Comparison of herbal-based and synthetic hemostatic agents for efficacy on a rat partial nephrectomy model

Experimental study

Murat Bagcioglu, *MD,* ***Emre Huri,*** *MD,* ***Mumtaz Dadali,*** *MD,* ***Hesna M. Astarci,*** *MD,* ***Mustafa F. Sargon,*** *MD, PhD,* ***Muzaffer Eroglu,*** *MD.*

ABSTRACT

الأهداف: مقارنة فعالية عامل مرقيء تركيبي معتمد على الأعشاب في اسئصال الكلية الجزئي.

الطريقة: أجريت دراستنا المخبرية في مستشفى مركز الأبحاث والتدريب، أنقرة، تركيا خلال الفترة من مايو حتى نوفمبر 2012م. تم تقسيم 20 جرذ بشكل عشوائي إلى 4 مجموعات. استخدمت غرز داخل الجسم مجموعة K، تاتشوسيل (مجموعة T)، هيموسير (H)، الدم المتوقف (A)، للوصول إلى الإرقاء في استئصال الكلية الجزئي. كما تم قياس وقت نقص التروية الدافيء ووقت الإرقاء وخسارة الدم في جميع المجموعات. فحصت العينات نسيجياً وباستخدام الالكترون المجهري بعد تضحية الفئران.

النتائج: ظهر أسرع إرقاء في مجموعة T، ثم مجموعة H ثم مجموعة A. اختلف وقت نقص التروية الدافيء ووقت الإرقاء بشكل إحصائي بين جميع المجموعات $p>0.001$. أكثر خسارة للدم كانت في مجموعة K. أظهر الاختبار النسيجي أن تفاعل الخلية الكبرى في مجموعة K أكثر بشكل إحصائي من مجموعة H و A $p>0.001$. لم تظهر نتائج نسيجية في الاختبار التركيبي لمجموعة K. لم نشاهد أي نتائج نسيجية في الاختبار التركيبي لمجموعة K. وفي الاختبار التركيبي لمجموعة T، كان هنالك خلايا أنبوبية بعدة فجوات في السيتوبلازم مع أدمة داخل الخلية. بينما نتائج التركيبية لمجموعة H و A كانت متشابهة.

خاتمة: في المستويات النسيجية والتركيبية، كان للعوامل المرقية المعتمدة على الأعشاب أثر إيجابي على الأنسجة الكلوية ودور الكبيبي عند عمل استئصال الكلية الجزئي.

Objectives: To compare the efficacy of herbal based and synthetic hemostatic agents in partial nephrectomy.

Methods: Our experimental study was carried out at the Ankara Training and Research Hospital, Ankara, Turkey between May and November 2012. Twenty rats were randomly divided into 4 groups. Intracorporeal sutures (group K), TachoSil® (group T), HaemoCer™ (group H), and Ankaferd Blood Stopper® (group A) were used to achieve hemostasis in partial nephrectomy. Warm ischemia time (WIT), hemostasis time (HT), and blood loss for all groups were measured. The specimens were examined histopathologically and electron microscopically after sacrificing the rats.

Results: The fastest hemostasis was detected in group T, followed by group H, and group A. The WIT and HT were significantly different for all groups ($p<0.001$). The greatest blood loss was found in group K. The histopathologic examination revealed that the giant cell reaction in group K was significantly more than in group H and group A ($p<0.001$). No pathologic findings were observed in the ultrastructural examination of specimens taken from group K. On ultrastructural examination of group T, tubule cells had many vacuoles in their cytoplasm with marked intracellular edema. Ultrastructural findings for groups H and A were similar.

Conclusion: At the histopathologic and ultrastructural levels, herbal-based hemostatic agents had a positive impact on renal tissue and glomerular function when applied during partial nephrectomy.

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From the Departments of Urology (Bagcioglu, Huri, Dadali, Eroglu), Pathology (Astarci), Ankara Training and Research Hospital, Ankara, and the Anatomy Department (Sargon), Hacettepe University, Hacettepe, Turkey.

