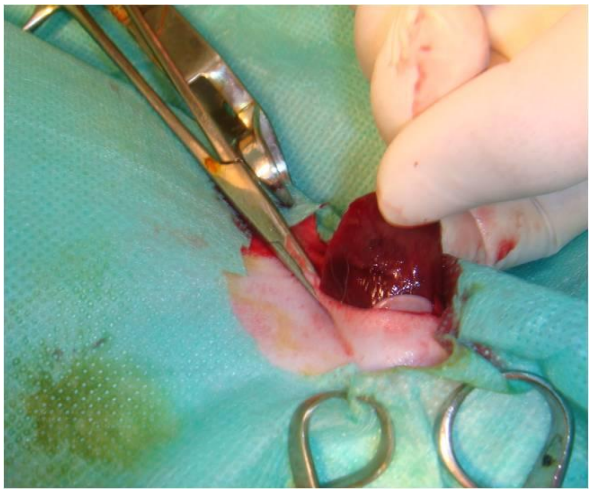

Fig. (1): Welded site immediately post laser exposure

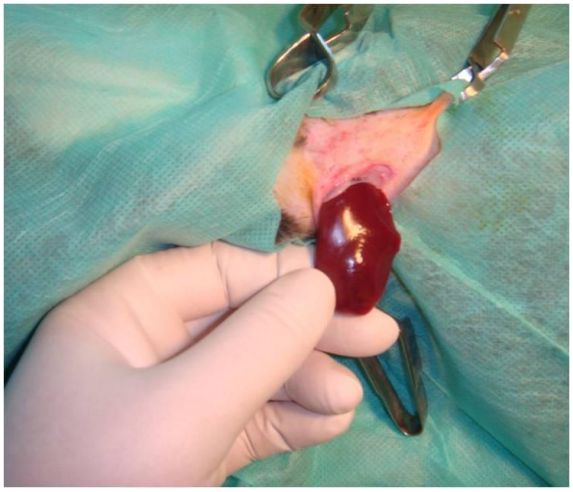

Fig. (2): Examination of the welded site 20 day post operative

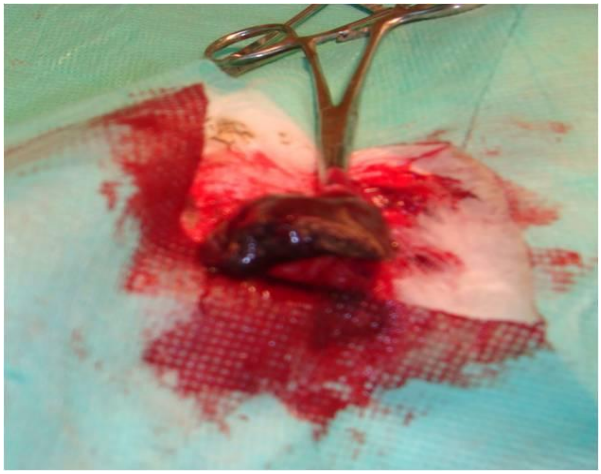

Fig. (3): Treated incision with surgicel across the operation

Fig. (4): Treated Incision with surgicel, 20 days post-operation

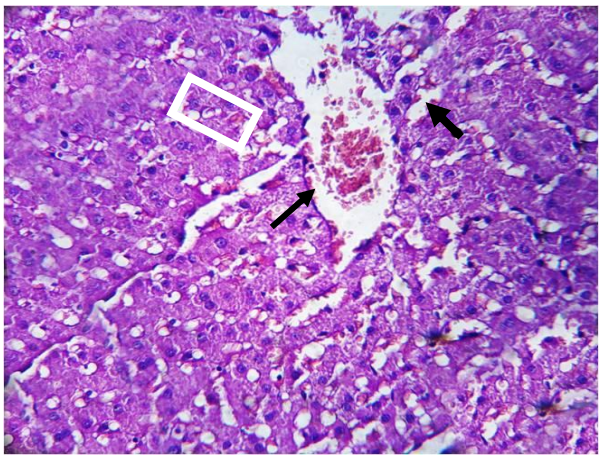

Fig. (5): Histopathological section in liver of rabbit showing slight congestion of central vein (→) and Sinusoids (●→) of group A (control group) (H & E \X 400).

