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Manuscript Draft

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Title: HAEMOSTATIC ROLE OF THE FOLKLORIC MEDICINAL PLANT EXTRACT ANKAFERD BLOOD STOPPER® IN RAT PARTIAL NEPHRECTOMY MODEL: CONTROLLED EXPERIMENTAL TRIAL

Article Type: Investigative Urology Article

Keywords: Ankaferd Bloodstopper®; partial nephrectomy; bleeding, hemostasis, invivo

Corresponding Author: Dr. Ali Ayyıldız, Associate professor

Corresponding Author's Institution: Ministry of Health Ankara Training and Research Hospital, Department of Second Urology Clinic

First Author: Emre Huri, Urologist, FEBU

Order of Authors: Emre Huri, Urologist, FEBU; Turgay Akgül, Urologist; Ali Ayyıldız, Associate professor; Hüseyin Üstün, Associate Professor; Cankon Germiyanoğlu, Associate Professor

Manuscript Region of Origin: TURKEY

Abstract: Purpose
Ankaferd BloodStopper (ABS); including five mixed plants in traditional Turkish medicine as hemostatic agent for external traumatic, postsurgical, dental bleedings. We investigated hemostatic efficacy of ABS in partial nephrectomy.

Materials and Methods
Twenty-four Wistar; divided into four groups. Group I (GI), partial nephrectomy (PN) with hilar control as conventional technique, Group II (GII), conventional technique with ABS, Group III (GIII), ABS application to renal parenchyma, Group IV (GIV), partial nephrectomy and ABS were performed without hilar control. Warm ischemia time (WIT), PN time (PNT), ABS application
number were recorded. Histopathologic evaluations were completed. Fisher test, Kruskal-Wallis and Mann-Whitney U tests were used for statistical analysis.

Results
Mean kidney size was 2x2.5x05 cm. Mean PN time (min) was 3.7 for GI, 2.7 for GII, 1.8 for GIII and 3.2 for GIV, between GI and GIII, difference was significant (p:0.007). WIT (min) in groups, GIII had a significant less WIT compared GI (p: 0.011). Number of ABS was higher in GII and GIV compared GII (p: 0.003). Glomerular necrosis was detected with higher rate in GI compared GIII and GIV (p:0.015). Calcification was formed significantly in GI compared GII, III and IV (p<0.05). Erythrocyte aggregation was confirmed higher in GII, III and IV than GI (p: 0.015). Giant cell reaction, fibrosis, inflammation, microvascular proliferation were not statistically different among groups (p>0.05).
Conclusions
ABS decreases PN and WI times, provides hemostasis. Erythrocyte aggregation confirmed haemostatic action of ABS, absence of glomerular necrosis and calcification may show positive relevance.

Suggested Reviewers:

Opposed Reviewers:

Response to Reviewers: Dear Editor and specific reviewers,
All corrections were indicated in revised manuscript as a bold mark.

Response to editor:
1) The word count of original article was checked.
2) The reference number was corrected
3) The first 6 authors were listed

Reviewer #1: The authors present work looking at a novel hemostatic agent, ABS, and its efficacy for use in partial nephrectomy using a rat model. The study is reasonably well done and introduces a novel agent that may be useful in the future. The authors have been reasonably cautious not to over state their case, in particular this will need to be moved into a larger animal model (such as a
porcine model) with bigger caliber vessels before it is tested in humans. The main issue with the paper is numerous grammatical issues throughout the text which will require editing prior to publication. Also, a point of clarification - does partial nephrectomy time refer to the entire operation time?

Response to Reviewer 1:
1) The comments were added to manuscript.
2) Partial nephrectomy time was described clearly
3) Numerous grammatical mistakes were edited carefully.

Reviewer #2: 1. General comments
This study showed that Ankaferd Blood Stopper (ABS) provide hemostasis action in partial nephrectomy of rats. This study is interesting, however, some parts are unclear.

2. Major problem
ABS is not a popular drug except in Turkey. The authors should show the detail of this drug, such as the proportion of five plants and making process.
P4, line 4,
The description of each group is not clear.
The authors wrote that intracorporeal suturing of parenchyma and collecting duct and hilar vascular control as conventional technique. It is not clear the difference between G2 and G3.