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Address correspondence and reprint request to: Dr. Murat Bagcioglu, Ugur Mumcu Mah 1649 Sok, Park Vadi Batievleri B-6/9 Batikent, Ankara 06370, Turkey. Tel. +903 122518536. E-mail: dr.muratbagcioglu@hotmail.com

Partial nephrectomy (PN) has become a common worldwide surgery, with the increase in the incidence of small-sized renal tumors in recent years. The oncologic results of PN are similar to those of radical surgery when performed for localized renal cell carcinoma (RCC).[1,2] Absolute indications for PN are bilateral RCC and cases with an anatomically or functionally solitary kidney. Relative indications for PN are when the patient has a unilateral renal tumor and a medical condition that can potentially threaten the function of the contra-lateral kidney, such as renal artery stenosis, renal stone disease, hypertension, or diabetics.[3] Patients with elective indications include those who undergo PN for the treatment of renal angiomyolipomas, atrophic and hydronephrotic duplicated collecting systems, and localized unilateral tumors with a normal contra-lateral kidney. The oncologic results of PN cases in elective indication are similar to radical nephrectomy for small renal tumors. (T1a).[4,5] The aim of PN is removal of the cancerous tissue, preserving the functional healthy renal tissue, and providing hemostasis rapidly and effectively. Many various surgical techniques, tools, and hemostatic agents have been tried to achieve hemostasis, and they are still being studied.[6] The purpose of this study is to compare the efficacy of herbal based and synthetic hemostatic agents that can be used in partial nephrectomy for rapid, efficient, reliable, and low-cost hemostasis.

Methods. We carried out our experimental study after the approval of the local ethics committee at Ankara Training and Research Hospital in Ankara, Turkey between May and November 2012. Twenty rats (Wistar, albino male, 20 weeks old) weighing 200-240 grams were used in the study. The animals were monitored in the preoperative and postoperative periods. Rats were individually fed in an iron cage, and the room temperature was maintained at 22°C. All the animals were treated with intramuscular ampicillin/sulbactam (20 mg/kg, Pfizer, Berlin, Germany) prior to the study. General anesthesia was achieved with Ketamine HCI (40 mg/kg, Parke Davis, Parke Davis, Detroit, USA). The rats were randomly divided into 4 groups. All surgical procedures were performed using a surgical optic loupe (x2.5 Heine™, Herrsching, Germany). Intracorporeal sutures (group K), TachoSil® (Nycomed, Zurich, Switzerland) (group T), HaemoCer™ (BioCer, Bayreuth, Germany) (group H), and Ankaferd BloodStopper® (Ankaferd Drug Cosmetics Inc. Co. Istanbul, Turkey) (group A) were used to achieve hemostasis during the procedures. The rats were incised with a midline incision to approach the right kidney. The renal pedicles were dissected. Rommel clamps were applied to the renal artery and warm ischemia was achieved. Lower pole partial nephrectomy (20% of the kidney was excised) was the performed for each case. Warm ischemia time (WIT) was defined as the period when the renal artery was controlled with a Rommel clamp. Then, either intracorporeal interrupted sutures or hemostatic agents were applied for hemostasis. The hemostasis time (HT) was defined as the period from the application of the hemostatic agent until no bleeding was detected. Sterilized one cm^2 sponges were used for measurement of blood loss. Rommel clamps were removed for 20-second intervals, and the bleeding was checked until the achievement of hemostasis.

