Original Article

Safety and Hemostatic Effect of Achillea millefolium L. in Localized Bleeding

Bleeding

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Running title : Safety and Hemostatic Effect of A. millefolium
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Abstract

Objective(s): To present the hemostatic effect of hydroalcoholic extract of *Achillea millefolium* L. in localized bleeding and evaluate the safety of this plant after topical usage in rat's liver.

Materials and methods: The aerial parts of this plant were macerated for 2 days using methanol. After anesthesia and laparotomy of twelve female Wister rats (120-220g), the liver was exposed and two incisions were performed for bleeding. One was packed by sponge with *A. millefolium* and another without *A. millefolium* as a control group. Animals were divided into 2 groups that *A. millefolium* (150mg/kg) was used in the first incision for one group and in the second incision for another. Liver biopsy was taken after 4, 6 and 8 weeks.

Results: We observed that usage of *A. millefolium* for liver incisions, either in the first incision or in the second incision; bleeding time decreases significantly (36.1% and 31.9% respectively). Histopathological evaluations revealed no signs of toxic and hepatic damage for periods 4, 6 and 8 weeks in the female rats.

Conclusions: This study confirmed the hemostatic effect of hydroalcoholic extract of *A. millefolium* in localized bleeding and also the safety of this plant for topical usage.

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

Bleeding is the most common and serious complications of surgery. Reducing blood loss is one of the main concerns of all surgical operations. Accomplishment of rapid hemostasis has many advantages such as improved hemodynamic stability and avoidance of deleterious effects of blood transfusion including infection and anaphylactic reaction.

Hemostatic agents, sealants and adhesives which have different functions are applied for achieving hemostasis. For examples, tranexamic acid is one of the hemostatic agents, carries a small risk of convulsion, renal failure and persistent atrial fibrillation and fibrin glue which is ideal for localized usage to secure hemostasis, conveys the potential risk of transmission of blood-borne infection including HIV and hepatitis B and C. Moreover, none of these materials are cost-effective.

\textit{Achillea millefolium} L. (Asteraceae) commonly known as yarrow is a flowering plant in the Asteraceae family and has been extensively used in folk medicine externally for wound healing and skin inflammations. It is also used internally against gastrointestinal and hepatobiliary disorders because of its anti-inflammatory, antimicrobial and spasmolytic properties. In addition, it has been used in classical times to stop internal bleeding. Several constituents of \textit{A. millefolium}, including essential oils, sesquiterpenes, and phenolic compounds such as flavonoids and phenolcarboxylic acids have been reported in phytochemical studies. \textit{A. millefolium} is widely used in Asia, North America and it also listed by the Council of Europe as a natural source of food flowering (essential oil, herb, flowers and other preparations). It is also a native plant called boomadaran. Besides anti-inflammatory, vasoprotective and anxiolytic-like effects, \textit{A millefolium} also plays roles in hepatoprotective, antiulcer and antioxidant activities. Herein, we evaluated the safety and effectiveness of hydroalcoholic extract of \textit{A. millefolium} in localized bleeding.
Material and Methods

2.1 Plant material and extraction procedure

The flowering aerial parts of *A. millefolium* were collected in June 2015 from the living collection. A voucher specimen with herbarium code of 83001-THE has been deposited at the herbarium of Faculty of Pharmacy. After proper cleaning, *A. millefolium* inflorescences were air-dried in an oven and then the dry plants were cut and grinded. The powdered plant material was macerated for two days using 80% methanol. After two days the mixture was filtered and then concentrated using a rotary vacuum evaporator (Heidolf, Germany) under reduced pressure and lyophilized.

2.2 Animal preparations and surgical techniques

Experiments were performed with twelve female Wister rats (120–220 g). Experimental procedures and housing were performed according to the policy guidelines of the National Guide for Institutes of Health for the Care and Use of Laboratory (NIH Publications No. 80–23). Animals were maintained at constant room temperature (22 ± 2 °C) under a 12 h light/dark cycles, with access to a standard pellet diet and water. The animals were acclimatized for 7 days before conducting experiments. Rats were anesthetized by intramuscular injection of Ketamine (75–100 mg/kg) and Xylazine (5–10 mg/kg). The liver was gently exposed through a midline laparotomy and two incisions were performed by 23-gauge needle through the right lobe of liver. One was packed by combination of gauze and *A. millefolium* extract (150 mg/kg powdered plant on gauze), another was packed only by gauze without plant extract as a control incision (Fig. 1A & B). Animals divided into two equal groups of six randomly. In group one, plant extract was applied on the first incision and in group two on the second incision. The gauze was removed every 10 seconds to check the condition of bleeding. Bleeding time is defined as the time between infliction of an incision and stopping of bleeding. After recording the bleeding time of each incision, the abdomen was closed and the rats were remained in infant warmer incubator for one hour post-operatively as a recovery unit in order to prevent animal hypothermia. At the end of the treatment, the animals were maintained until natural death. The University Ethical Committee approved all the interventions. (No. 16804–94–01–91)

2.3 Histopathological Study

Liver samples were obtained randomly from two rats in each group at 4, 6 and 8 weeks after
interventions. In each period of time, a wedge incision from the lacerated parts of right lobe of rat's liver was performed (Fig. 1C) and fixed into 10% buffered formalin (Merck, Darmstadt, Germany). After dehydrating through graded alcohol, samples were subsequently embedded in paraffin, then sectioned at 5 μm and cleared in xylene. For histopathological evaluation, all sections were stained with Hematoxylin and Eosin (H&E). Masson's Trichrome staining also was performed to identify better structural integrity. Two expert pathologists who are blind to the design examined the histologic slides under a light microscope. Histopathological changes including disruption of the parenchymal architecture accompanied by congestion, presence of inflammatory cells, granuloma formation or fibrosis and focal necrosis were considered as liver damage.

