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Assessment of the hemostatic activity of "hemoben" in the model of liver damage on hypocoagulation

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***Abstract*---**In the article, the authors present the results of experimental studies on the hemostatic properties assessment of the new domestic hemostatic implant Hemoben under artificial hypocoagulation using medications on a model of a planar wound in the liver of rats. An attempt was made to stop bleeding using gauze strips and balls and a hemostatic sponge in the control group of animals. Experimental and morphological studies have convincingly proved that under drug hypocoagulation, Hemoben provides fine and stable hemostasis, which significantly exceeds the parameters of a hemostatic sponge.

***Keywords*---**hypocoagulation, hemostatic, hemoben.

Relevance

Treatment aspects of parenchymal organs damage and performing operative interventions on the abdominal organs at present is that the number of people who are forced to systematically use drugs that reduce blood clotting in the background of concomitant diseases of the cardiovascular system has increased significantly [3, 4, 5]. Also the consequence of the COVID-19 pandemic is the use of drugs that lower the function of the blood coagulation system [1, 6]. Under these conditions, stopping bleeding and its control subsequently becomes an urgent task requiring a solution. Most topical agents are ineffective in conditions of hypocoagulation [2, 7, 8].

Materials and Methods

Studies were carried out to assess the hemostatic properties of a new domestic hemostatic implant Hemoben under conditions of artificial hypocoagulation using medications. The study groups included experiments on white outbred laboratory rats weighing 220-280 g. Animals were kept in separate cages of 4 individuals in a vivarium with good nutrition and a constant water supply. The aspirin drug was used to reduce blood coagulation in the experiment, which laboratory animals took for two weeks at the rate of 2.5 mg of the drug per 250.0 g of animal weight three times a day with meals.

Animals of the pilot batch received aspirin at the above dose by dissolving an aspirin tablet in water. Animals of the control group received a placebo. Experiments with the simulation of a planar wound of the liver were carried out two weeks after the preparatory phase. The technique consists of a planar wound formation of the left lobe of the liver along the anterior surface using an abrasive material to a depth of 1-2 mm. Given the size of the rat's liver, we can assume that the bleeding was of a mixed nature with a sufficient degree of intensity. Hemoben hemostatic powder was used to stop bleeding. It was used until permanent hemostasis. In the control group of animals, an attempt at bleeding control was made using gauze strips and balls.

The observation was carried out after hemostasis for 10 minutes to control its stability. Subsequent studies at the macro and microscopic level of healing and change processes of the liver wound in laboratory parameters were carried out 1, 3, 5, 7, and 14 days after the operation.

The experiments were carried out on an empty stomach in the morning. Anesthesia was performed with inhalation of anesthetic isoflurane. The left lobe of the liver was removed into the wound after a median laparotomy up to 3 cm long. After the damage to the liver surface with an abrasive material, bleeding of a mixed nature occurred.