After PN, intracorporeal suturing was applied to the incised surface of the kidney in group K using 6/0 Vicryl to achieve hemostasis. The rats in group T received TachoSil, which was applied to the surface of the kidney after PN to achieve hemostasis (**Figure 1**). The rats in group H received an application of HaemoCer by an applicator, and it covered the surface of kidney followed by compression with sterilized 1 cm^2 sponges (**Figure 2**). The sponges were moisturized with isotonic NaCl. Ankaferd BloodStopper was used to achieve hemostasis for the rats in group A, with 0.05 cc of Ankaferd applied per one cm^2 of the renal surface. The WIT, HT, and blood loss for all the groups were measured, and statistically compared among the groups. Three weeks later, the rats were sacrificed under anesthesia. A right nephrectomy procedure was performed on all rats, and the resected specimens were blindly evaluated by pathologists via histopathologic and electron microscopic examination. A histopathologic examination was performed and giant cell reaction, glomerular necrosis, acute inflammation, calcification, fibrosis, adhesions, fistulas, tubular thyroidization, fibroblast activation, erythrocyte aggregation, microvascular proliferation, and siderophages were evaluated. The electron microscopic examination was undertaken with a Jeol JEM 1200 EX (Jeol LTD, Tokyo, Japan) brand transmission electron microscope.

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) for Windows 15.0 was used for statistical analyses. Kruskal-Wallis, Fisher Exact, and Mann Whitney U tests were used for comparisons, and $p<0.05$ was regarded as significant.

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Results. The mean WIT and HT were measured for all groups (**Table 1**). The fastest hemostasis was detected in group T followed by groups H and A. The mean WIT and HT for all groups were significantly different ($p<0.001$). The surgical procedure with intracorporeal suturing that was performed in group K took a longer time than the other groups to which hemostatic agents were applied. When the groups were compared according to blood loss, the greatest loss was found in group K, and the least loss was found in group T.

On macroscopic examination, it was observed that all the materials in group A resorbed well, and there was only minimum tissue reaction to the environment. Severe adherence was observed in 2 cases. It was observed that the hemostatic material took the place of the excised lower pole in group T. For group H, the material was completely resorbed, and there was almost no tissue reaction, but severe adherence was observed in neighboring tissue. The histopathologic examination revealed the presence of giant cell reaction in group K was

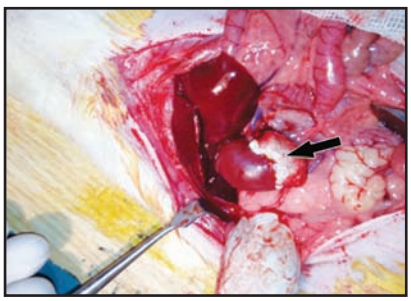

Figure 1 - Hemostasis with TachoSil® application.

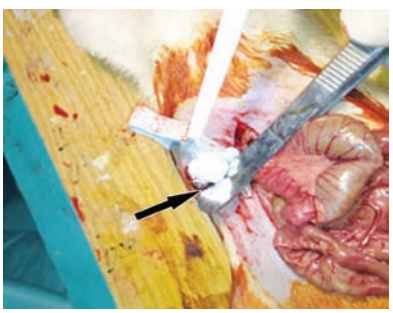

Figure 2 - Application of HaemoCer™ .

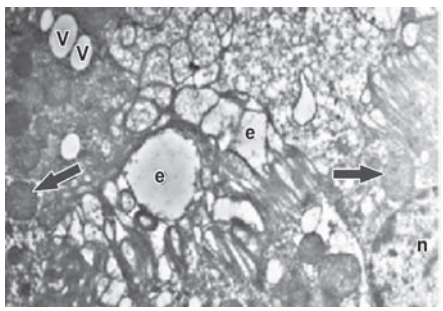

Figure 3 - Transmission electron microscopic image of a tubule cell (Group T). e - intracellular edema, n - nucleus of a tubule cell, v - vacuoles in the tubule cell cytoplasm, arrows - normal mitochondria structure

Table 1 - The comparison of the different groups in terms of hemostasis.

Groups	Mean warm ischemia time (seconds)	Mean hemostasis time (seconds)
Group K	152.25	141.24
Group T (the fastest)	39.67	32.17
Group H (second)	67	56.5
Group A (third)	82.25	69.75

Group K - received intracorporeal sutures, Group T - received TachoSil®, Group H - received an application of HaemoCer™ , Group A - received Ankaferd BloodStopper®

Table 2 - Statistical analyses of histopathologic parameters among the groups.

