

Evaluation of Efficacy and Potential Complications of the Plant-Based Nanoparticles Hemostat Powder on Controlling Hemorrhage in Rat Models of Liver Injury: An Experimental Study

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Abstract

Background

Liver resection requires time management due to the high risk of bleeding. Plant-based materials are superior to other agents in reducing complications during hemostasis. This present study aimed to investigate the effect of plant-based topical hemostatic nanoparticles from the rosaceous as an innovative hemostatic agent.

Materials and Methods

Twenty male Wistar rats were randomly divided into two study groups. All rats were anesthetized and their anterior lobe of the liver was amputated. The incision site was directly pressed with a dry gauze for 2 minutes in the control group and repeated every 30 seconds until hemostasis was achieved. Homeostatic material was compressed on the incision site and clotting time was recorded in the intervention group. The mean hemostasis time was compared in both groups using Paired Samples Test. $P < 0.05$ was considered statistically significant.

Results

Hemostasis was successfully achieved in both groups. The hemostasis time were 6.7 ± 1.33 and 183 ± 26.26 in Nanoparticles and control groups, respectively and their differences were significant ($P < 0.001$). Histopathology evaluation indicated a slight increase in vascularity, fibrosis, and polymorphonuclear neutrophil (PMN) infiltration in one case. Mild to moderate lymphocyte infiltration was detected in 2 cases.

Conclusion

The produced plant-based Nanoparticles could significantly reduce the hemostasis time in the intervention group compared to that of the control one.

Keywords: Hemostasis, Liver injury, Plant-based nano-particles, Powder Nanoparticles (Tetraethyl orthosilicate (TEOS), (3-Aminopropyl) Triethoxysilane (APTES), perylene-3, 4, 9, 10-tetracarboxylic dianhydride (PTCDA) and Trimethoxy (octadecyl) saline (TMODS)

Introduction

Bleeding and thrombotic complications are the leading causes of death in patients undergoing liver surgery. Timely and appropriate hemostasis in urgent cases plays an important role in surviving patients with threatening bleeding (1). Also, uncontrolled post-traumatic bleeding is one of the leading causes of death among the military and the second one among civilians (2).

Hemostasis can be accomplished by different methods such as chemical and mechanical agents including direct pressure, pressure dressing, and wound packing (3).

Several hemostatic agents and dressing have been

developed over years for controlling hemorrhage such as QuikClot, HemCon, WoundStat, Celox, and Combat Gauze (4).

These hemostatic agents are superior to classical techniques such as manual pressure and tourniquet, especially in controlling pre-hospital hemorrhage (3).

However, these hemostatic agents are produced from various products such as collagen, fibrinogen, and thrombin of humans and animals which cause viral infection and are time-consuming and expensive to produce. Therefore, producing more effective, safer, easier, and cheaper hemostatic products is essential (5).

Many recent studies have demonstrated that Quik

Clot takes precedence over standard gauze in controlling bleeding which contains inert minerals granules. Nevertheless, side effects such as exothermic reactions are still possible (6-8).

The present study aimed to investigate the effect of innovative topical hemostatic Nanoparticles using rosaceous extract.

Material and Methods

Animals

A total of 20 male Wistar rats weighing 240 ± 10 g were randomly divided into two groups of 10 rats. The animals were kept at 22 ± 2 °C, with a humidity of $54 \pm 2\%$, and a 12 h light/dark cycle a week before the study. Rats were given ad libitum access to food and water. All experimental protocols have been approved by the Animal Research Ethics Committee, Mashhad University of Medical Sciences, Mashhad, Iran.

Surgery and procedure

General anesthesia was induced by intraperitoneal (IP) injection of a Ketamine/Xylazine mixture (Ketamine 100 mg/kg and Xylazine 10 mg/kg).

The liver was exposed after shaving the hair from the abdomen section and opening the peritoneal cavity. A 2-cm long and 5-mm deep incision was made in one of the anterior lobes. The bleeding continued for 5 seconds and then the accumulated blood was collected by pressing gauze. The animals were divided into two groups after bleeding.