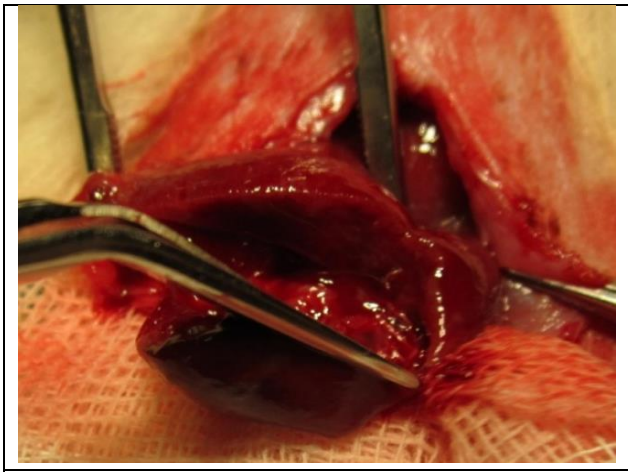

Fig. 1. Performing liver resection in a rat

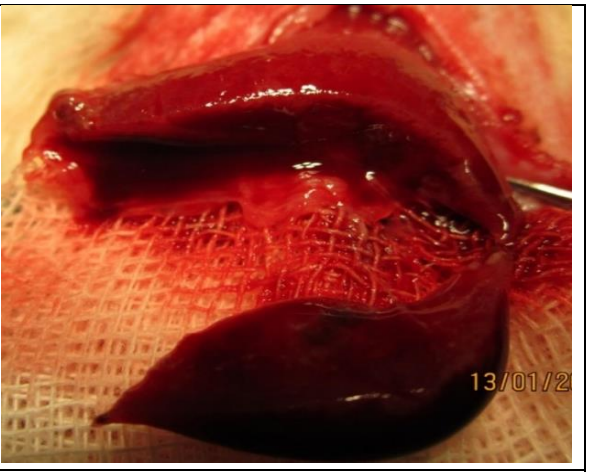

Fig. 2. Voluminous bleeding surface of the resected part of the liver

Bleeding in the control group practically did not stop without gauze balls. A short-term bleeding control occurred in the background of blood pressure lowering, but the bleeding resumed after a short period. Liver wound meticulous hemostasis could be achieved using up to 4-8 gauze balls 1x1 cm in size. The average time for a meticulous hemostasis was 430-600 sec. Blood loss, measured by weighing gauze balls before and after bleeding control, was 2-3 ml. Monitoring the condition of the liver wound for 10 minutes revealed episodes of rebleeding in 50% of cases.

Fig. 3. Application of hemostatic powder on the resected part of the liver

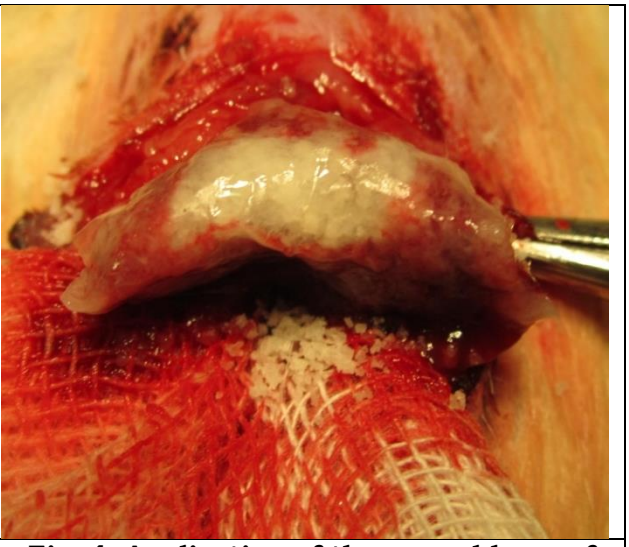

Fig. 4. Application of the second layer of hemostatic film on the resected surface of the liver

In animals of the pilot batch, bleeding was stopped by powdering Hemoben hemostatic powder on the wound immediately after the surface was dried with a

gauze ball (Fig. 1-7). Results of the studies showed that it was necessary to use a 2-fold volume of the powder to stop the bleeding, which, according to the instructions, was 1 g per 100 cm2 of the wound surface.

