

## Research Article

### Hemostatic Effect of Three Different Doses of *Justicia Adhatoda* Leaf Extract on Bleeding Time in Mice

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#### Abstract

**Background:** Hemorrhage can occur as a consequence of innumerable conditions. It can lead to hemodynamic compromise and death unless treated promptly. Various traditional medicinal plants are being studied for their effects on hemostasis. *Justicia adhatoda* is commonly used in Ayurvedic medicine to control both external and internal bleeding such as peptic ulcers, piles, bleeding gums, and menorrhagia.

**Objective:** The objective of this study was to observe the effect of *Justicia adhatoda* leaf extract on bleeding time in mice.

**Methods:** An experimental study was conducted at PGMI, Lahore. Twenty Swiss albino mice were randomly divided to make four groups. Each group had five animals. Normal saline was administered to Group (I). Group (II) was given 50 mg/kg dose of leaf extract. Group (III) received 100 mg/kg dose of extract and Group (IV) was given 200 mg/kg dose of extract. One hour later, the tail was amputated after anesthetizing the animals. Bleeding time was estimated by gently placing Whatman filter paper on the edge of the incision every 30 seconds. The observation of no adherent blood was defined as bleeding time.

**Results:** Bleeding time decreased in all the extract-treated groups. A maximum decrease was recorded in the group that received 200 mg/kg extract (1.9±1.03 minutes) versus normal control (6.1±2.36 minutes), with  $p < 0.01$ . Bleeding time decreased to 2.6±1.39 minutes with 100 mg/kg dose, with  $p < 0.05$ .

**Conclusion:** According to the results of this study, *Justicia adhatoda* leaf extract is effective in controlling excessive bleeding. However, further work needs to be performed to elucidate the underlying mechanism.

**Received** | 09-11-2018: **Accepted** | 12-12-2019

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**Keywords** | Hemostasis, *Justicia adhatoda*, bleeding time.

#### Introduction

Hemorrhage can pose a serious threat to health and life unless managed timely and appropriately. It can manifest as external loss or maybe internal, concealed within the organ systems. The severity of hemorrhage may vary from minor, requiring no intervention, to major, resulting in hemodynamic compromise and even death.<sup>1</sup> Hemorrhage can

occur as a consequence of innumerable conditions. Trauma and accidental injuries constitute one of the important causes of bleeding in otherwise healthy people.<sup>2</sup> Likewise, menorrhagia, epistaxis, peptic ulcer disease, and dengue can cause massive bleeds in patients without any underlying coagulation disorder.<sup>3</sup>

The other extremely significant cause of hemorrhage

is a coagulation disorder, either genetic or acquired. Common genetic disorders include Hemophilia A and B, Von Willibrand's Disease and some platelet disorders.<sup>4</sup> Amongst the acquired disorders, liver disease, disseminated intravascular coagulation and drug-induced coagulopathy are the most important causes.<sup>5</sup> Management of bleeding in these cases includes intravenous fluids, blood, fresh frozen plasma or surgical intervention if required.<sup>6</sup>

*Justicia adhatoda* is a perennial, evergreen shrub that grows naturally in India, Nepal, and the Pothohar region of Pakistan.<sup>7</sup> It is popular in Ayurvedic medicine for the treatment of various diseases. The leaves of *Justicia adhatoda* contain several alkaloids including vasicine, vasicinol, adhatodine, adhavasione, flavonoids, saponins, tannins, sterols, polyphenols, glycosides and other metabolites like steroids, carbohydrates and alkanes.<sup>8</sup> Leaf juice and extract have been used for many years, in folk medicine, for treating conditions like bronchitis, asthma, tuberculosis, blood disorders, and dysentery.<sup>9</sup> Various scientific studies have demonstrated its antimicrobial,<sup>10</sup> anti-ulcer<sup>11</sup> and wound healing property.<sup>12</sup>

In traditional medicine, *Justicia adhatoda* has been used for many years to control bleeding due to idiopathic thrombocytopenic purpura, local bleeding due to peptic ulcer, piles and menorrhagia.<sup>13</sup> However, no scientific work has been done so far to observe this effect.