The incision site of the first group was directly pressed with a dry gauze for 2 minutes and then repeated every 30 seconds until hemostasis was achieved.

Homeostatic material was applied on the incision site and hemostasis time was recorded in the second group. In all cases, a standard weight of 20 g was put onto the bleeding point (Figure 1).

If the bleeding failed to stop after 3 times, it was considered a hemostatic failure. Additional blood pressure or blood vessel ligation was required for hemostasis in these cases. Hemostasis time was defined as the interval between the incision and complete cessation of bleeding. The incision was closed again by suturing after controlling the bleeding. The animals were similarly anesthetized and the liver was exposed a week later. Livers were removed and immediately fixed in 10% formalin and sent to the pathology laboratory.

Based on the hepatic pathological grading system of Nouri et al. liver inflammation is classified into 6 grades: 0= No change; 1= Minor inflammatory infiltration without edema; 2= Mild to moderate inflammatory infiltration with mild edema; 3= Mild to moderate inflammatory infiltration with moderate

edema; 4= Moderate inflammation with diffusion of neutrophils and edema; 5= Severe inflammation of the tissue and edematous changes, fibrosis, and hemorrhage.

Histological evaluation

Different parts of livers were removed from all rats and washed with saline. Then, they were fixed for 24-72 h in 10% neutral buffered formalin. The tissues were embedded in paraffin after dehydration and cut into 5 μ m sections using a microtome. The paraffin-embedded sections were stained with Hematoxylin-Eosin stain (H&E stain) and Masson's trichrome. Sections were examined under a light microscope (magnification of x40). The slices were examined for cell infiltration including neutrophils, lymphocytes, eosinophils, edema, hemorrhage, fibrin deposition, and germination, and collagen fiber growth (Figure 2).

Preparation and characterization

Tetraethyl orthosilicate (TEOS), (3-aminopropyl) triethoxysilane (APTES), perylene-3, 4, 9, 10-tetracarboxy dianhydride, and trimethoxy (octadecyl) silane (TMODS) were purchased from Sigma Aldrich. Aqueous ammonia (25%) was obtained from the Merck index. All chemicals and solvents had AR grades and were used without further purification (17).

Nuclear magnetic resonance (^1H NMR) spectra were recorded on Bruker Avance AV300 (300 MHz) NMR instrument using CDCl_3 solvent. Absorption and emission spectra were measured by Shimadzu UV-1601PC and Shimadzu RF-5301PC. Bruker FT-IR spectrometer ALPHA was used for recording Fourier transform infrared spectroscopy (FTIR analysis). Thermogravimetric analyses (TGA) were conducted using a TA instrument SDT 2960. All samples were heated from 25 °C to 800 °C at a heating rate of 10 °C/min in a nitrogen atmosphere. FT-IR spectra were recorded in the range of 3800–400 cm^{-1} using a Varian Excalibur 3100. The samples were mixed with KBr powder and grounded in an agate mortar and pestle before being pressed into a disk for recording the spectrum (17).

Statistical analysis

All data were given as mean \pm standard deviation. Data were analyzed using SPSS software (version 22.0). Kolmogorov-Smirnov test was used for evaluating the normal distribution of data. Hemostasis times were compared in nanoparticle and control groups using Paired Samples Test. The differences were considered statistically significant at $P < 0.05$.

Results

The hemostasis time of the two groups is shown

in Table 1. Hemostasis was successfully achieved in both groups. Hemostasis times were 6.7 ± 1.33 and 183 ± 26.26 in Nanoparticles and control groups, respectively and their differences were significant ($P < 0.001$).

Slight increases in vascularity, fibrosis and polymorphonuclear neutrophils (PMN) infiltration were observed in one case. Mild to moderate

lymphocyte infiltration was detected in 2 cases. Mild edema occurred in 7 cases and 6 cases were associated with mild bleeding.

