Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 56 (2): 29-38. Jan. 2018
DOI: 10.5455/ajvs.291349

The Adhesive Properties of Fibrin Glue Extracted from Bovine Plasma on Liver Wound Healing in Male Rats

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Key words:
liver, fibrin glue, wound, Haemostasis, Hemorrhage

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ABSTRACT

Fibrin sealant, or fibrin "glue," is a unique surgical haemostatic**,** sealant and adhesive material that is being used in a variety of surgical situations. It is a two-component system in which a solution of concentrated fibrinogen and factor XIII are combined with a solution of thrombin and calcium to form a coagulum, simulating the final stage of the clotting cascade. The present study was designed to examine the adhesive effect of ethanol extracted fibrinogen of bovine origin on rat liver wound healing. In the present study fibrinogen was extracted from bovine plasma using cold ethanol and prothrombin was extracted from bovine plasma by exposure to barium chloride. The study was conducted on sixteen mature white male rats. They were allocated into two equal groups (8 rats /group) and a surgical liver wound was induced in the left liver loop of each rat. In the first group (control) the wounds were exposed to slight manual compression with a sterile cotton tampon and then left for natural healing and in the second group (fibrin glue treated) the wounds were sealed using a combination of (bovine fibrinogen, prothrombin and thromboplastin calcium). After 3 and 6 days of wound induction four rats of each group were euthanized under light ether anesthesia, their abdomens were excised and livers were collected, washed by cold saline, grossly examined and fixed in neutral formalin for histopathological examination. Also blood samples were collected at the days 3 and 6 of experiment to obtain serum for biochemical estimations of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate Aminotransferase (AST), total protein, albumin , globulin , cholesterol ,high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL).Fibrin glue treated liver wound ,exhibited a grossly marked uniform healing which was confirmed by histopathological examination indicated by the presence of organized fibrous tissue at the site of wound and the adjacent part of liver tissue, normally appeared without degeneration. While in control group the site of liver wound showed uniform healing and there histopathological examination revealed unorganized fibrous tissue formation and the adjacent part of liver tissue exhibits variable degrees of degeneration. The liver function parameters in control group were adversely affected significantly compared to fibrin glue treated group.

1. INTRODUCTION

Fibrin glue is a biological tissue adhesive which imitates the final stages of the coagulation cascade when a solution of fibrinogen is activated by thrombin (the two components of fibrin glue) (Le Guéhennec et al., 2004)

Naturally, fibrinogen and fibrin play a key role in blood clotting .After a vessel injury, fibrin is formed as the lead of the clotting cascade, and blood cells are trapped to fibrin mesh. The fibrinogen molecule is comprised of two sets of three polypeptide chains, termed α, β, and γ, which are joined together in the N-

terminal E domain by five symmetrical disulfide bridges (Mosesson, 2005)

Fibrin also acts as a vector for delivering growth factors. Growth factors play an essential role in cell proliferation, migration, differentiation, and tissue regeneration. Fibrin is a modulator of macrophages activity and changer rate between wound inflammation and tissue repair. Endothelial cells injury stimulates the synthesis of platelet-activating factor (PAF) as a primary hemostasis process and wound healing (Lewis et al., 1988)

Stephen, 2008 reported that fibrin glue has the advantage of reducing time to hemostasis in vascular surgery compared with conventional manual compression.

In recent years, a range of topical hemostatic agents and techniques became available that led to surgical success. These products, which are classified as topical hemostats, sealants, and adhesives, would be fast-acting, non-antigenic, easily applied and removed, inexpensive, stable and transportable with few side-effects (Gruen et al., 2012 & Zentai et al., 2014)

Traumatic injuries are the primary cause of death in 1 to 34 years-old people and are the fifth leading cause of death (Holcomb, 2004). In trauma handling, hemorrhage management plays an important role in surgery success and saving patients' lives (Paydar et al., 2014& Zentai et al., 2014)

Approximately, 25 percent of liver injuries lead to mortality. After liver injuries, when internal bleeding occurs, coagulopathy can happen as a result of hypothermia, dilution of clotting factors, and thrombocytopenia (Navarro & Brooks, 2015). Thus, the current investigation was designed to evaluate the adhesive effect of ethanol extracted fibrinogen of bovine origin on liver wound healing against natural healing.