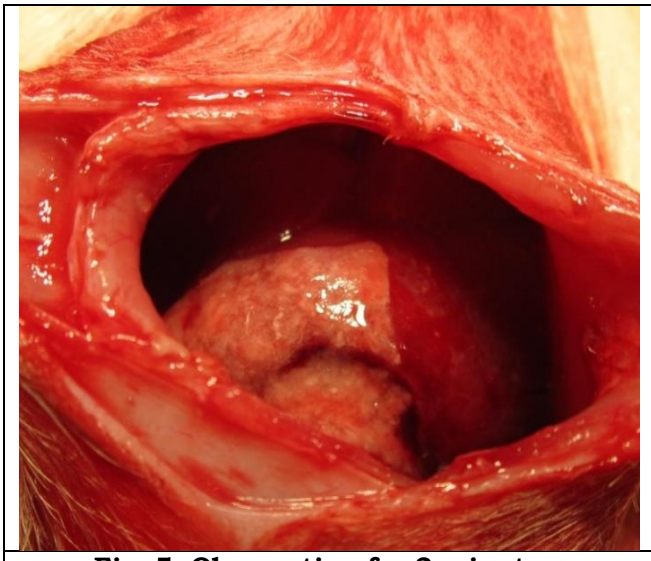

Fig. 5. Observation for 2 minutes, no rebleeding

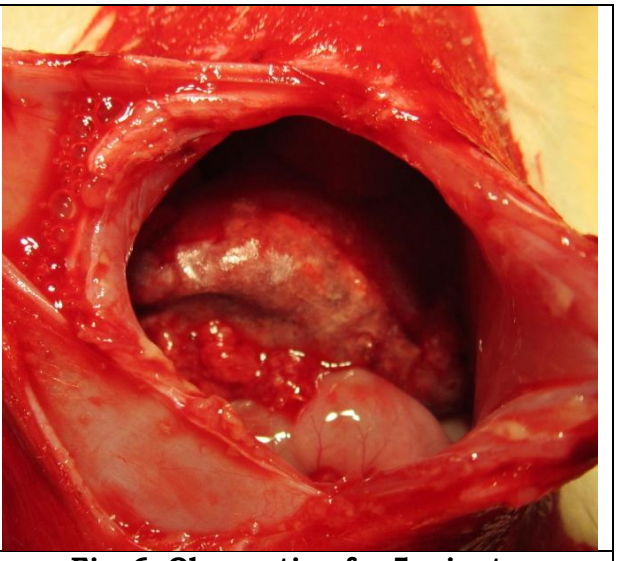

Fig. 6. Observation for 5 minutes, no rebleeding

Our observations showed that for 1 cm2 of the liver wound surface, it was necessary to use the powder 2 or 3 times at the rate of 10 mg per 1 cm2 of the liver wound. The average drug consumption was 35.5±2.3 mg/cm2. The research results showed that hemostasis after applying the hemostatic powder occurred in a short time. The adhesion and strength of the powder contribute to the mechanical stop of bleeding vessels. Meticulous hemostasis after using Hemoben was observed within 90-210 seconds, on average 175.0±23.3 seconds. Blood loss, calculated by weighing gauze balls, was mere 1.2 ml. It should be noted that the time of hemostasis with the use of Hemoben powder was significantly reduced when applying gauze strips sprinkled with Hemoben powder. It was enough to apply strips 1 or 2 times so that hemostasis was achieved within 30-80 seconds after pressing the strip to the wound. Blood loss in this case was insignificant and amounted to less than 0.5 ml.