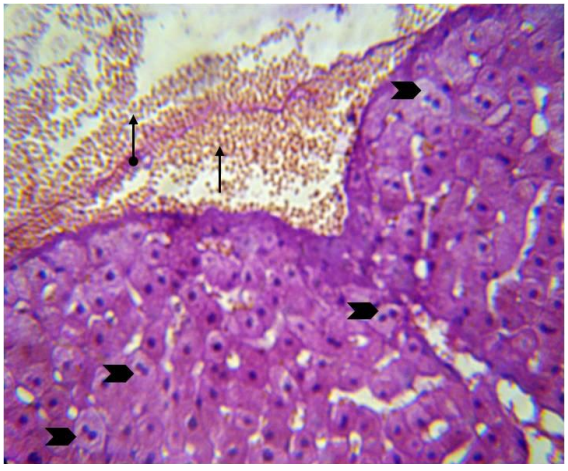

Fig. (6): Histopathological section in liver of rabbit showing the albumin fusing the two edges of the incision with red blood cells at the base of albumin (●►) On the outer area (►) with increase in mitosis of hepatocytes (▰) (**4 days** post-operation of group C (H & E \X400

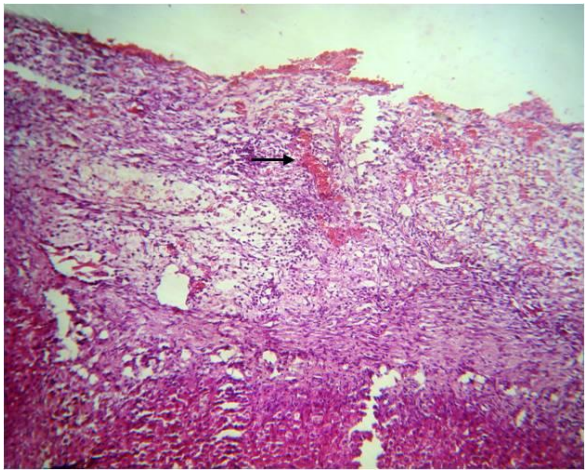

Fig. (7): Histopathological section in liver of rabbit showing proliferation of granulation tissue containing large number of congested blood capillaries (→) (**8 days** post-operation of group C (H & E \X 100)

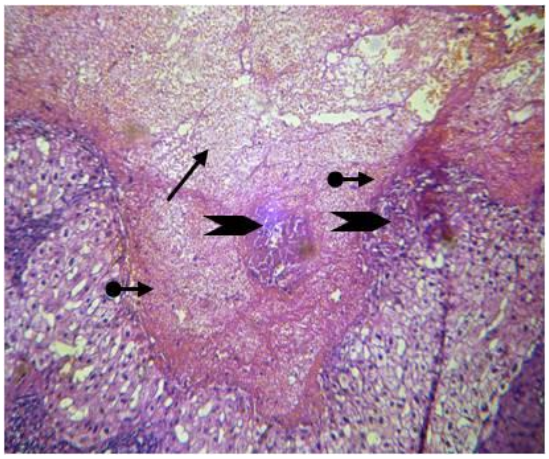

Fig. (8): Histopathological section in liver of rabbit showing presence of fibrouse exudate (→) and hemorrhage (●→) with infiltration of Neutrophils forming microabcess (➤) (**4 days** post-operation of group B (conventionally treated group)) (H & E \X00).

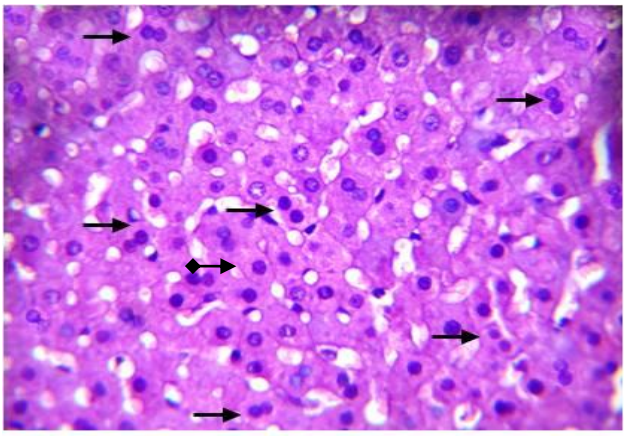

Fig. (9): Histopathological section in liver of rabbit showing increase in mitosis of hepatocytes, large number of mitotic figure and enlargement of hepatocytes (→) with congested sinusoids (◆→)(**4 days** post-operation of groupc) (H & E \ X 400)

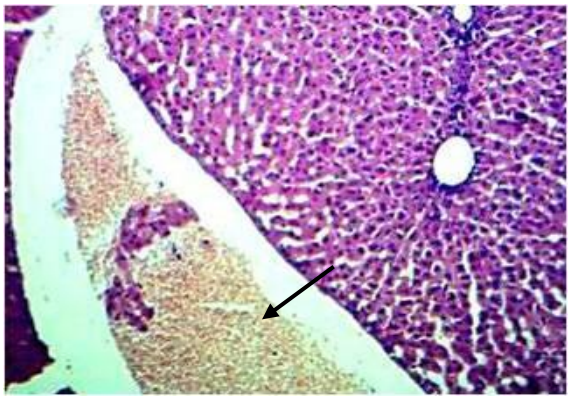

Fig. (10): Histopathological section in liver of rabbit showing liver sections appears nearly as control with area of hemorrhages up on the surface (←) (**20 days** post-operation of group B (conventionally treated group) (H & E \X 100).