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2. MATERIALS AND METHODS

2.1. Materials, chemicals, buffers and reagents

Ethylene Diamine Tetra-acetic Acid (EDTA), 372.24(M.W) and Ethanol 97%, El- Gomhoria Pharmaceutical Chemicals Company, Egypt. Barium chloride, 208.23 g (M. W) , and Sodium citrate,258 (M.W), Delta Laboratory Chemical, Egypt L-Lysine Hydrate, 145.188, (M.W) and ammonium sulfate crystal reagent, 132.14 (M.W), Dop Organik Kimya San.VE_TIC.LTD.STI. Tris (Tris (Hydroxymethyl) Amino methane), 121.14 (M.W), Bio Shop ® Canada. Phenyl Methyl Sulfonyl Fluoride and ammonium hydroxide, 40 (M.W), Sigma Aldrich USA ،BioMed-Liquiplastin for prothrombin time (P.T) determination , Diamond Diagnostic Co., Egypt. Resuspension buffer was prepared as follow: 2.42g tris-base, 15.68g sodium citrate, 3.92 g lysine and distilled water to one liter. The pH was adjusted to 6.8.

Dialysis buffer was prepared as follow: 5.16g sodium citrate, 5.89g sodium chloride and distilled water to one liter, The pH was adjusted to7.4.

2.2. Extraction of fibrinogen rich protein:

Fibrinogen rich protein from bovine plasma samples was extracted by precipitation using cold ethanol according to the method of Burnouf-Radosevich et al. (1990).

2.2.A. Blood collection:

Fresh bovine blood was taken from a local slaughterhouse ,and 10% volume of 3.8%trisodium citrate **(w/v)** was added. The blood was centrifuged for 30 minutes at 3000 r.p.m. The plasma was stored at -80°C.

2.2.B. Fibrinogen extraction by ethanol :

450 ml frozen plasma at -80 °C was thawed slowly for two days at 4 °C and centrifuged at 4000 r.p.m. for 20 minutes at 4 °C. Then the precipitate was resuspended in the resuspension buffer containing 20 mM tris-base, 55 mM sodium citrate, 27 mM lysine, pH 6.8. 50 ml of 10% ethanol was added to 450 ml plasma. The Sample was incubated on ice overnight then centrifuged in cooling centrifuge at 4000 rpm for 20 min at 4 °C. The supernatant was discarded and the precipitate was resuspended again in resuspension buffer. A second 10% ethanol precipitation step was carried out at 4 °C for two hours on ice. The precipitate collected from the second ethanol precipitation was resuspended in resuspension buffer and dialyzed against dialysis buffer -Dialysis buffer containing 20 mM sodium citrate, 100 mM sodium chloride, pH 7.4 at 4°C. The dialyzed protein was collected and centrifuged for 20 min at 4000 rpm at 25 °C.

The precipitate rich in extracted fibrinogen was lyophilized in freeze dryer (Alpha 2-4 LD plus, Sigma USA) and stored at 4 °C till use.

2.3. Extraction of prothrombin:

The aim of this procedure was to first purify prothrombin from plasma and then its activation to thrombin by thromboplastin. The procedure was mainly modified from the methods of Bajaj & Mann, 1973 and Bajaj et al., 1981.

2.3.A. Bovine blood collection:

Fresh bovine blood was taken from a local slaughterhouse and 10% volume of 4% trisodium citrate (w/v) was added. The blood was centrifuged twice for 30 minutes at 2000 x g at 4'C. The plasma was used immediately or stored at -2O C for later use.

2.3. B. Enrichment of prothrombin:

120 ml of 1 M BaCl2 was added to 1 liter of citrated plasma at a rate of one drop per second (with gentle stirring to adsorb prothrombin onto barium citrate so that the prothrombin was separated from the plasma. Stirring was continued for 20 minutes. The mixture was allowed to stand for 30 minutes and centrifuged for 30 minutes at 3600 x g. The barium citrate precipitate was dissolved in 320 ml of 0.9% NaCl (w/v), 0.02 M trisodium citrate by slowly stirring while 80 ml of 1 M BaCl2 was added drop wise. Stirring was continued for 20 minutes. After adding all BaC12 the mixture was allowed to stand for more than 1 hour and then centrifuged for 30 minutes at 3600 x g. By stirring, the barium citrate precipitate was suspended in 120 ml of cold 0.2 M Na2EDTA (pH 7.4) in which the barium was effectively removed from the barium citrate complex, freeing the prothrombin. The suspension was dialyzed for 40 minutes against 15 volumes of 0.02 M Na2EDTA, pH 7.4, 0.02 M trisodium citrate, 0.9% NaCl with gentle stirring. Dialysis was continued against 15 volumes of 0.02 M trisodium citrate, 0.09% NaCl for more than 5 hours with the dialysate changed every 1 hour, and the dialysis bag was gently mixed to ensure complete mixing at all times. The sample was centrifuged for 30 minutes at 3600 x g and the precipitate was discarded. Saturated ammonium sulphate solution (pH 7.4, adjusted with concentrated NaoH) was added drop wise to the supernatant to a final concentration of 40% saturation (v/v) while stirring slowly. Stirring was continued for 20 minutes. The sample was centrifuged for 30 minutes at 3600 x g. Saturated ammonium sulfate was added drop wise to the supernatant under gentle stirring until 60% saturation (v/v). Stirring was continued for 20 minutes. The mixture was allowed to stand for 1 hour and was then centrifuged for 30 minutes at 3600 x g. The precipitate was dissolved in 20 ml of 0.025 M trisodium citrate buffer, pH 7.4, containing Phenyl Methyl Sulfonyl Fluoride (PMSF) (1 mg/ml). The suspension was dialyzed against 15 volumes of 0.025 M trisodium citrate buffer (pH 7.4) overnight with two changes. The dialyzed sample was centrifuged for 30 minutes at 7200 x g to remove the precipitate. The supernatant containing crude prothrombin was used for hydrolyzing (activating) to thrombin.

