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Safety and hemostatic effect of *Achillea millefolium* L. in localized bleeding

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Abstract

Background and Aim: This study aims to demonstrate the hemostatic effect of the hydroalcoholic extract of *Achillea millefolium* L. in localized bleeding and to assess the safety of its topical application on rat liver.

Materials and Methods: The aerial parts of *A. millefolium* were macerated in methanol for two days. Twelve female Wistar rats, weighing 120–220 g, underwent anesthesia and laparotomy. The liver was exposed, and two incisions were made to induce bleeding. One incision was treated with a sponge soaked in *A. millefolium* extract, while the other served as a control. The animals were divided into two groups: in one, *A. millefolium* (150 mg/kg) was applied to the first incision, and in the other, to the second incision. Liver biopsies were collected after 4, 6, and 8 weeks.

Results: Application of *A. millefolium* to liver incisions, whether first or second, significantly reduced bleeding time (by 36.1% and 31.9%, respectively). Histopathological analysis showed no signs of toxicity or hepatic damage after 4, 6, and 8 weeks in the female rats.

Conclusion: The study confirms the hemostatic effect of the hydroalcoholic extract of *A. millefolium* in localized bleeding and establishes its safety for topical use.

Keywords: *Achillea millefolium*; hemostatic; safety; bleeding; localized; liver.

Introduction

Bleeding is a common and significant complication of surgery. Minimizing blood loss is a critical concern in all surgical procedures.[1] Rapid achievement of hemostasis offers numerous benefits, such as enhanced hemodynamic stability and the avoidance of adverse effects associated with blood transfusions, including infections and anaphylactic reactions.[2,3]

Various hemostatic agents, sealants, and adhesives, each with distinct functions, are utilized to achieve hemostasis.[1] For example, tranexamic acid, a hemostatic agent, poses a minor risk of convulsion, renal failure, and persistent atrial fibrillation.[4] Similarly, fibrin glue, effective for localized hemostasis, carries a potential risk of transmitting bloodborne infections like HIV and hepatitis B and C.[5] Additionally, these materials are not cost-effective.[1]

Achillea millefolium L. (Asteraceae), commonly known as yarrow, is a flowering plant in the Asteraceae family, widely used in traditional medicine. It has external applications in wound healing and treating skin inflammations[6] and is taken internally for gastrointestinal and hepatobiliary disorders due to its anti-inflammatory, antimicrobial, and spasmolytic properties.[7,8] Historically, it has been used to halt internal bleeding.[8] Phytochemical studies of *A. millefolium* have identified essential oils, sesquiterpenes, and phenolic compounds such as flavonoids and phenolcarbonic acids.[9] It is popular in Asia and North America and is recognized by the Council of Europe as a natural food source (essential oil, herb, flowers, and other preparations).[10] Known locally as boomadaran,[11] *A. millefolium* also exhibits anti-inflammatory, vasoprotective, and anxiolytic-like effects[12–14] and is associated with hepatoprotective, antiulcer, and antioxidant activities.[15–17] This study aims to evaluate the safety and effectiveness of the hydroalcoholic extract of *A. millefolium* in localized bleeding.

Materials and Methods

Plant Material and Extraction Procedure

The flowering aerial parts of *Achillea millefolium* L. were gathered in June 2015 from a living collection. The herbarium code 83001-THE was assigned to a voucher specimen, deposited at the Faculty of Pharmacy herbarium. Post-cleaning, the *A. millefolium* inflorescences were air-dried in an oven, subsequently cut, and ground. This powdered plant material was macerated using 80% methanol for two days. Following filtration, the mixture was concentrated under reduced pressure using a rotary vacuum evaporator (Heidolf, Germany) and then lyophilized.

Animal Preparations and Surgical Techniques

The study utilized twelve female Wistar rats (120–220 g), conforming to the National Institutes of Health guidelines for laboratory animal care (NIH Publications No. 80–23). Maintained at a steady room temperature (22±2 °C) with a 12-hour light/dark cycle, the animals had access to standard pellet food and water. They were acclimatized for seven days

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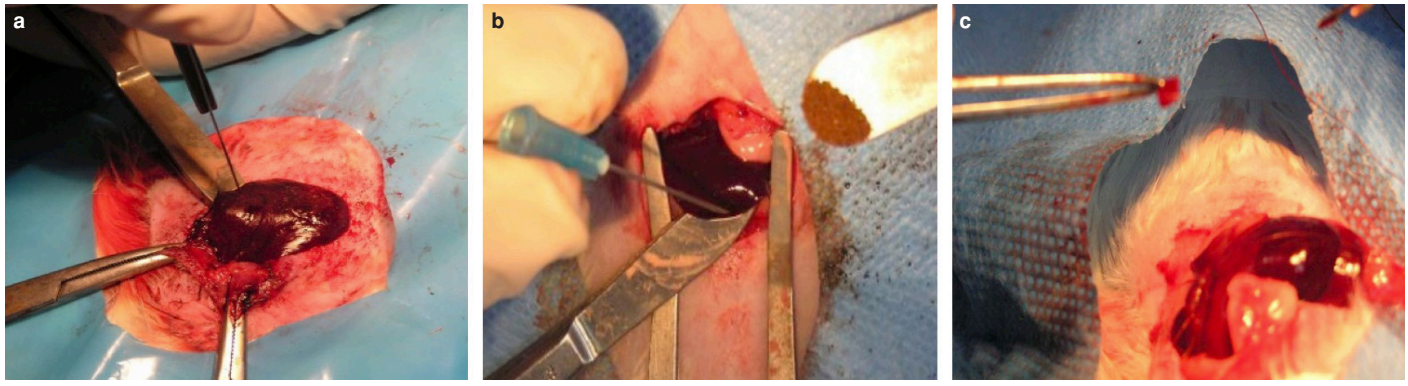