3. Minor problem
Table 2, figure 1,6B are not necessary.
In the figure 2, if there is the significant difference in the group, please show in the figure.

Response to Reviewer 2:

1) The proportion of Ankaferd was added.
2) The description of groups were clarified. Especially, the difference between G2 and G3 was clarified.
3) Table 2, Figure 1 and Figure 6B were excluded from the manuscript.
9) In Fig 2, significance between G1 and G3 was demonstrated.
Dear Editor,

It will be a pleasure to submit an article of “**Haemostatic role of the folkloric medicinal plant extract Ankaferd Bloodstopper® in rat partial nephrectomy model: controlled experimental trial**” to your journal. We confirm the following items:

1. All authors have made a significant contribution to the findings and methods in the paper.
2. All authors have read and approved the final draft.
3. Financial or commercial interests has been acknowledged.
4. The work has not already been published and has not been submitted simultaneously to any other journal.
5. The corresponding author takes on the above responsibilities with his/her signature.

Best regards!

Emre Huri, MD, FEBU

Ankara Training and Research Hospital
2nd Urology Clinic
ESRU Past Secretary
Ankara /Turkey

dremrehuri@yahoo.com
Running Title: BloodStopper® (Ankaferd) in Partial Nephrectomy

HAEMOSTATIC ROLE OF THE FOLKLORIC MEDICINAL PLANT EXTRACT ANKAFERD BLOOD STOPPER® IN RAT PARTIAL NEPHRECTOMY MODEL: CONTROLLED EXPERIMENTAL TRIAL

Emre HURİ*, MD, Urologist, FEBU
Turgay Akgül*, MD, Urologist
Ali Ayyıldız*, MD, Urologist, Assos. Prof.
Hüseyin Üstün**, MD, Pathologist, Assoc. Prof.
Cankon Germiyanoğlu*, MD, Urologist, Assoc. Prof.

*Ankara Training and Research Hospital, 2. Urology Department
** Ankara Training and Research Hospital, Pathology Department

Corresponding Author:
Emre Huri, MD, FEBU
Address: Hilmi Barlas Arinnapark Sitesi 1808.sokak
NO:2/A/24
Çayyolu/ANKARA/TURKEY
Telephone: +90 312 240 04 68
Fax: +90 312 485 36 76
E-mail: dremrehuri@yahoo.com, emrehuri@gmail.com

Key Words: Ankaferd Bloodstopper®; partial nephrectomy; bleeding; hemostasis; invivo
ABSTRACT

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Ankaferd BloodStopper (ABS); including five mixed plants in traditional Turkish medicine as hemostatic agent for external traumatic, postsurgical, dental bleedings. We investigated hemostatic efficacy of ABS in partial nephrectomy.

Materials and Methods
Twenty-four Wistar; divided into four groups. Group I (GI), partial nephrectomy (PN) with hilar control as conventional technique, Group II (GII), conventional technique with ABS, Group III (GIII), ABS application to renal parenchyma, Group IV (GIV), partial nephrectomy and ABS were performed without hilar control. Warm ischemia time (WIT), PN time (PNT), ABS application number were recorded. Histopathologic evaluations were completed. Fisher test, Kruskal-Wallis and Mann-Whitney U tests were used for statistical analysis.

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Conclusions
ABS decreases PN and WI times, provides hemostasis. Erythrocyte aggregation confirmed haemostatic action of ABS, absence of glomerular necrosis and calcification may show positive relevance.
Introduction

Haemostatic agents play an important role during the laparoscopic or open renal surgery. It could be used as a stand-alone agent in many conditions. Up to now, many materials has been described to stop the hemorrhage in kidney surgery. For renal parenchymal haemostatic aids, glues and things were reviewed by Thompson et. al\(^1\). At first, they identified advances in tissue sealants, selective renal ischemia and haemostatic cutting modalities\(^1\). Bergel firstly described the use of dry plasma to facilitate hemostasis in 1909\(^2\). Afterward, fibrin glues, absorbable fibrin adhesive, synthetic hydrogel polymer, liquid albumin-indocyanine green solder were developed to use with the same purpose as a sealant agent\(^3-6\).