2.3. Animals and experimental design:-

Thirty two mature white male rats aged 6 months and with an average weight 200 ± 20g were allocated into two groups (16 rats/group).Rats were provided with food (crushed corn grain, bread and milk) and water ad-libitum. Each rat was housed in a separate cage throughout the experimental period.

1. 1st group (16 rats): served as a control group, the surgically induced wound was left for natural healing.
2. 2nd group (16 rats): the surgically induced wound was treated by ethanol extracted fibrin glue of bovine origin,1000 mg of lyophilized fibrinogen rich protein with fibrinogen concentration of 397 mg/dl was firstly suspended in 4 ml saline as a stock solution (addition of equal amounts of fibrinogen , prothrombin and thromboplastin calcium solutions with a ration of 1:1:1).

Following induction of inhalation anesthesia using ether, the abdomen was opened by a medial skin incision wound of 3 cm length. The topography of liver was determined and a portion of the main left liver lobe was resected using a scalpel to induce a wound of approximately 1 cm length. Sterile gauze was used to remove blood leaking from the fresh wound. The wound was then completely covered with fibrin glue, and hemostasis or blood loss was determined over the 5 minutes from wound induction. In our present study one application was quite sufficient to achieve heamostasis. No other method was employed to halt blood loss from the cut surface. After hemostasis was established, the liver was repositioned into the abdomen and irrigated with 5 ml sterile saline solution. The abdomen was then closed using standard surgical procedures. In control group the same procedure was performed without the use of fibrin glue and the bleeding control was achieved only by using a slight manual compression with a sterile cotton tampon.

2.4. Tissue sampling:-

At days 3 and 6 of the experiment half of the rats from each 1st group and 2nd group were euthanized under light ether anesthesia, their abdomens were excised and livers were collected, washed by cold saline 0.9%, grossly examined and fixed in neutral buffered formalin 10% for histopathological examination.

2.5. Blood sampling:-

Blood samples were collected (at days 3 and 6) from the retro-orbital venous plexus of each rat before being euthanized under light ether anesthesia. The blood samples (about 3 ml of blood) were placed in plain centrifuge tubes, left 30 minutes in slope position at room temperature to clot and centrifuged for 15 at 3000 rpm minutes for separation of serum. The clear serum was carefully separated, then transferred into clean dry epindorffs and kept frozen at –20°C until used for biochemical analysis.

2.6. Serum biochemical parameters:-

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured calorimetrically according to method of (Reitman and Frankle, 1957) using (kits from Bio-labo, France). Serum alkaline phosphatase (ALP) activity was measured kinetically according to Belfield and Goldberg (1971). The level of serum total proteins, serum albumin, serum total and direct bilirubin, serum total cholesterol (TC) and triglycerides (TG), High density lipoprotein-cholesterol (HDL) were determined according to the previously described methods of Doumas et al., 1981; Doumas and Biggs, 1972; Walter and Gerade, 1970; Friedman and Young, 1997; Burstein et al., 1980; respectively, using commercial diagnostic kits supplied by Vitro Scient. Co. Egypt.

2.7. Histopathologic examination:-

Following necropsy, small specimens of liver containing the site of wound were collected from control and treated rats, and rapidly fixed in 10% neutral buffered formalin for 24 hrs. After fixation, tissue specimens were dehydrated in ascending grades of alcohol, cleared by xylene, then were processed through the conventional paraffin embedding technique. 5 µm thick sections were obtained from paraffin blocks, stained with hematoxylin and eosin (H&E) (Harries, 1989) and examined under light microscope.