Therefore, this study was aimed to observe the hemostatic activity of *Justicia adhatoda* leaf extract in mice.

## Methods

**Study design:** Experimental study

**Study setting:** Postgraduate Medical Institute, Lahore

**Preparation of *Justicia adhatoda* extract:** Fresh leaves of *Justicia adhatoda* were collected from Bagh-e-Jinnah, Lahore. They were identified by the Botany Department of Government College University, Lahore (Voucher # GC Herb. Bot. 2980). Leaves were washed with water thoroughly. They were air-dried at approx. 32°C (room temperature). The weight

of dried leaves was recorded to be constant after 3 days. Dried leaves were coarsely powdered and immersed for one week in 1:10 (w/v) ethanol (80%). Whatman filter paper was used to filter the supernatant twice, followed by air drying at approx. 32 °C (room temperature). The weight of the crude extract was recorded and it was stored at 4°C until further use. It was dissolved in normal saline before administration.<sup>10</sup>

## Animals

Healthy male Swiss albino mice were chosen for the study. The weight of animals was within 25-35 grams. They were placed, under hygienic conditions, in the animal house of PGMI, Lahore. Five mice were placed in one cage (30 × 30 × 45 cm). The animals were acclimatized for one week before beginning the study. They were allowed access to water and food ad libitum. The care and handling of animals was done according to the 'Guide for the care and use of laboratory animal'. Ethical approval was obtained from the Institutional Review Board before commencement of the study.

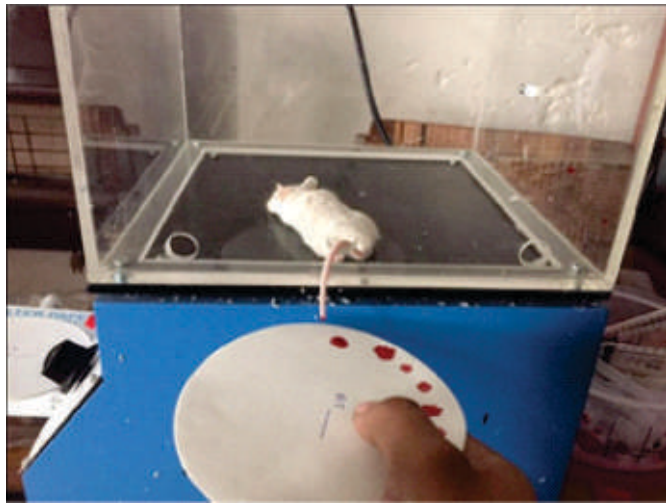
Twenty mice were divided into four separate groups by simple random sampling. Five mice were placed in each group. Single-dose of normal saline was given to Group (I), in an amount equal to that used in the experimental groups. Group (II), (III) and (IV) were given single dose of 50 mg/kg extract, 100 mg/kg extract and 200 mg/kg extract respectively.

## Laboratory procedures:

All the doses were administered to mice by intraperitoneal injection using a 1 ml disposable syringe. One hour after the intraperitoneal administration of *Justicia adhatoda* extract or normal saline, ketamine was injected intraperitoneally at the dose of 80 mg/kg total body weight to anesthetize the mice.<sup>14</sup>

Mice were placed one by one on the hot plate, set at a fixed temperature of 38°C, in a way that the tail dropped almost 2 cm down from the upper surface of the platform. Then, the tail was passed through a paper template of fixed-sized hole. The tail was transected using a no. 21 surgical blade. For estimation of bleeding from the incision, filter paper (Whatman International Ltd, Maidstone, UK) was gently placed on the edge of the clot every 30 seconds

(Fig. 1), taking care not to dislodge the clot. The observation of no adherent blood was defined as the bleeding time.<sup>15</sup>



**Fig. 1:** Measurement of bleeding time by tail cut method

#### Statistical analysis:

All the data was transcribed into GraphPad Prism 5.0 for statistical analysis. Data was checked for normality with Shapiro Wilk test. Normal distribution was observed in all the data sets. Significance of outcome in all the study groups was tested with One-way ANOVA (Analysis of Variance). For analysis of difference between the group means, post hoc Tukey's test was used.  $P < 0.05$  was considered significant.