Histopathologic examination	Group K and Group T	Group K and Group H	Group K and Group A	Group T and Group H	Group T and Group A	Group H and Group A
Giant cell reaction	0.650	0.04*	0.04*	0.740	0.740	-
Glomerular necrosis	0.370	-	-	0.370	0.370	-
Acute inflammation	0.561	0.317	-	0.893	0.796	0.741
Calcification	-	-	-	-	-	-
Fibrosis	0.007*	0.127	0.127	0.005*	0.005*	0.008*
Tubular thyroidization	-	0.127	0.008*	0.091	0.005*	0.127
Fibroblast activation	0.025*	0.495	0.04*	0.091	-	0.127
Fistulas	-	-	-	-	-	-
Erythrocyte aggregation	0.65	0.317	0.495	0.176	0.777	0.127
Microvascular proliferation	-	-	-	-	-	-
Siderophages	0.371	0.317	-	0.866	0.371	0.317

Group K - received intracorporeal sutures, Group T - received TachoSil®, Group H - received an application of HaemoCer™, Group A - received Ankaferd BloodStopper®, *$p<0.05$ was regarded as significant for all analyses

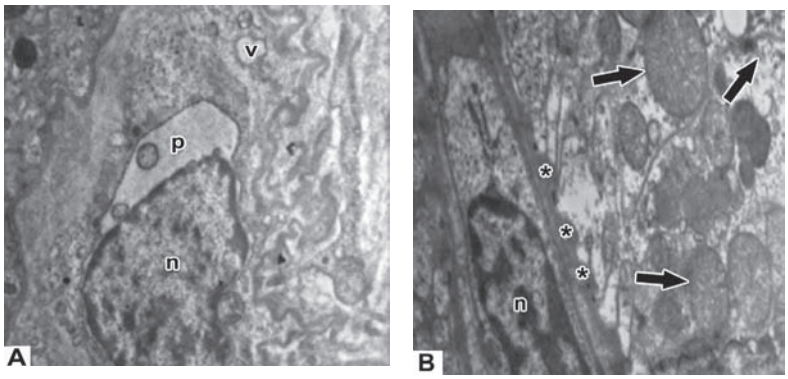

Figure 4 - Transmission electron microscopic images of podocyte (Group H) showing: **A**) enlarged perinuclear cistern (P) and vacuole (v), and **B**) nucleus of podocyte (n). *pedicel, arrows - normal mitochondria structure

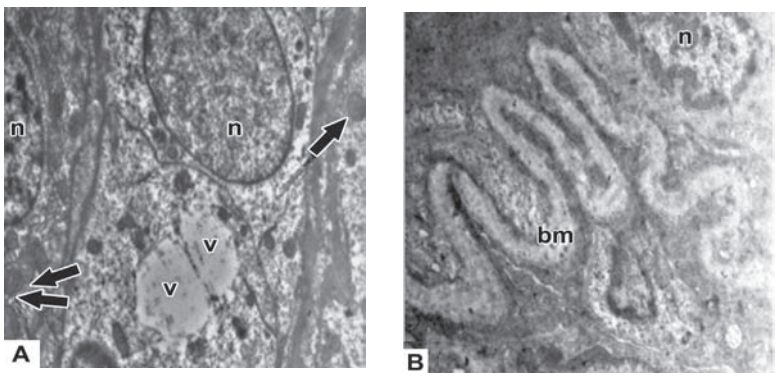

Figure 5 - Transmission electron microscopic images of tubule and glomerular basal membrane (Group A) showing: **A**) nucleus of tubule cell (n), vacuoles in tubule cell cytoplasm (v), normal mitochondria structure (arrows), and **B**) basal membrane (bm).