2.8. Statistical analysis:-

Data were statistically analyzed using the Statistical Analysis System software (SAS, 2011). Effect of treatments on biochemical parameters was performed by the analysis of variance. Means were compared using Duncan's Multiple Range test at a significance level of $P \leq 0.05$. Values are presented as means $\pm$standard errors.

3. RESULTS

In fibrinogen treated group, all animals survived the surgical procedures (mortality rate =0%).but in control group, only 50% of animals survived the surgical procedure and 50% died during 1st 24 hours after surgical operation. .

3.1. Serum biochemical assays

As recorded in Table (1), There was insignificant decrease in level of AST (U/L) in fibrin glue treated group and natural healing group after 6 days compared to 3 days (22.08 ± 1.31 VS 27.33 ± 2.88 and 32.43 ± 2.35 VS 35.53 ± 4.16 , respectively). and also there was a significant decrease in its value in fibrin group compared to natural healing group after 3 and 6 days(27.33 ± 2.88 VS 35.53 ± 4.16 and 22.08 ± 1.31 VS 32.43 ± 2.35 ,respectively).

The serum ALT (U/L) activity showed a significant decrease in fibrin glue treated group after 6 days of surgical liver wound induction compared to 3 days (89.9 ± 4.64 VS 108.73 ± 7.51, respectively) and also there was a significant increase in its value in natural healed group after 6 days compared to 3 days (146.33 ± 4.33 VS 134.38 ± 5.89, respectively). Moreover there was a significant decrease in its value in fibrin glue treated group compared to natural healing group after 3 and 6 days (108.73 ± 7.51 VS 134.38 ± 5.89 and 89.9 ± 4.64 VS 140.33 ± 4.33) , respectively).

There was a significant decrease in AlP (U/L) activity after 6 days compared to 3 days in both fibrin group and natural healing group (156.3 ± 3.57 VS 185.0 ± 5.93 and 178.0 ± 5.72 VS 198.2 ± 4.52, respectively). No significant differences in activity of ALP in fibrin group compared to natural healing group after3 (185.0 ± 5.93 VS 198.2 ± 4.52) but a significant decrease in its activity in treated group after 6 days (156.3 ± 3.57 VS 178.0 ± 5.72, respectively).

Data presented in table (2) showed a significant increase in the level of serum total protein (g/dl)in fibrin glue treated group after 6 days compared to 3 days (7.45± 0.62 VS 5.77 ± 0.52) and a significant increase in its value in fibrin glue treated group compared to natural healing group after 3 days (5.77 ± 0.52 VS 5.34 ± 0.14) .Moreover there were insignificant increase in its value in fibrin glue treated compared to natural healing group after 6 days and also in natural healing group after 6 days compared to 3 days (7.45 ± 0.26 VS 6.25 ± 0.29 and 6.25 ± 0.29 VS 5.34 ± 0.14, respectively).

There was insignificant increase in level of serum albumin (g/dl) in fibrin glue treated group after 6 days compared to 3 days (4.63 ± 0.44 VS 3.83 ±

0.57). Moreover there were in significant increase in its value in fibrin glue treated group compared to natural healing group after 3 days and significant increase after 6 days (3.83 ± 0.57 VS 2.78 ± 0.13 and 4.63 ± 0.44 VS 3.05 ± 0.09), respectively).

Serum globulin (g/dl) showed a significant increase in fibrin glue treated group after 6 days compared to 3 days (2.82 ± 0.29 VS 1.93 ± 0.21).Moreover there were significant decrease in its value in fibrin glue treated compared to natural healing after 3 days (1.93 ± 0.21 VS 2.57 ± 0.02) and in significant decrease after 6 days (2.82 ± 0.29 VS 3.20 ± 0.32).

There were a significant decrease in total bilirubin level (mg/dl) in fibrin glue treated group after 6 days compared to 3 days (0.50 ± 0.02 VS 0.67 ± 0.04) and in significant decrease in its value in natural healing group after 6 days compared to 3 days (0.64 ± 0.04 VS 0.71 ± 0.04).Moreover there were in significant decrease in its value in fibrin glue treated group compared to natural healing group after 3 days (0.67 ± 0.04 VS 0.71 ± 0.04) and significant decrease after 6 days (0.50 ± 0.02 VS 0.64 ± 0.04).