Figure 1. Liver incisions were performed by 23-gauge needle. One was packed only by gauze and another was packed by combination of gauze and A. millefolium powder **(a, b)**. Liver biopsies were taken through a wedge incision from the lacerated parts of rat's liver **(c)**.

Table 1. Effect of hydroalcoholic extract of *A. millefolium* in localized bleeding (group one)

Rats	First incision	Second incision
	BT (s) with *A. millefolium*	BT (s) without *A. millefolium*
1	60	70
2	80	120
3	70	90
4	60	100
5	50	90
6	70	140

BT: Bleeding time; s: Seconds.

before experimentation. Anesthesia was administered via intramuscular injections of Ketamine (75–100 mg/kg) and Xylazine (5–10 mg/kg). A midline laparotomy exposed the liver, where two incisions were made with a 23-gauge needle through the right lobe. One incision was packed with a gauze and *A. millefolium* extract (150 mg/kg powdered plant on gauze) combination, while the other was treated only with gauze as a control (Fig. 1a, b). The rats were randomly divided into two groups of six. In the first group, the plant extract was applied to the first incision, and in the second group, to the second incision. Gauze was removed every 10 seconds to assess bleeding. Bleeding time, defined as the duration from incision infliction to bleeding cessation, was recorded for each incision. Post-operation, the rats were placed in an infant warmer incubator for one hour to prevent hypothermia. The animals were maintained until natural death, with all interventions approved by the University Ethical Committee (No. 91-01-94-16804).

Histopathological Study

Liver samples were randomly obtained from two rats in each group at 4, 6, and 8 weeks post-intervention. A wedge incision was made in the lacerated parts of the right liver lobe (Fig. 1c) and fixed in 10% buffered formalin (Merck, Darmstadt, Germany). Following dehydration through graded alcohol, the samples were embedded in paraffin, sectioned at 5 µm, and cleared in xylene. For histopathological examination, all sections were stained with Hematoxylin and Eosin (H&E), and Masson's Trichrome staining was performed to better assess structural integrity. Two expert pathologists, blind to the experimental design, examined the histologic slides under a light microscope. Histopathological changes such as parenchymal architecture disruption, congestion, inflammatory cell presence, granuloma formation, fibrosis, and focal necrosis were indicative of liver damage.

Statistical Analysis

Data were expressed as means (M) and standard deviation (SD). The Shapiro-Wilk test checked data normality, rejecting normal distribution for p-values less than 0.05. The Student's t-test analyzed the data, with p-values less than 0.05 indicating statistical significance. All statistical analyses were conducted using SPSS version 16 (SPSS Inc., Chicago, IL, USA).

Results

Distribution of Bleeding Time Scoring

The distribution of bleeding time scores for each incision in both groups was analyzed. All scores displayed a normal distribution, with all p-values >0.05.

Effect of Hydroalcoholic Extract of *A. millefolium* on Localized Bleeding

The study results revealed a significant reduction in mean bleeding time with the application of *A. millefolium*. In group one, the mean bleeding time with İ was 65.00 seconds (SD=10.49), significantly lower than the control group's mean bleeding time of 101.67 seconds (SD=24.83); t(5)=-4.35, p=0.007 (Table 1). Similarly, in group two, the mean bleeding time with *A. millefolium* was 78.33 seconds (SD=17.22), significantly less than the control group's 115.00 seconds (SD=21.68); t(5)=-3.99, p=0.010 (Table 2).

Biopsy and Histopathology

Histopathological examination of the liver samples revealed no evidence of liver damage. This included the absence of congestion, parenchymal architecture disruption, and focal necrosis. Additionally, there was no presence of inflammatory cells such as polymorphonuclear cells and lymphocyte infiltration, and no signs of granuloma formation or fibrosis were observed in samples treated with the hydroalcoholic extract of *A. millefolium*. Masson's Trichrome staining demonstrated an intact collagenous structure and a normal vascular pattern (Fig. 2).