The main action in renal parenchyma during the hemostasis, producing fibrin which is facilitated by fibrinogen and thrombin. These products generally support this cascade to ensure hemostasis. Very special features of kidney are to receive one-fifth of the cardiac output each minute, therefore any injury to renal parenchyma can result in potentially hemorrhage\(^7\). That is the reason for why so much important to secure renal parenchymal hemostasis during the nephron-sparing surgery and renal trauma. In both of open and laparoscopic partial nephrectomy, increased warm ischemia time due to uncontrollable bleeding is a restricted factor to preserve renal function sufficiently. However, the most difficult aspect of laparoscopic partial nephrectomy is to achieve sufficient hemostasis and reconstruction of the kidney within the time constraints of warm ischemia\(^7\).

In this article, the main topic is what the place of haemostatic agent of Ankaferd BloodStopper® (ABS) in nephron-sparing surgery in animal model. As a first time in urology field, we used ABS as a haemostatic agent in partial nephrectomy model. ABS is a unique folkloric medicinal plant extract, which has historically been used in Turkish traditional medicine. Mixture of five plants was included to ABS, and each of them has some effect on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics and cell mediators\(^8\). We aimed to evaluate the efficacy and histopathologic effect of ABS in partial nephrectomy model with and without hilar clamping and intracorporeal suturing.

Materials and Methods

Study design. This study was approved by the Animal Review and Local Ethic Committee and the research council at our institution. A total of 24 Wistar rats,
divided into four groups, weighing 1-2 gram underwent right lower pole partial nephrectomy. The targeted excised tissue was determines as an approximate 1 cm² for each rat. Partial nephrectomy techniques of the groups were differed from each other. **Group I (GI)** underwent right partial nephrectomy (PN) with hilar vascular control- intracorporeal suturing of renal parenchyma and collecting duct as an conventional PN, for **Group II (GII)**, underwent conventional partial nephrectomy with application of Ankaferd Blood Stopper® (ABS), **Group III (GIII)**, received ABS application to renal parenchym and collecting duct with hilar control (non-sutured group) and in **Group IV (GIV)**, partial nephrectomy and application of ABS were performed without hilar control (non-sutured group). At first month, following the sacrifice, right nephrectomy was performed to evaluate histopathologic results. Gross specimens and histological sections were evaluated in a blind fashion by one pathologist (H.Ü.) stained with haemotoxylen-eozine (H&E).

**Operation technique.** Following the administration of prophylactic single dose broad-spectrum antibiotics, a midline incision was made in the abdomen after steril prep and draping. The right kidney was completely mobilized. The right renal artery and vein (hilar control) were then occluded with Rommel vascular clamp, and the lower 1/3 of the right kidney was resected in guillotine fashion with a single stroke of an amputating knife. The surgeon with assistant immediately began one of four techniques, randomly determined, reparative procedures. After the procedure, sponges were used to collect the all visible clothes and blood. And then kidney was replaced in the renal fossa.

**Haemostasis techniques.** As a conventional therapy, bimanual pressure was applied to the amputated renal margin by the surgical assistant. Segmental vessels and collecting system were repaired with absorbable sutures. Sponges or Surgicel ® were not used. As an alternative to conventional method, injectable form of Ankaferd (2cc) was applied to the amputated renal margin slowly until the bleeding had stopped. In just one group (GIV), investigating the effect of ABS on active renal parenchyma bleeding, hilar vessels was not occluded. Electrocautery was not used. If the bleeding was not stopped in GII, III, IV, extra dose ABS was applied.

**Objective parameters.** Warm ischemia time (WIT) was measured from initial occlusion of hilar vessels until final release of the right renal vessels. Partial nephrectomy time (PNT) and number of ABS application were recorded. PNT
referred the entire operation time. Weight and measures of partial nephrectomy specimens were determined. Urine extravasation, adherence to adjacent organs, infection in renal operated margin was evaluated. Pathologic specimens were evaluated with emphasis on presence or absence of giant cell reaction, intestinal metaplasia, acute inflammation, foreign material reaction, fibrosis, adhesions, necrosis, fistula, erythrocyte aggregation, microvascular proliferation, fibroblastic activation, cyderophahge, glomerular necrosis and calcification. We could not find any standardized pathological scoring system in the literature for this type of surgery. Therefore, we compared the groups according to the most important pathologic features, as shown in Table I. in the partial nephrectomy specimens.

Statistical Analysis.