Data presented in table (3) showed insignificant decrease in total cholesterol (mg/dl) level in fibrin glue treated after 6 days compared to 3 days (90.9± 2.59 VS 95.1 ± 4.36) and a significant decrease in its value in natural healing after 6 days compared to 3 days (77.6 ± 1.98 VS 96.7 ± 2.57).Moreover there were insignificant decrease in its value in fibrin glue treated group compared to natural healing group after 3 days and significant increase in its value after 6 days (95.1 ± 4.36 VS 96.7 ± 2.57 and 90.9 ± 2.59 VS 77.6 ± 1.98 , respectively).

Triglyceride (mg/dl) level revealed a significant decrease in fibrin glue treated and natural healing group after 6 days compared to 3 days (167.3 ± 15.2 VS 239.0 ± 42.5) & (130.7 ± 2.49 VS 138.7 ± 8.36),respectively. Moreover there were significant increase in its value in fibrin glue treated compared to natural after 3 and 6 days (239.0 ± 42.5 VS 138.7 ± 8.36) and (167.3 ± 15.2 VS 130.7 ± 2.49) respectively.

There were a significant increase in level of HDL (mg/dl) in fibrin glue treated group after 6 days compared to 3 days (37.5 ± 4.99 VS 15.7 ± 1.88) .Moreover there was a significant increase in its value in fibrin glue treated compared to natural healing after 6 days (37.5 ± 4.99 VS 23.4 ± 4.11), but insignificant decrease after 3 days (15.7 ± 1.88 VS 26.4 ± 3.75).

There was a significant decrease in level of low density lipoprotein (mg/dl) in fibrin glue treated after 6 days compared to 3 days (28.0 ± 2.35 VS 51.4 ± 6.57) and insignificant decrease in natural healing group (19.8 ± 3.37 VS 21.5 ± 6.37).Moreover there were significant increase in fibrin glue treated compared to natural healing group after 3 and 6 days (21.5± 6.37 VS 51.4 ± 6.57 and 28.0 ± 2.35 VS19.8 ± 3.37, respectively).

There was no significant difference in level of VLDL(mg/dl) in fibrin glue treated group after 6 days compared to 3 days (26.1 ± 1.10 VS 26.3 ± 0.91) and significant decrease in its value in natural healing group (33.4 ± 3.04 VS 47.8 ± 8.50) . Moreover there were significant decrease in its value in fibrin glue treated compared to natural healing group after 3 and 6 days(26.3 ± 0.91 VS 47.8 ± 8.50 and 26.1 ± 1.10 VS 33.4 ± 3.04, respectively).

3.2. Histopathological results:

Liver of the control group, Fig. 1 (on the left) showing fibrosis at site of wound (un organized fibrous tissue) and hepatocytic degeneration in the remaining part of liver after 3 days. While Liver of treated, Fig.1 (on the right) showing hepatocytic regeneration at site of wound and the remaining part of liver healthy showing normal histological appearance after 3 days.

Liver of the control group**,** Fig. 2 (on the left) showing large area of fibrosis (un organized fibrous tissue) and replacement of injured tissue by a collagenous scar, hepatocytic degeneration and cirrhosis after 6 days.

Liver of treated group Fig. 2 (on the right) showing fibrosis (organized fibrous tissue) only at the site of wound and the remaining part of liver was not affected (healthy) showing normal histological appearance after 6 days.

Table 1: Effect of fibrin glue treatment in surgically induced liver wounds versus natural healing on activities of Aspartate Aminotransferase (AST) (U/L) , Alanine Aminotransferase(ALT) (U/L) and alkaline phosphatase (ALP) (U/L) after two periods (3 and 6 days) of treatment in rats.

Parameter	Group	3 days	6 days
AST(U/L)	Fibrin glue treated	27.33 ± 2.88 a	22.08 ± 1.31 a
	Natural healing	35.53 ± 4.16 * a	32.43 ± 2.35 * a
ALT(U/L)	Fibrin glue treated	108.73 ± 7.51 *b	89.9 ± 4.64 a
	Natural healing	134.38 ± 5.89 b	146.33 ± 4.33 * a
ALP(U/L)	Fibrin glue treated	185.0 ± 5.93 a	156.3 ± 3.57 b
	Natural healing	198.2 ± 4.52 a	178.0 ± 5.72 *b

Values are means ± standard errors.
* Groups within period differ significantly (P<0.05).
Means within group (row) followed by different letters differ significantly (P<0.05).