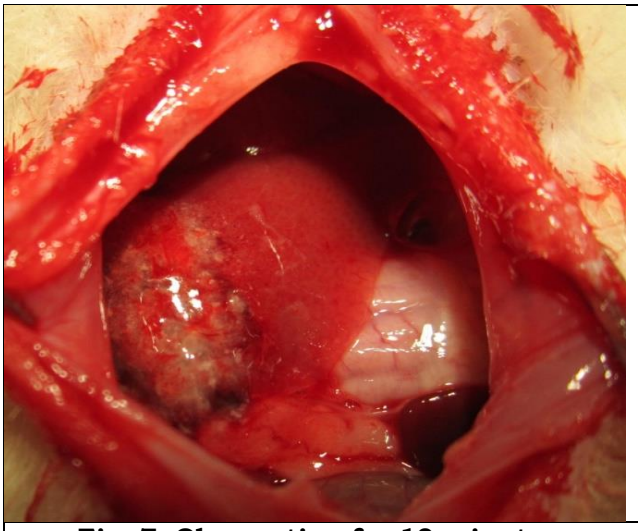

Fig. 7. Observation for 10 minutes, no rebleeding

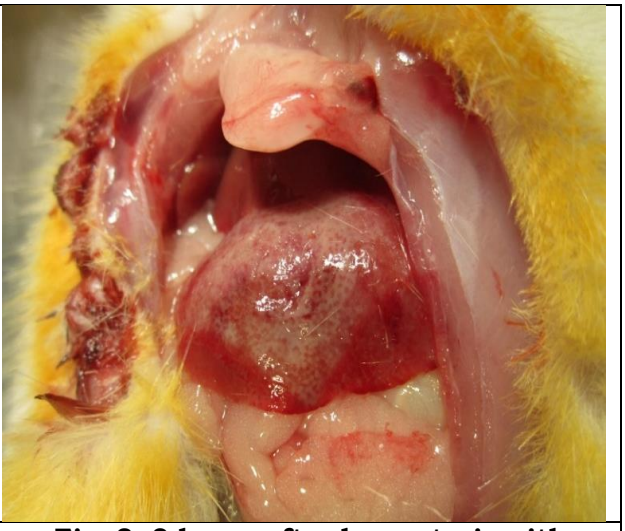

Fig. 8. 3 hours after hemostasis with Hemoben powder

Rebleeding after hemostasis during the observation period for 10 minutes was 15%. As a rule, rebleeding occurred in those cases when the wound was covered with a loose gel-like thrombus. It should be expected that in conditions of effusion from the abdominal cavity, the number of rebleeding may increase. In this regard, we have proposed a method for the permanent hemostasis by adhesion of the omentum to the liver wound surface using dry Hemoben powder. The adhesive force of Hemoben is sufficient to hold the omentum on the wound surface and prevent the process of clot erosion from the wound surface. Similar experiments were performed on six laboratory rats, and in no case did we observe rebleeding or the omentum discharge off the liver wound.

Subsequent observations of the animals showed that 1 and 3 hours after the surgery, the implant remained on the liver surface as a white coating and did not discharge from the wound surface. There were no signs of bleeding. The abdominal cavity remained intact. In subsequent periods, we noted the replacement of the powder with fibrin without a significant inflammatory reaction. On the 30th day after the surgery, the liver wound turned out to be completely healed without cicatrization and infiltration of the edges. Complications and lethal outcomes of animals during and after the experiments were not observed.

After 12 hours, the presence of free fluid in the abdominal cavity was not detected. A loose adhesive process involving the anterior abdominal wall was noted. There was a dense fibrin coating on the surface of the wound. Macroscopic examination on the 1st day in the abdominal cavity showed a loose adhesive process involving the greater omentum and a pronounced covering of the wound surface with fibrin.

On the 3rd day in the abdominal cavity, there was a moderate adhesive process with areas of the greater omentum. The small intestine loops were not involved in the process. A slight decrease in fibrin plaque was noted (Fig. 9).