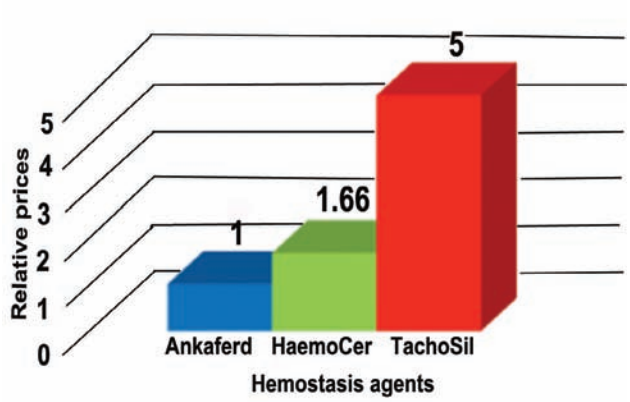

Figure 6 - Comparison of prices among Ankaferd®, HaemoCer™, and TachoSil®.

significantly more than in groups H and A (p<0.001). There was no significant difference between groups T and group K. There was no significant difference between all groups in the assessment of glomerular necrosis, calcification, fistula, erythrocyte aggregation, microvascular proliferation, and acute inflammation (p>0.05). In group T, the presence of fibrosis was significantly more than the other groups (p<0.001). There was no significant difference among the other groups (p>0.05) (**Table 2**). No pathologic findings were observed in the ultrastructural examination of the specimens taken from group K. The specimens taken from all groups showed normal endothelial cells of glomerular capillaries, mesangial cells, secondary extensions (pedicels) from podocytes, and basal membranes. The tubule cells in group T had many vacuoles in their cytoplasm with marked intracellular edema (**Figure 3**). The ultrastructural findings for groups H and A were similar. The perinuclear cisterns of the podocyte cells in glomerular capillaries were enlarged (**Figures 4A & 4B**). There were also vacuoles in the podocytes' cytoplasm. The tubule cells in these groups also had large vacuoles in their cytoplasm (**Figures 5A & 5B**).

Discussion. Bergel used topical fibrin to achieve hemostasis in modern surgery for the first time in 1909.[7] After that, hemostatic agents were used widely.

Hemostasis was the first indication for the use of hemostatic agents; additionally, they are often used in the areas of tissue adhesion and tissue engineering. Minimal invasive surgery is the basic grounds for this technology.

In the past, patients with RCC were mostly diagnosed in the metastatic stage with little chance for cure. However, nowadays, most of cases are being identified at a stage when the disease is localized to the kidney, and nephron sparing surgical techniques have had satisfactory results.[8] The improvement in imaging techniques is the reason for the increased occurrence of incidental renal mass, and also the reason for the increased number of small renal masses. The common goals of all the surgical methods are preserving more functional renal tissue, and achieving sufficient hemostasis. Hemostatic agents and fibrin sealant materials are used systematically for this purpose in PN. TachoSil® is a fixed, single-use ready combination, and this collagen patch is covered by human plasma components, fibrinogen, and thrombin. TachoSil® assembles the hemostatic and adhesive features of coagulation factors I and Ia for the mechanical support of collagen leaf material, and it creates a fibrin coagulum on the bleeding surface to support hemostasis. TachoSil® can be applied to hard accessible surgical areas.[9]

In our study, the shortest HT and WIT recorded in the TachoSil® group illustrates the rapid effects of the product. Similarly, as the TachoSil® group also had the least amount of recorded blood loss, it is also superior in that aspect in comparison with the other groups. Examination of the specimens revealed that the resected kidney part was filled by TachoSil®, showing the good biological harmony of the product. However, it can be stated as a drawback of the product, as it was not completely absorbed during the 3-week period. Residual material was detected macroscopically on the incised kidney surface. Histopathological examination revealed that fibrosis was more than the other groups, and intracellular pathological changes were apparent on the electron microscopic views. Several vacuoles were noticed in the cytoplasm of tubule cells, and there was also intracellular edema in the tubule cells in this group. Ankaferd is an herbal extract used as a hemostatic agent in traditional Turkish medical science. Ankaferd consists of the standardized mixture of thyme (*Thymus vulgaris*), licorice (*Glycyrrhiza glabra*), unripe grape (*Vitis vinifera*), galingale (*Alpina offiōcinarum*), and nettle (*Urtica dioica*). Each of these herbs and plants is indicated to have hemostatic effects on endothelium cells, blood cells, vessel formation (angiogenesis), vascular dynamics, and mediators. The results in the Ankaferd group showed that HT was shorter than the suture group, but higher than the other agents. The material was completely absorbed, and there was minimal residue in the macroscopic examination. However, severe adhesions and tissue reaction were seen in the bowels. Upon microscopic examination, foreign body reaction, inflammation, and fibrosis were not observed, and the material was found to have high histocompatibility. The electron microscopic view showed no pathological changes to the normal anatomic structure.