Table 2: Effect of fibrin glue treatment in surgically induced liver wounds versus natural healing on levels of serum total protein(g/dl) , Serum albumin(g/dl) , Serum globulin(g/dl) and total bilirubin (mg/dl)after two periods (3 and 6 days) of treatment in rats.

Parameter	Group	3 days	6 days
Total protein(g/dl)	Fibrin glue treated	5.77 ± 0.52 b	7.45 ± 0.62 a
	Natural healing	5.34 ± 0.14 a	6.25 ± 0.29 a
Serum albumin(g/dl)	Fibrin glue treated	3.83 ± 0.57 a	4.63 ± 0.44 a
	Natural healing	2.78 ± 0.13 a	3.05 ± 0.09 *a
Serum globulin(g/dl)	Fibrin glue treated	1.93 ± 0.21 b	2.82 ± 0.29 a
	Natural healing	2.57 ± 0.02 a	3.20 ± 0.32 a
Total bilirubin(mg/dl)	Fibrin glue treated	0.67 ± 0.04 a	0.50 ± 0.02 b
	Natural healing	0.71 ± 0.04 a	0.64 ± 0.04 *a

Values are means ± standard errors.
* Groups within period differ significantly (P<0.05).
Means within group (row) followed by different letters differ significantly (P<0.05).

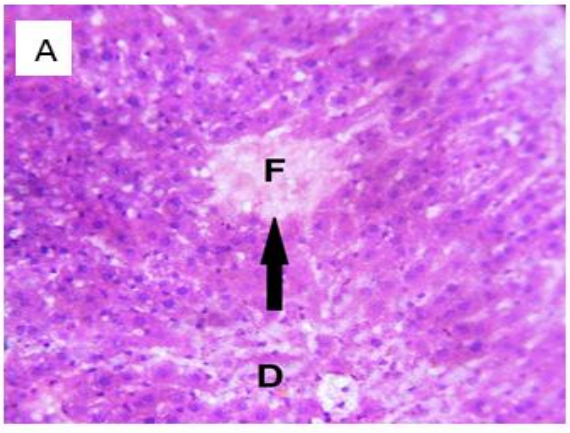

Fig. 1.A. Photomicrograph of control 3 days old rat liver surgical wound left for natural healing showing fibrosis at site of wound (un organized fibrous tissue) (F and arrow) and hepatocytic degeneration (D) (H &E, x 400)

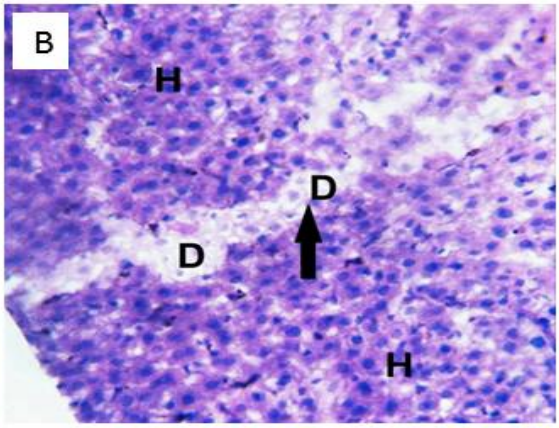

Fig. 1.B. Photomicrograph of treated 3 days old rat liver surgical wound using ethanol extracted bovine fibrin sealant showing hepatocytic regeneration at site of wound (D) and the remaining part of liver tissue surrounding the wound is healthy (H) (H & E,x 400)

Table 3: Effect of fibrin glue treatment in surgically induced liver wounds versus natural healing on total cholesterol(mg/dl) ,triglycerides(mg/dl) , high density lipoprotein (HDL) (mg/dl),low density lipoprotein (LDL)(mg/dl) and very low density lipoprotein (VLDL) (mg/dl) after two periods (3 and 6 days) of treatment in rats.

Parameter	Group	3 days	6 days
Total cholesterol(mg/dl)	Fibrin glue treated	95.1 ± 4.36 a	90.9 ± 2.59 a
	Natural healing	96.7 ± 2.57 b	77.6 ± 1.98 *a
Triglycerides(mg/dl)	Fibrin glue treated	239.0 ± 42.5 *a	167.3 ± 15.2* b
	Natural healing	138.7 ± 8.36 a	130.7 ± 2.49 b
HDL(mg/dl)	Fibrin glue treated	15.7 ± 1.88 a	37.5 ± 4.99 *b
	Natural healing	26.4 ± 3.75 a	23.4 ± 4.11 a
LDL(mg/dl)	Fibrin glue treated	51.4 ± 6.57 a	28.0 ± 2.35 b
	Natural healing	21.5 ± 6.37 *a	19.8 ± 3.37 a
VLDL(mg/dl)	Fibrin glue treated	26.3 ± 0.91 a	26.1 ± 1.10 a
	Natural healing	47.8 ± 8.50 *a	33.4 ± 3.04 b

Values are means ± standard errors.
* Groups within period differ significantly (P<0.05).
Means within group (row) followed by different letters differ significantly (P<0.05).