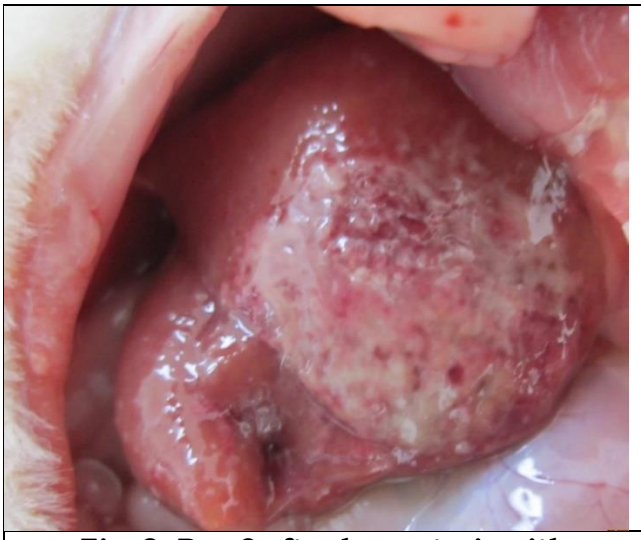

Fig. 9. Day 3 after hemostasis with Hemoben powder

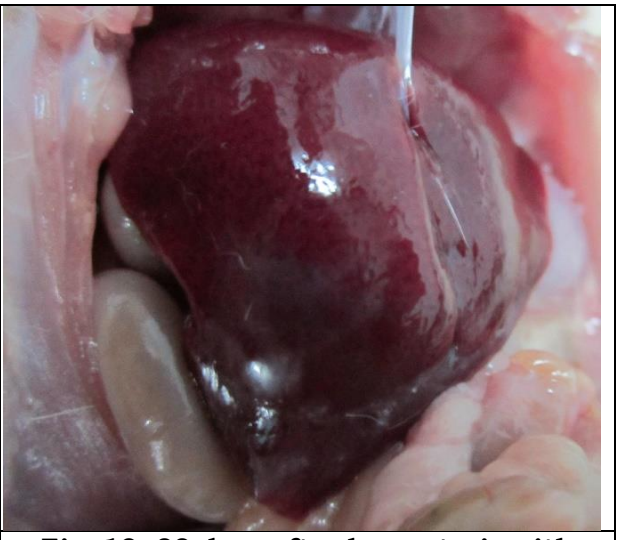

Fig. 10. 30 days after hemostasis with Hemoben powder

On the 7th day of the experiment, the adhesive process with the surface of the liver, omentum, and xiphoid process persisted in the abdominal cavity. A decrease in fibrin plaque is noted. On the 14th day of the experiment, no pronounced adhesive process was noted in the abdominal cavity. On the surface of the liver there was a thin transparent fibrin film. On the 30th day of the experiment, a moderate adhesive process was noted in the abdominal cavity (Fig. 10). The surface of the liver was smooth, soft, without signs of inflammation.

In the group of animals where a hemostatic sponge was used on the 1st day after the surgery, the sponge on the liver surface was a loose mass imbibed with blood. There were signs of a rebleeding event from a liver wound in the abdominal cavity. There was tissue edema and a loose adhesive process in the area of the liver wound covered with a sponge.

Histologically, there were hemorrhages around the wound, necrosis of hepatocytes, sinusoid edema, vascular congestion. With macro and microscopic assessment on days 3, 7, and 14 there was a gradual increase in signs of inflammation and the formation of a dense adhesive process in the control group (Fig. 11-12).

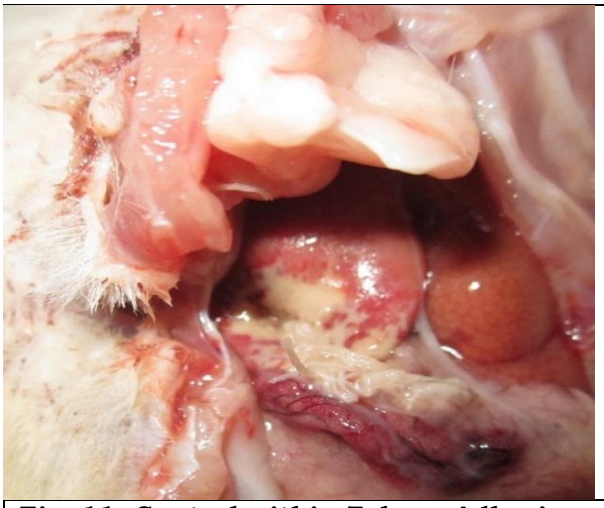

Fig. 11. Control within 7 days. Adhesions in the area of hemostasis with a sponge