A week after surgery, 6 wounds were grade 2, two wounds remained unchanged, one was grade 1 and 5, and no samples of grade 3 and 4 wounds were observed (Table 2).

Table 1- Hemostasis Time of Nanoparticles and control groups

group	Mean hemostasis time (seconds)	P-value
Nanoparticles (n=10)	6.7 ± 1.33	$P < 0.01$
Control (n=10)	183 ± 26.26	

Table 2- Pathological changes and Grades one week after surgery

	PMN	Lymph	Edema	Bleeding	Fibrosis/ Fibroblast	Vascularization	Grade
1	-	-	-	-	-	-	0
2	-	-	-	-	-	-	0
3	-	+1	1+	-	2+	1+	5
4	-	-	1+	1+	-	-	2
5	-	-	1+	1+	-	-	2
6	-	-	1+	1+	-	-	2
7	-	-	1+	1+	-	-	2
8	-	-	1+	1+	-	-	2
9	-	-	1+	1+	-	-	2
10	-	+1	-	-	-	-	1

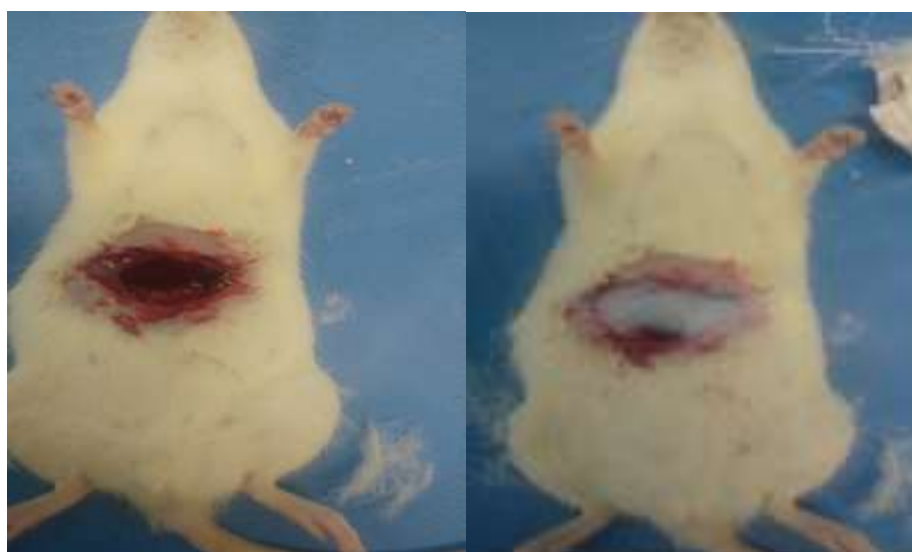


Fig 1- The incision site and place of hemostatic material

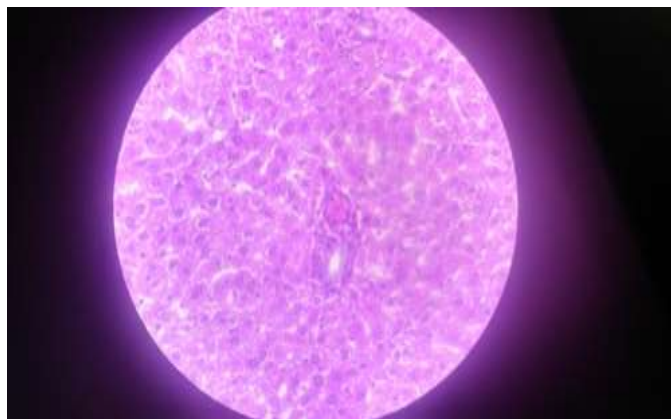


Fig 2- Histological examination of the liver in rats

Discussion

Major hemorrhage without any effective hemostasis is life-threatening. Various hemostatic agents have been recently introduced such as generating immunological reactions and being expensive, unavailable, and inefficient which bear some limitations.