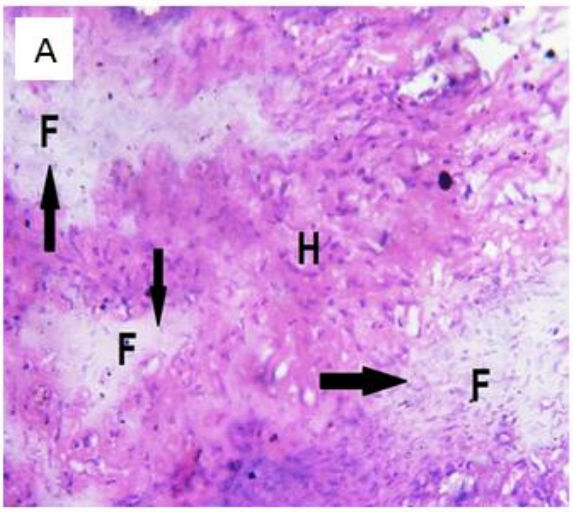

Fig.2.A. Photomicrograph of Six days old control rat liver surgical wound (left for natural healing) showing large area of fibrosis (un organized fibrous tissue) (F), hepatocytic degeneration (arrows) and cirrhosis (H&E,x 400).

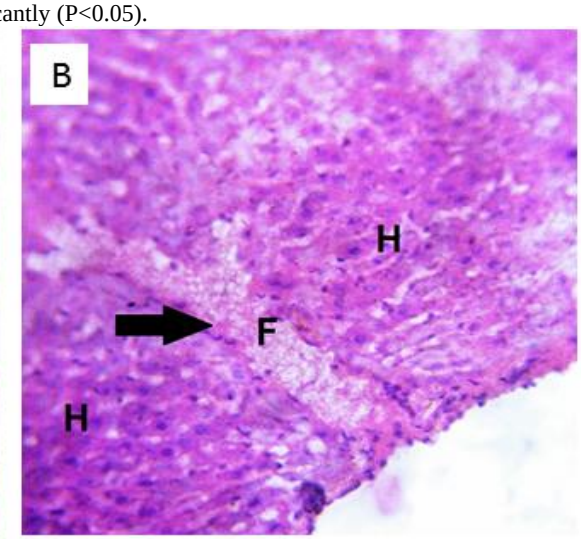

Fig. 2. B. Photomicrograph of Six days old rat treated liver surgical wound using ethanol extracted bovine fibrin sealant showing fibrosis (organized fibrous tissue) only at the site of wound (F) and the remaining part of liver tissue is not affected showing healthy hepatocytes and normal liver architecture (H) (H &E,x400)

4. Discussion

Liver and other parenchymatous organs are at the risk of various types of abdominal trauma, with a mortality rate greater than 60% ,Uncontrollable hemorrhage, coagulopathy, multiple organ failure, and sepsis are the consequences of abdominal injuries, which cause a high mortality rate (Pusateri et al.,2003).

Fibrin is a natural substance with a high potential for application in tissue engineering and wound healing (Mendez et al., 2015).

The findings of this study showed a 50%mortality rate in control group left for natural healing while no mortalities were recorded in treated group (fibrin glue treated liver wound). The highly significant decrease in mortality rate could be a consequence of the observed decrease in blood loss during the procedure in test group in comparison to the control agree with the findings of (Mehrzad et l., 2017).

The mortality rate in control groups could be probably caused by internal hemorrhage and liver trauma, which was successfully controlled by fibrin glue treatment of liver wounds in test group. Fibrin activities showed cell proliferation, migration, differentiation, and tissue regeneration as reported by (Banihashemi et al., 2015) and other studies have shown successful hemorrhage control with fibrin sealants in the presence of coagulopathy and in a trauma setting (Holcomb et al., 1999& Pusateri et al., 2004).

The serum AST, ALT, ALP and bilirubin are the most sensitive biochemical markers employed in the diagnosis of hepatic dysfunction (Nnodim et al., 2010).