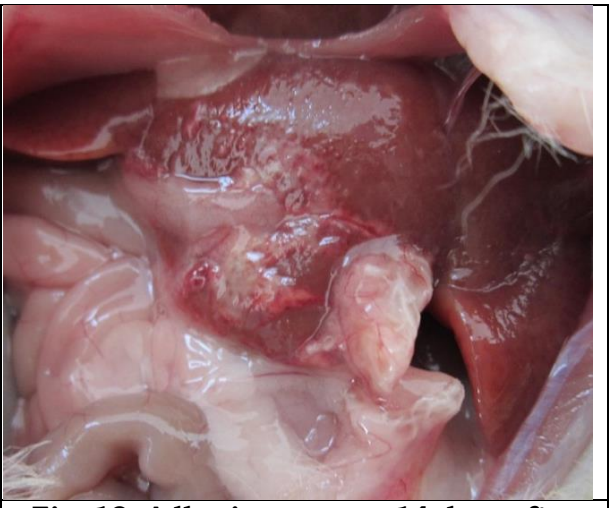

Fig. 12. Adhesive process 14 days after stopping the bleeding with a sponge

Table 1
Complete Blood Count

INDICATORS	Before	3 days after	7 days after	10 days after	14 days after
Leukocytes	5,0	2,7	4	6	8
Leukocytes	5,1	6,8	7	8	4
Percentage of lymphocytes	61,1	68,7	65	58	71
Monocytes	31,9	17,2	25	30	20
Eosiophils	1,8	7,0	2,5	5,5	3,5
Basophils	0,1	0,3	0,5	0,5	1,5
Red blood cells	6,9	6,2	7	5	6
Hemoglobin	140	131	148	120	130
Hematokit	39,6	35,9	41	35	36
Mean corpuscular volume	56,9	57,4	58	56	57
Mean corpuscular hemoglobin	20,1	21,0	21,5	20,6	20
Mean corpuscular hemoglobin concentration	354	365	375	340	350
RDW	12	11,4	10	11	12
RDW standard deviation	26	25,1	25	24	26
Platelets	152,7	128	138	143	160
MPV	7,0	7,2	7,8	6,7	7,2
Platelets distribution width	14,6	17,9	15	18	16

On the 30th day, the process of resorption of the connective tissue was observed and a connective tissue capsule was formed around the remnants of the collagen coating. We can conclud that the Hemoben implant causes a morphological reaction of the liver in the form of inflammation and proliferation of connective

tissue on the 1st day, but these processes quickly remit. On the 7-14th day, complete resorption of the implant was observed. Regenerative processes were observed in the liver parenchyma by the 30th day after implantation, especially in the damaged area, which indicates the restoration of liver tissue after Hemoben application. An increase in signs of inflammation was observed at the same time in the control group. The sponge did not resolve on the 14th day. There was a pronounced adhesive process and the formation of a purulent abscess around the sponge. The data of laboratory blood tests in the experiment before and after the surgery are given in Tables 1-2.

Table 2
Biochemical indicators

INDICATORS	Before	3 days after	7 days after	10 days after	14 days after
Glucose	5,0	5,9	6,9	7,0	5,9
Blood amylase	1300	1330	1300	1300	1300
Total bilirubin	27	11	13	24	12
Conjugated bilirubin	0	0	0	0	0
Unconjugated bilirubin	24	9			
Creatinine	52	44	73	34	87
Urea	3,7	4,0	4,2	4,6	4,8
Total protein	83	72	79	69	80
Potassium			8,2	6,5	8,0
Sodium			143	128	142
АсАт	255	573	346	238	521
ALT	45	108			
Phosphatase			245	225	517

Discussion

The conducted studies allowed us to establish that on the background of taking drugs that reduce the blood clotting time, the superficial wound of the liver has a weak tendency to spontaneous hemostasis. The use of a Hemoben hemostatic implant allows effectively stop bleeding. However, the drug consumption increases by 2-3 times comparing with the specified instructions. Studies at the microscopic level did not reveal significant negative consequences of large doses of the hemostatic implant. To reduce the consumption of hemostatic powder and form stable hemostasis, we suggest covering the formed thrombus with a strand of the greater omentum, which adheres when dry Hemoben powder is used. If it is not possible to use an omentum, it is possible to accelerate the formation of blood clot retraction by applying hot (100 degrees) blowing of a loose thrombus for 2 minutes.

A formation method of drug-induced hypocoagulation in an experiment has been developed. It sufficiently reflects similar conditions in humans. It has been established that the Hemoben hemostatic implant has a high adhesiveness to the wet surface of objects and biological tissues and significantly exceeds those of a collagen sponge. Under drug hypocoagulation, Hemoben provides fine, and stable

hemostasis, which significantly exceeds the parameters of a hemostatic sponge. If necessary, you can increase the consumption of Hemoben hemostatic powder by 3-4 times, which does not lead to complications when stopping parenchymal organ bleeding. Omentopexy is indicated using the adhesive properties of Hemoben dry powder to enhance its hemostasis during drug hypocoagulation to prevent rebleeding.

Conclusion

Thus, the experimental studies have shown that the developed new hemostatic material Hemoben in the form of a powder can be used to stop parenchymal bleeding. In conditions of hypocoagulable bleeding, the use of a hemostatic sponge made of collagen is less effective, and more time is spent on application of pressure on a blood vessel to arrest bleeding, while at the same time, the risk of rebleeding from a liver wound remains high.

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