The results were analyzed with a programme of SPSS 12.0/Windows. Definitive statistics were determined as a mean, standard deviation, minimum, maximum and percentage values. Fisher test, Kruskal-Wallis and Mann-Whitney U tests were used for evaluating significance among the groups. Posthoc Bonferroni test was also used for correction of significance level in subgroup comparison.

Results

Initial Groups Evaluation. A total of 24 open right lower-pole partial nephrectomy were performed, and at least major bleeding was induced in every case. The rats used for this experimental study had similar morphometric characteristics in body and kidney shape. The mean kidney size was 2x2.5x0.5 cm. The resected lower pole kidney tissues were also similar in size and shape, approximate 0.5µ². All animals survived during the operation and postoperative period.

Operative findings. Mean partial nephrectomy time (min) was 3.7 (SD: 0.8) for GI, 2.7 (SD: 0.5) for GII, 1.8 (SD: 0.8) for GIII and 3.2 (SD: 0.8) for GIV, between GI and GIII, the difference was significant statistically (p: 0.007) (Figure.1). Warm ischemia time (WIT) (min) in groups excluded GIV, 2.5 (SD: 0.5) for GI, 1.8 (SD: 0.4) for GII, 1.3 (SD: 0.5) for GIII. GIII had a significant less WIT compared with GI (p: 0.011). The number of application of ABS was 2.7 (SD: 0.8) for GII, 4.5 (SD: 0.8) for GIII, 6.0 (SD: 1.1) for GIV, significantly higher in GIII and GIV compared with GII (p: 0.003). In ABS groups, the formation of aggregate (protein network) was observed macroscopically onto the resected area following the active
Haemostasis (Figure 2). Haemostasis was detected macroscopically in all rats. The blood loss could not be evaluated objectively due to the small kidney size and resected tissue. There were no significant intraoperative complications.

**Sacrificial findings.** Macroscopic view of the kidneys was evaluated consecutively. In GI, 2 of 6 kidneys (33.3%) revealed the adherence to the adjacent organ, in 6 specimens, the resected renal surfaces shown the irregular shape and hardness (Figure 3A). In GII, although the traditional suture technique was used with ABS to provide haemostasis, no adherence and irregular shape were detected (Figure 3B). ABS kidneys, with or without warm ischemia (GIII, IV) were all in a good shape especially nearly to resected area, however, gelatinous, redness and wealthy tissue were observed in a macroviews at transected kidney (Figure 3C). There were no hematoma, urinoma and urine leakage in any of the groups, however, in GI, the adherence to adjacent organ and foreign material reaction was confirmed. Weight of kidneys were statistically not different among groups (p>0.05)

**Histopathologic evaluation:** Glomerular necrosis was detected with higher rate significantly in GI compared with GIII and GIV (p: 0.015) (Figure 4). Calcification was formed significantly in GI compared with GII, III and IV separately (p<0.05). Erythrocyte aggregation was confirmed significantly higher in GII, III and IV than GI (p: 0.015) (Figure 5). Giant cell reaction, acute inflammation, fibrosis, adhesion, thyroidization, fibroblast activation, microvascular proliferation were not statistically different among groups (p>0.05) (Table I).

**Discussion**

Ankaferd Bloodstopper®, a medicinal product, has been approved by Ministry of Health, in the management of external hemorrhage and dental surgery bleedings in Turkey based on safety and efficacy reports indicating its sterility and nontoxicity ([www.ankaferd.com](http://www.ankaferd.com)). It comprises a standardized mixture of the plants 5 mg *Thymus vulgaris*, 9 mg *Glycyrrhiza glabra*, 8 mg *Vitis vinifera*, 7 mg *Alpinia officinarum* and 6 mg *Urtica dioica* in 100 ml Ankaferd solution. The basic mechanism of action for ABS is the formation of an encapsulated protein network that provides focal points for vital erythrocyte aggregation. The protein network induced by ABS is formed rapidly (<1s), however, blood cells, particularly erythrocytes participates in protein network formation. It was shown that ABS-induced protein network was capable of regulating further coagulation and haemostatic reactions.
Hence, normal haemostatic elements were spared during formation of the protein network, the blood clotting process being driven by protein agglutination. The restricted number of studies has been performed regarding the efficacy of ABS in visceral organs. While in one article, ABS was used in a case with upper gastrointestinal bleeding successfully, in another one, therapeutic potential for the management of hemorrhage in open heart surgery was confirmed. This study design was based on the evaluation efficacy of ABS to achieve hemostasis in partial nephrectomy models.