In the present study), the serum ALT(U/ml) and AST(U/ml) activity level were significantly decrease in fibrin glue treated group after 6 days of surgical liver wound induction compared to 3 days after surgery and also compared to control group in which ALT level showed a significant increase after 6 days these findings agree with the fact that Serum AST and ALT activities are used as very sensitive markers for necrotic lesions within the liver due to their easy liberation from the hepatocyte cytoplasm into the blood stream (Kirchain and Gill, 1997) as a result of membrane lipid peroxidation (Wendel et al., 1979 & Knight et al.,2003). Activity of serum ALT can be used as a marker for the assessment of hepatobiliary damage in rats (Boone et al., 2005).

There was a significant decrease in AlP (U/ml) level after 6 days compared to 3 days in both fibrin group and natural healing group, Moreover there was in significant decrease in level of ALP in fibrin group compared to natural healing group after3 days. While a significant decrease in its value after 6 days in fibrin treated compared to control this agree with (Demirel et al., 2008) they found AST, ALT and ALP activates were lower in fibrin sealant group on the 3rd, 10th and 20th as compared to the primary suture group. Also agrees with fact that alkaline phosphatases are present in many tissues, including bone, intestine, kidney, liver, placenta and white blood cells. Damage to these tissues causes the release of ALP into the blood stream and increases its value more than normal (Kaplan &Marshall, 1972).

The total proteins and albumin showed significant high values in fibrin glue treated rats compared to natural healing group (control), also there was a significant decrease in level of total bilirubin in fibrin glue treated after 6 days compared to control. These findings could be attributed to the significant liver damage in control group as in case of advanced cirrhosis they have hypoalbuminemia caused both by decreased synthesis by the hepatocytes and water and sodium retention that dilutes the content of albumin in the extracellular space. Other factors likely contribute to the development of hypoalbuminemia, including an increased transcapillary transport rate (Henricksen et al., 2001). Moreover, bilirubin and albumin values are associated with the function of hepatic cells (Muriel et al., 1992).

The results of the present study indicated that serum lipids profile of total cholesterol, triglycerides, and LDL values were significantly lower in control group compared to treated these finding could be attributed to that total cholesterol, HDL and LDL levels decrease gradually with progression of cirrhosis and also this is reasonably expected as liver biosynthesis has been reduced (Phukan et al., 2013).

Liver has a crucial role in lipid metabolism, many stages of lipid synthesis and transportation. Presumably, it is reasonable to expect an abnormal lipid profile in cases of severe liver dysfunction (Halsted, 2004). HDL, LDL, total cholesterol and TG were significantly lower in patients with liver cirrhosis compared to control, with the amount of decrease in the serum values of HDL, LDL, and total cholesterol had a positive correlation with the severity of liver damage (Ghadir et al., 2010) which agree the histological findings in the present study, where control livers showed excessive fibrosis and variable degrees of hepatic tissue degeneration.

Histopathology sections from the fibrin glue treated liver wound specimens showed that the site of the previous incision was completely repaired, and a dense organized fibrous septum was observed in treated group. On the other hand control livers showed a significant formation of fibrous tissue with large area of fibrosis (un organized fibrous tissue and replacement of injured tissue by a collagenous scar. Concluding better healing in treated groups and this agree with the findings of (Mehrzad et al., 2017).

Excessive liver fibrosis in control liver could be resulted from the perpetuation of the normal wound healing response leading to abnormal continuation of fibrogenesis (connective tissue production and deposition (Schiff et al., 2003). The histological findings obtained also showed that hepatic healing was at higher rates in the fibrin sealant group than in the other group, These results of this study are in agreement with (Bezerra et al., 1999 & Bezerra et al., 2001) who reported that fibrin sealant stimulates plasminogen activators had an important additive role in liver regeneration by their contribution on remodeling of the liver.

Fibrin sealant mimic the final stages of the blood haemostasis cascade to produce a stable fibrin clot, and are widely used to assist in the repair and stabilization of wounds and to support physiological healing mechanism (Jackson, 2001). Fibrin also acts as a vector for delivering growth factors. Growth factors play an essential role in cell proliferation, migration, differentiation, and tissue regeneration.

Fibrin is a modulator of macrophages activity and changer rate between wound inflammation and tissue repair. Endothelial cells injury stimulates the synthesis of platelet – activating factor (PAF) as a primary hemostasis process and wound healing (Lewis et al., 1988)

In conclusion : In the present study ,sealing of liver wound using fibrin glue managed to give a better hemorrhage control , uniform wound healing with less formation of fibrous tissue and reduced the negative impact of liver trauma caused by wound on liver function, recommending its use in veterinary surgery as a lifesaving procedures .

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