Self-assembled silica Nanoparticles were investigated as hemostatic agents. These plant-based Nanoparticles can reduce hemostasis time in animal models of liver injury. The Nanoparticles were composed of a plant in the Rosaceae family which includes numerous genera with different ethnomedicinal uses including *Rosa*, *Prunus*, *Rubus*, *Crataegus*, *Pyrus*, *Malus*, *Geum*, *Fragaria*, *Cotoneaster*, *Spiraea*, *Sorbaria*, *Amygdalus*, *Agrimonia*, *Eriobotrya*, *Cydonia*, *Duchesnea*, *Comarum*, *Poterium*, *Filepedula*, *Padus*, and *Poterium* (9). Different species can be used for treating constipation, diabetes, diarrhea, rheumatism, hepatitis, cough, abdominal pain, and even obesity (10). One of the most beneficial properties of these drugs in sources is their ability in healing wounds, suppressing inflammation, and curing infections (11). Their good efficiency urged us to use them as a hemostatic property in controlling hemorrhage after liver injuries and treating hepatic injuries (12, 13).

The average bleeding time was 183 seconds in the control group after causing hepatic injury by an incision. However, the average time was higher than those of other studies. In their study of hemostatic plants in liver injury, Satar et al. reported a hemostasis time of 223 s in the control group (14). Ozdemiret al. reported a hemostasis time of 377 seconds in the control group in another similar study on carbon dioxide as a hemostatic agent (15). However, Midi et al. reported an average time of 220 and 410 seconds for other plant hemostatic agents in non-heparinized and heparinized rats, respectively (16). Despite all the above studies performed on Sprague-Dawley rats, Wistar rats were analyzed in

the present study and the differences can be interpreted with this judgment.

A similar study was conducted on Algan Hemostatic Agents (AHA) using 64 5-7 week-old Sprague Dawley rats. Different forms of these AHA including gel, liquid, and powder were used. Their results showed that all types of Algan Hemostatic Agents could significantly reduce the hemostasis time in rat models with hepatic injury compared to that of the saline-impregnated sponge as the control group. Furthermore, they proposed that powder was superior to all other materials in this case (16). In the above-mentioned study, the mean hemostasis time was 8 seconds in the intervention group. In this case, the present study indicates a better result of 6.7 seconds.

Another study was conducted on solid carbon dioxide powder which was reported a mean time of 377, 203, and 176 seconds for the control, suture, and solid carbon dioxide groups, respectively. The differences were significant in this study (15); however, the results show that plant compositions give better results.

In the present study, a slight increase in vascularity, fibrosis, and polymorph nuclear neutrophils (PMN) infiltration were observed in one case due to pathologic assessments. Mild to moderate lymphocyte infiltration was detected in 2 cases. Mild edema was observed in 7 cases and 6 cases were associated with mild bleeding. Mild fibrosis was also detected in the Algan study, but there was no sign of necrosis. Furthermore, portal inflammation was detected. They also reported that the hemostatic surface was in between Algan hemostatic gel and hepatic surface that hemostatic gel was resolved a week later and hemostasis surface remained (16).

Abajy et al. (17) also assessed the hemostatic property of *Potentilla erecta* from rosaceous. They reported that topical use of the plant had a significant hemostatic property in reducing bleeding time. They

believed this property may be due to the astringent effect of this plant.

The present study was conducted using a new plant-based hemostatic product which indicated better hemostatic results compared to those of other

Conclusion

Genera of Rosaceae has shown hemostatic properties which may be due to their astringent effect. A powder of plant-based nanoparticles was produced (13, 14) as a hemostatic product that could effectively reduce the hemostasis time from 183 seconds in the control group to 6.7 in the intervention group.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of the present study.

Acknowledgments

All experimental protocols have been approved by the Animal Research Ethics Committee, Mashhad University of Medical Sciences, Mashhad, Iran.

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