Partial nephrectomy is now acceptable approach for the management of localized small renal tumors. Although the complication rates are slightly higher that those of open radical nephrectomy, the advantages in terms of renal preservation have become more apparent. As a result, partial nephrectomy being performed more frequently, even in patients with a normal contralateral kidney. However, the expansion of the indications for partial nephrectomy has been shown in publications, especially larger tumors up to 7 to 10 cm. Recently, the development of laparoscopic partial nephrectomy has gained more popularity in the world, including hilar control, suture repair of collecting system, suture ligation of blood vessels, and capsular closure over Surgicel bolsters. Most investigators were limited reconstruction time to 30 minutes while some felt that it could be extended to as long as one hour. Bleeding and ischemic renal damage due to warm ischemia period are the most important complications following the surgery. Decrease of WIT and PNT, various tissue sealant and haemostatic agents has been developed to replace tissue suturing. Several agents have been investigated for their haemostatic potential in managing vascular injury, and many have also been evaluated for their efficacy on repairing the collecting system injury. In our results, ABS application onto the transected kidney area provided active hemostasis in partial nephrectomy with significant decrease in WITs and PNTs, comparable with suture group. With more application number of ABS in GIV, active hemostasis was also observed without hilar occlusion, however, PNT in GIV was not different from GI (p>0.05). However, the absence of urinoma in groups applied ABS may reveal the effect of ABS on collecting system repair following one month. These favorable findings resulted the active haemostasis and regular healing of transected kidney without adherence and urinoma around the kidney. In our observation, we demonstrated the foreign material reaction in conventional PN model, however, it could be hypotised that use of ABS
onto the sutured line in GII prevent this reaction against the suture by the way of antibacterial effect of ABS\(^\text{16}\).

A various tissue sealants and haemostatic agents were produced in use of urological practice. Pursifull et.al. reviewed the use of fibrin sealant and gelatin matrix to support hemostasis during open or laparoscopic partial nephrectomy\(^\text{17}\). Desai and colleagues\(^\text{18}\) demonstrated the effectiveness of gelatin matrix in a porcine hand-assisted LPN model without hilar occlusion. This provided the avoidance of warm ischemia and its associated complications. Gill demonstrated that gelatin matrix dramatically decreases complication rates of renal reconstruction after LPN\(^\text{19}\). Recent studies have reported the potential efficacy of these materials in reducing haemorrhage and urinary leakage in LPN\(^\text{20}\). Briefly, decrease of blood loss, WIT and PNT were shown as a main target in partial nephrectomy procedures with using haemostatic agents and glues. However, the molecular and histologic effects of these products have not been absolutely determined up to now in the literature. Generally, these agents were used as supportive products on sutured kidney during PN, in our study; ABS was applied to stop bleeding without suturing renal parenchyma and collecting duct system.

**Histopathologic findings of tissue sealant were evaluated in porcine laparoscopic partial-nephrectomy model\(^\text{17}\).** Nonspecific changes were noted in all specimens, which included foreign-body giant cell reaction, intestinal metaplasia of collecting duct system, lymphoid follicles and microcalcifications. The sutured kidneys were noted to have strong host reaction and more acute inflammation as in our study. Contrastly, we demonstrated specific findings in ABS groups histopathologically. The exact mechanism of erythrocyte aggregation for haemostatis action was significantly confirmed in ABS groups. However, absence of glomerular necrosis and calcification significantly in ABS groups could be the sign of positive effect of Ankaferd on renal tissue and glomerular function.

It could be stressed that the ideal topical haemostatic agent for partial nephrectomy procedure should be easy to use, show its effect within minutes, be effective in arterial and venous bleeding, preserving the glomerular structure and renal parenchyma, providing the closure of collecting duct system and not to be toxic and anaphylactic. We knows that it is too early to declare ‘ABS is an ideal haemostatic agent for partial nephrectomy in human’, however, the preclinical results has given an expectation regarding the efficacy of ABS on haemostasis during partial
nephrectomy in rats. However, using ABS in a larger animal model with bigger caliber vessels should be tried before it is tested in human.