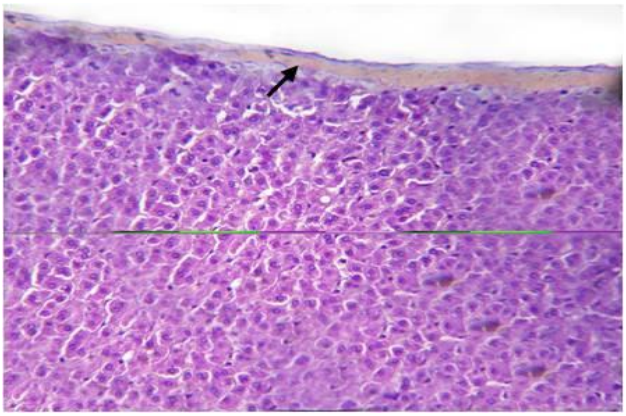

Fig. (11): Histopathological section in liver of rabbit showing the liver parenchyma appears like the control with slight subcapsular hemorrhage (→) (**20 days** post-operation of group C (H & E\ X 100)

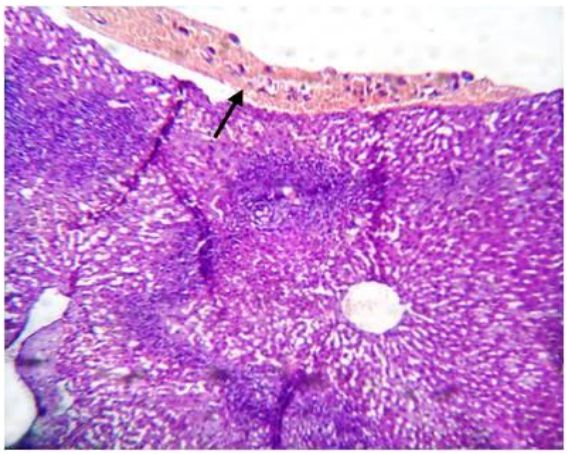

Fig. (12): Histopathological section in liver of rabbit showing a thick layer hemorrhage on the capsule (→) (**4 days** post-operation of group C (H & E\ X100)

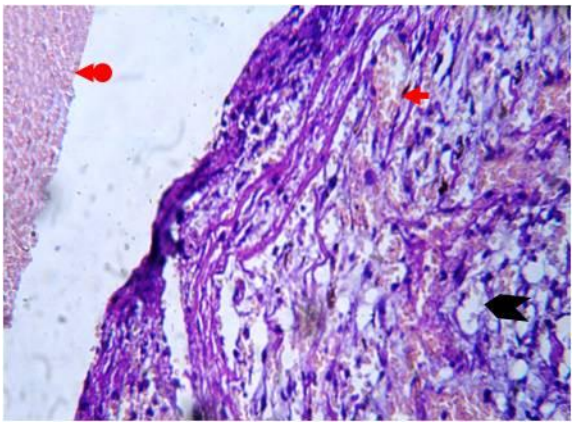

Fig. (13): Histopathological section in liver of rabbit showing proliferation of granulation tissue containing large numbers of congested sinusoids ( ) the surface is covered with hemorrhage ( ) . Vacuolar degeneration of hepatocytes ( ) (8 **days** post-operation of group C (H & E \ X400)

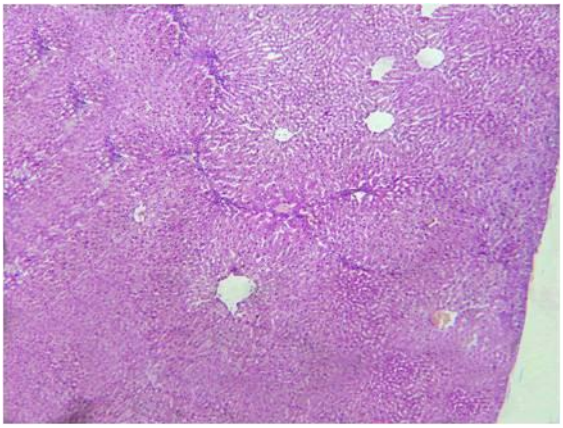

Fig. (14): Histopathological section in liver of rabbit showing that the liver appear like control (20 **days** post-operation of group C (H & E\ X 100)

Discussion and Conclusions

Results confirm that Q-Switched Nd:YAG laser with wavelength 532nm , 3.68 J/cm^{2}and 4Hz with 20% Human Serum Albumin Soldering, can produce better welding process on the incised liver tissue, with increase in mitosis of hepatocytes in compare with conventional treated group. This wavelength is readily absorbed by hemoglobin with deeper tissue penetration and thereby displays greater hemostatic capability (Yasmin, et al., 2000 and Kamegaya, et al., 2005).