Microporous polysaccharide hemospheres (MPH) were made from an herbal-based absorbable polysaccharide shaped into hemostatic globules. It is in powder form and is used to achieve hemostasis in cases of bleeding that are difficult to stop with a tourniquet, by compression, or other methods.[10] The MPH used in our study was HaemoCer and is produced in Germany by Bio-Cer. HaemoCer's activity is not well document in the literature. Its mechanism of action and clinical features were similar to Arista, a hemostatic agent in the same class of materials. We aimed to use this MP hemostatic agent in our rat partial nephrectomy model to study the effects of a new herbal-based agent. HaemoCer was not problematic in terms of storage conditions and it was ready for use; however, it does require an applicator. It also requires extra care to apply a sufficient amount of HaemoCer to the application area and to protect the neighboring tissues from unnecessary material contamination. It achieved hemostasis as the second agent in terms of average HT. HaemoCer was completely absorbed and no residual material was detected; however, adhesions were seen on macroscopic examination. The reason for the adhesions was the excessive material in neighboring bowel tissue, which was caused by the applicator use. HaemoCer had high biocompatibility as there was minimal tissue reaction. The material was completely absorbed. Inflammation and foreign body reaction were statistically insignificant, which can be regarded as an advantage for HaemoCer as the capillary endothelium cell was ultra-structurally normal. Pedicels and enlarged perinuclear cisterns in the podocyte cytoplasm were detected in the electron microscopic views.

An advantage was observed for herbal-based hemostatic agents compared to other chemical hemostatic agents histopathologically. There was no inflammation, foreign body reaction, glomerular necrosis, and calcification on histopathological examination. The anatomic structures were normal at the ultrastructural level. A positive impact at

histopathologic level was indicated in another study performed in our clinic.[11] The reason for this may be attributed to the fact that herbal-based hemostatic agents contain antioxidant aromatic herbs Some of the herbal-based natural products are very popular nowadays due to their various pharmacologic effects, such as antitumor and antioxidant activity.[12,13] Specially, it is reported that thyme has a powerful systemic antioxidant effect.[14] The results of this study also indicate that these natural products have a local antioxidant effect.

The limitation of our study is in the small number of rat used. The effects of the hemostatic agents should be compared in larger samples using histopathologic and electron microscopic examination.

When cost-effectiveness is considered, the herbal-based hemostatic agents proven to be effective in prior studies on partial nephrectomy, were cheaper than the other synthetic agents used in our study. When the prices were compared, TachoSil was 5 times more expensive than Ankaferd, and 3 times more expensive than HaemoCer (**Figure 6**).

It is critical to achieve hemostasis in all surgical procedures with same purpose: minimal blood loss and successful surgical technique. Previous studies on this issue are limited to case reports, retrospective analyses, and personal experiences, retrospective analysies and personal experiences. There is a need for studies comparing one agent to another. Probably, there will be more studies in this area in the future due to the advancements in technology and laparoscopy techniques. The aim should be to discover an ideal hemostatic agent. The ideal hemostatic agent must be highly efficient, used easily, reliable, histocompatible, and cost-effective.

In conclusion, the use of hemostatic agents in PN is observed to be more efficient and faster in achieving hemostasis when compared with primary suturing. TachoSil® achieved hemostasis faster and in a more efficient manner when compared with the other agents. The results of HaemoCer™, previously reported for partial nephrectomy, were similar to the results obtained with Ankaferd®. Histopathologically, and at the ultrastructural level, herbal-based hemostatic agents showed no pathologic findings, with normal anatomic structure of renal tissue, and glomerular function when applied during PN.

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