Conclusion
This preclinical experimental study is the first experience for using Ankaferd as a haemostatic agent in renal hemorrhage model. We concluded that ABS facilitated effective hemostasis with decreasing partial nephrectomy time and warm ischemia time in various partial nephrectomy models. Demonstrating the erythrocyte aggregation in our ABS groups is compatible with the basic mechanism of action for ABS that appears to be the formation of protein network providing focal points for erythrocyte aggregation. Although preservation glomerular histology is not main effect of ABS, we demonstrated the absence of glomerular necrosis and calcification which should be evaluated afterward with controlled study, comparable with conventional group. In the future, preclinical and clinical studies are recommended to provide evidence based medicinal findings regarding the routine application of ABS in renal surgical procedures.
References

between 4 and 7 cm results in outcome similar to radical nephrectomy. J Urol, 171:1066, 2004


Table

Table I. Important histopathologic features of groups and comparison with each other
Figure Legends.

Figure 1. Mean partial nephrectomy times of groups

Figure 2. Formation of Ankaferd induced protein network and aggregate onto the resected area of kidney

Figure 3A. Macroscopic appearance of Group I: needle shown the adherence surface to the adjacent organ

Figure 3B. Macroscopic appearance of Group II: well-healing of sutured tissue without adherence

Figure 3C. Macroscopic appearance of Group III (ABS): flat, wealthy and redness area without the presence of infection, urinoma and hematoma

Figure 4. Glomerular necrosis in area of resected section following traditional partial nephrectomy. H&E, reduced from X20

Figure 5. Histologic confirmation of erythrocyte aggregation in area applied haemostatic agent of Ankaferd, H&E reduced from X40
Abbreviations:
ABS: Ankaferd Bloodstopper®
WIT: warm ischemia time
PNT: partial nephrectomy time
GI,II,III,IV: Group I,II,III,IV
LPN: laparoscopic partial nephrectomy
<table>
<thead>
<tr>
<th>Histopathologic Parameters</th>
<th>Group I (n=6)</th>
<th>Group II (n=6)</th>
<th>Group III (n=6)</th>
<th>Group IV (n=6)</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of Giant Cell Reaction</td>
<td>3 (50%)</td>
<td>4 (66.7%)</td>
<td>4 (66.7%)</td>
<td>4 (66.7%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Absence of Glomerular Necrosis</td>
<td>0 (0.0%)</td>
<td>4 (66.7%)</td>
<td>5 (83.3%)</td>
<td>5 (83.3%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Absence of acute inflammation</td>
<td>3 (50%)</td>
<td>6 (100%)</td>
<td>5 (83.3%)</td>
<td>5 (83.3%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Absence of Calcification</td>
<td>0 (0.0%)</td>
<td>6 (100%)</td>
<td>5 (83.3%)</td>
<td>5 (83.3%)</td>
<td>&lt;0.05**</td>
</tr>
<tr>
<td>Absence of fibrosis</td>
<td>3 (50%)</td>
<td>4 (66.7%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Absence of adhesions</td>
<td>4 (66.7%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Absence of tubular thyroidization</td>
<td>6 (100%)</td>
<td>4 (66.7%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Absence of fibroblast activation</td>
<td>2 (33.3%)</td>
<td>4 (66.7%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Absence of fistula</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Presence of eritrocyst aggregation</td>
<td>1 (16.7%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>&lt;0.05**</td>
</tr>
<tr>
<td>Presence of microvascular proliferation</td>
<td>4 (66.7%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Presence of cyderopahge</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

*p value was measured by comparison GI, III, IV with GI

**significant level
INFORMATION FOR AUTHORS

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TITLE: HEMOSTATIC ROLE OF THE FOLKLORE MEDICINAL PLANT EXTRACT ANAKHEED
Authors: BLOOD STREAM IN RAT PRACTICE. ARIDHECO GLYCEMAR: CONTINUED EXPERIMENTAL TRIAL

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☐ Please check here if, based on the criteria listed above, you have no disclosures to report.

Signature: [Signature]

Date: [August 28, 2008]

See AUA.org for complete text of the AUA Disclosure/Conflict of Interest Policy.

[January 2008]