2.4 Statistical analysis
The results were expressed as means (M) and standard deviation (SD) for quantitative variables. The Shapiro-Wilk test was used to verify the normality of the data, which rejects a normal distribution for p-value less than 0.05. The data were analyzed by Student’s t-test and differences were considered statistically significant when P < 0.05. All the statistical analyses were performed by SPSS version 16 (SPSS Inc. Chicago, IL, USA).
Results

3.1 Distribution of the bleeding time scoring
We measured the distribution of bleeding time scoring for each incision in both groups that all the scores showed a normal distribution (all p-values > 0.05).

3.2 Effect of hydroalcoholic extract of A. millefolium in localized bleeding
The results indicated that the mean bleeding time with A. millefolium (M=65.00 seconds, SD=10.49) was significantly lower than the mean bleeding time without it (M=101.67 seconds, SD=24.83) ; t(5)=−4.35, p=0.007 in group one (table 1).
Moreover, there was a significant difference in the scores of bleeding time with A. millefolium (M=78.33 seconds, SD=17.22) and without it (M=115.00 seconds, SD=21.68) ; t(5)=−3.99, p=0.010 in group two (table 2).

3.3 biopsy and histopathology
Histopathological evaluation reveals no liver damage such as congestion, disruption of the parenchymal architecture and focal necrosis. It also shows lack of inflammatory cells like polymorph nuclear cells and lymphocyte infiltration and no evidence of granuloma formation or fibrosis in all samples which used hydroalcoholic extract of A. millefolium. Masson Trichrome staining shows well an intact collagenous structure and normal vasculature pattern (Fig. 2).
Discussion

In the present study, we investigated the hemostatic effect of the hydroalcoholic extract of *A. millefolium* in localized bleeding in an animal model. Specifically, our result suggests that usage of *A. millefolium* for liver incisions, either in the first incision or in the second incision, bleeding time significantly decreases (36.1% and 31.9% respectively). These findings support the efficacy of *A. millefolium* to stop bleeding in classical times. Intravenous injection of *A. millefolium* reduced the blood clotting time by 32% in rabbits and persistent hemostasis was observed for 45 minutes with no toxic effect\(^8,10\). Intravenous administration has general distribution to all the organs and this deleterious effect has not been clearly defined. But in our study *A. millefolium* only used topically and no systemic distribution was observed. Recently, algan hemostatic agent as a multi-herbal extract containing *A. millefolium* was used to control localized bleeding in rat liver model. However, the efficacy of *A. millefolium* alone was not determined. On the other hand, the safety of this extract was unclear due to lack of follow up\(^18,19\). Our study also indicates that *A. millefolium* made no significant changes in the structure of liver tissue with no signs of toxic and hepatic damage after 4, 6 and 8 weeks. It means *A. millefolium* is safe in localized usage. The safety of this plant was observed after chronic exposure in either female or male rats\(^16\).

The reason for choosing liver in this study relates to the organ's friable nature and its dual blood supply (portal vein and hepatic artery) which may make it more susceptible to bleeding than other organs. However, the outcome of these studies can be applicable during the surgery of other organs. Bleeding might happen even from an injury by the suturing tissue and lead to the most important complication during surgery. Using materials such as *A. millefolium* that reduce blood loss with no hemostatic ligature on liver is the best way to stop bleeding in this situation.

Because of different physiological and biological characteristics of rats (even rats of the same race), we decided to perform both incisions in each rat's liver in order to decrease the confounding factors and compare the bleeding time more accurately.

In order to eliminate the possible effect on the coagulation cascade which could influence bleeding times of the second, *A. millefolium* was used for group one and two
in the first and second incisions respectively.
We suggest that further studies should be done with greater number of rats or using larger animals to investigate the safety and hemostatic effect of hydroalcoholic extract of *A. millefolium* in localized bleeding before using in human. It seems that some tests including allergic reactions and also the sterilization effect on the efficacy of hydroalcoholic extract of *A. millefolium* must be performed in order to identify the side effects of this drug that can be used in human being as a new hemostatic drug in the future.

**Conclusion**
In conclusion, the present findings confirm that hydroalcoholic extract of the flowering aerial parts of *A. millefolium* has hemostatic effect in localized bleeding. Moreover, our observation shows the safety of this plant in an animal model.

**Conflict of interest**
The authors have no conflicts of interest to declare.

**Financial Disclosure**
The author declare that the study has received no financial support.
References


12. Goldberg AS, Mueller EC, Eigen E, Desalva SJ. Isolation of the anti-


### Tables

**Table 1.** Effect of hydroalcoholic extract of *A. millefolium* in localized bleeding (group one)
BT = bleeding time; s = second

<table>
<thead>
<tr>
<th>Rats</th>
<th>First Incision</th>
<th>Second Incision</th>
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<td>BT(s) with <em>A. millefolium</em></td>
<td>BT(s) without <em>A. millefolium</em></td>
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<td>1</td>
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<td>6</td>
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**Table 2.** Effect of hydroalcoholic extract of *A. millefolium* in localized bleeding (group two)
BT = bleeding time; s = second

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<td>BT(s) with <em>A. millefolium</em></td>
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Figure Legend 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 and B). Liver biopsies were taken through a wedge incision from the lacerated parts of rat's liver (C).
Figure Legend 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 (A1 and A2), six weeks (B1 and B2) and eight weeks (C1 and C2) after using hydroalcoholic extract of *A. millefolium*. 