In the present study the histopathological examination findings confirm that the laser irradiated group shows the fusing of the two edges of incision filled with red blood cells between the closely approximated cut surface of the liver, and Increase in mitosis of hepatocyte & Proliferation of granulation tissue containing large numbers of congested blood capillaries appear clearly after 72 hours post-operation without ischemic changes. After 20 days the liver appears like the control Group (A).

Quiescent hepatocytes become proliferative and restore liver functional capacity. Thermally denatured albumin near the surface of the incised surface was clear in the early stages of treatment. In the later stages, the repaired area of the liver is completely covered with early fibrous tissue and some residual denatured albumin is present in the outer surface that was being removed by phagocytes with infiltration of fibroblasts in the soldered site, finally the soldered liver surface had healed, covered by a layer of mature fibrous tissue and an outer capsule of dark elastic fibers walled off the entire region. The liver has a marked regenerative capacity; regeneration is mainly a result of hyperplasia and has a rapid onset. The onset of hepatocytes synthesis occurs 12-16 hours post-operatively, followed by a peak of mitosis at 32-34 hrs; the mass of liver is restored within 8-20 days. If laser soldering can indeed reduce the morbidity and mortality associated with bleeding, biliary leakage and sepsis following liver surgery, then it may be possible to resect directly invading tumors, and primary hepatomas buried deep within the parenchyma, because the raw liver surfaces could be soldered with the laser. As a conclusion from this work it can be said that this laser soldering technique has great promise, and could potentially reduce morbidity and mortality associated with liver injury.

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أصـــــلاح الكبـــــد في الأرانب بأستخدام ليزر النيديميوم ياك بطول موجي 532 نانومتر : دراســـة في الجســـم الحـــي

صبا يوسف تنو **علي شكر محمود**

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الخلاصة

إن الغرض من هذه الدراسة هو التقييم السريري والنسيجي لتأثير إستخدام أشعة الليزر من نوع النيديميوم ياك وبطول موجي 532 نانومتر مع استخدام مصل الألبومين البشري بتركيز 20% (كعامل مساعد في عملية اللحام) على أنسجة الكبد الحي .وقد استخدم في هذه الدراسة 21 أرنبا" ذكرا" بعد أن تم تقسيمهم الى ثلاث مجاميع رئيسية هي : مجموعة السيطرة ، مجموعة العلاج بإستخدام الطرق التقليدية و مجموعة العلاج بإستخدام أشعة الليزر . إن كلا من المجموعتين التي تمت معالجتها بالطريقة التقليدية وبإستخدام الليزر إحتوت على ثلاث مجاميع فرعية تم تقييمها بالإعتماد على مدى سرعة الإستجابة والتقبل للعلاج وخلال ثلاث فترات زمنية حددت حسب المتطلبات العلمية . أما مجموعة السيطرة فتركت بدون إجراء أي علاج عليها لغرض الحصول على القيم الطبيعية للفحوصات على الارانب المحلية . وكذلك تم إجراء دراسة نظرية أولية على كبد الأرانب بإستخدام قيم مختلفة الطاقة من اشعة الليزر للوصول إلى وتقييم أفضل إستجابة لعملية لحام أنسجة الكبد . إن مجاميع العلاج بالليزر أستخدم فيها الليزر من نوع (Q-switched Nd:YAG) بطول موجي قدره 532 نانومتر بعد إضافة مصل الألبومين البشري على الجرح الذي عمل في خلايا الكبد بإستخدام شعاع و بكثافة طاقة 3.68 جول/سم^2 و بتردد 4 هرتزومدة تشعيع بين 60-90 ثانية بوضع نهاية مباشر لقطعة اليد على الكبد . أما العلاج بالطريقة التقليدية فتم بإستخدام (surgciel) . وقد تمت متابعة المجاميع كافة وسريريا و أخذت نماذج من الكبد خلال ثلاث أوقات زمنية بعد إجراء العمليات على الحيوانات المعالجة ، حيث كانت الأوقات بعد أربعة أيام ، ثمانية أيام وعشرون يوماً من إجراء العملية لكل منها. وقورنت نتائج كلا المجموعتين الرئيسيتين المعالجتين بنتائج مجموعة السيطرة . بينت النتائج السريرية على سلامة وتفوق تقنية إستخدام الليزر في علاج الجرح الذي تم إحداثه في الكبد . أما إختبارات تحليل الأمراض النسيجي أظهرت مؤشرات لقابلية توليد كبيرة في الإنقسام الاعتيادي .