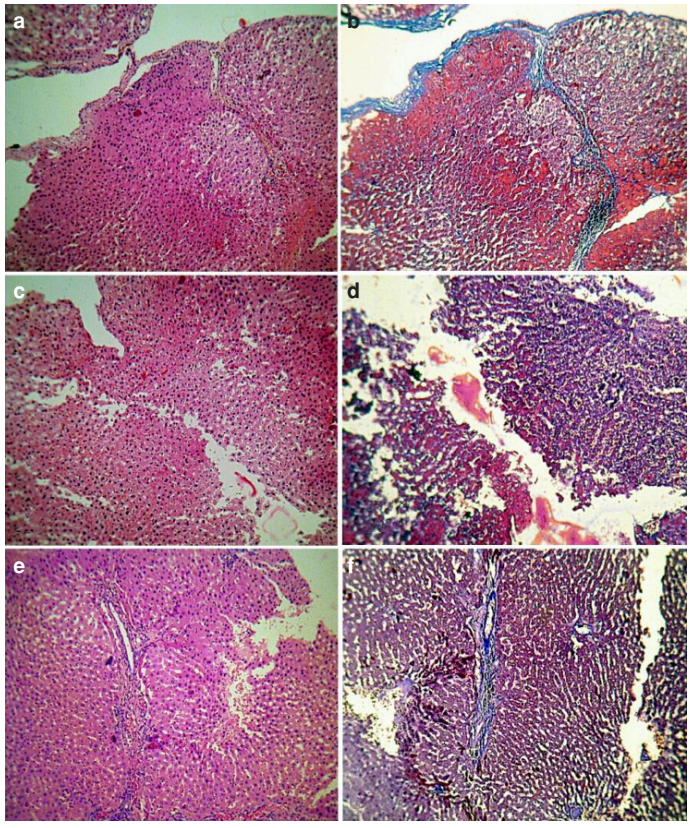

Figure 2. Histological image of the liver sections stained with H&E (left) and Masson's Trichrome (right), magnification 100x. The normal parenchymal architecture with no evidence of congestion, focal necrosis and inflammatory cells with normal collagenous pattern four weeks **(a, b)**, six weeks **(c, d)** and eight weeks **(e, f)** after using hydroalcoholic extract of *A. millefolium*.

Discussion

In this study, we evaluated the hemostatic effect of the hydroalcoholic extract of *Achillea millefolium* L. in localized bleeding using an animal model. The results indicate a significant decrease in bleeding time with the application of *A. millefolium* on liver incisions, by 36.1% and 31.9% for the first and second incisions, respectively. These findings corroborate historical uses of *A. millefolium* as a hemostatic agent. Previous studies demonstrated a 32% reduction in blood clotting time in rabbits following intravenous injection of *A. millefolium*, with sustained hemostasis for 45 minutes and no toxic effects.[8,10] However, unlike the general distribution observed with intravenous administration, our study focused on topical application, showing no systemic distribution or deleterious effects.

A recent study used a multi-herbal extract containing *A. millefolium* to control localized bleeding in a rat liver model, but the specific efficacy of *A. millefolium* alone was not isolated, and safety data were lacking.[18,19] Our study adds to this body of knowledge by confirming the safety of *A. millefolium*, showing no significant changes in liver tissue structure or signs of toxicity and hepatic damage after 4, 6, and 8 weeks. This aligns with previous findings on the safety of *A. millefolium* after chronic exposure in rats.[16]

The liver was chosen for this study due to its friable nature and dual blood supply, which can make it more susceptible to bleeding than other organs. However, these findings may be applicable to surgeries involving other organs. Bleeding during surgery, even from suturing injuries, can be a significant complication. Materials like *A. millefolium* that reduce blood loss without the need for hemostatic ligatures are crucial in managing such bleeding.

Table 2. Effect of hydroalcoholic extract of *A. millefolium* on localized bleeding (group two)

Rats	First incision	Second incision
	BT (s) without *A. millefolium*	BT (s) with *A. millefolium*
1	110	80
2	130	60
3	90	70
4	140	80
5	130	110
6	90	70

BT: Bleeding time; s: Seconds.

To mitigate physiological and biological variances among rats, even within the same strain, both incisions were performed on each rat's liver. This approach aimed to decrease confounding factors and allow for more accurate bleeding time comparisons. We also alternated the use of *A. millefolium* between the first and second incisions in the two groups to eliminate potential effects on the coagulation cascade that could influence bleeding times.

Future studies should involve a larger sample size or larger animals to further investigate the safety and hemostatic effectiveness of *A. millefolium*'s hydroalcoholic extract in localized bleeding before considering its application in humans. Additionally, tests to assess allergic reactions and the sterilization effect on the efficacy of *A. millefolium*'s extract are crucial to understand potential side effects, paving the way for its use as a new hemostatic drug in humans.

Conclusion

In summary, this study confirms that the hydroalcoholic extract of the flowering aerial parts of *A. millefolium* effectively reduces bleeding in localized areas. Additionally, the study establishes the safety of this plant extract in an animal model, suggesting potential for future human applications.

Ethics Committee Approval: The Tehran University of Medical Sciences Ethical Committee granted approval for this study (date: 01.14.2012, number: 91-01-94-16804).

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