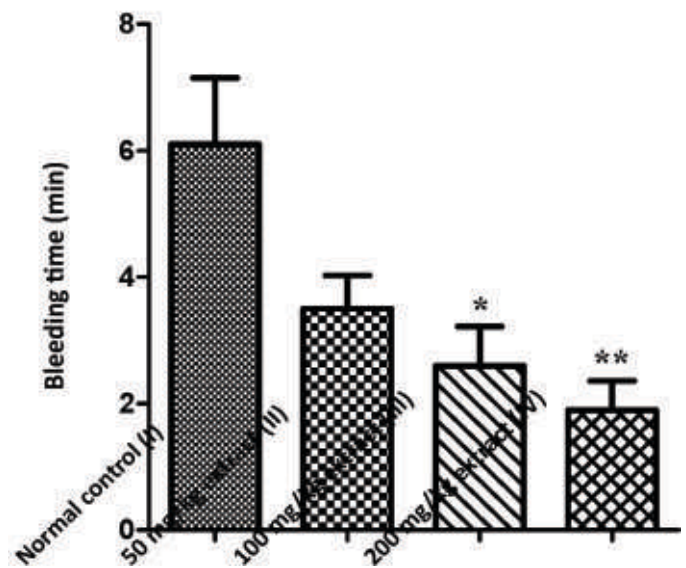
#### Results

Numerically, a decrease in bleeding time was documented in all of the extract-treated groups. Mean bleeding time of  $6.9 \pm 2.36$  minutes was observed in normal control (I). In comparison, bleeding time decreased to  $3.5 \pm 1.17$  minutes,  $2.6 \pm 1.39$  minutes and  $1.9 \pm 1.03$  minutes in 50 mg/kg (II), 100 mg/kg (III) and 200 mg/kg (IV) extract-treated groups, respectively. Mean difference was statistically significant between group (I) and (III), with  $p < 0.05$  and group (I) and (IV), with  $p < 0.01$ .

Therefore, *Justicia adhatoda* extract was observed to effectively decrease bleeding time at 100 mg/kg and 200 mg/kg dose.

However, difference amongst the groups (II), (III) and (IV) was not significant (Fig. 2).

#### Dose response effect of *J. adhatoda* on bleeding time



**Fig. 2:** Effect of different Doses of *Justicia Adhatoda* on Bleeding Time (mean  $\pm$  SE) in mice (n=5)

\* =  $p < 0.05$  (vs. normal control)

\*\* =  $p < 0.01$  (vs. normal control)

Comparison of means of the four groups by ANOVA revealed that the difference between the group means was significant, with a p value of 0.0036.

**Table 1a:** Effect of different Doses of *Justicia Adhatoda* on Bleeding Time in Mice (n=5)

Bleeding time (minutes)					
Groups	Mean	SD	Min.	Max.	p-value
Normal Control (I)	6.1	2.36	2.5	9.0	0.0036
Extract 50 mg/kg (II)	3.5	1.17	2.0	4.5	**
Extract 100 mg/kg (III)	2.6	1.39	1.0	4.5	
Extract 200 mg/k (IV)	1.9	1.03	1.0	3.0	

\*\* =  $p < 0.01$

**Table 1b:** Comparison of Effect of Different Doses of *Justicia Adhatoda* Leaf Extract on Bleeding Time in Mice by Post hoc Tukey's test (n=5)

Tukey's Multiple Comparison Test		Mean Diff.	Significance
Normal control (I)	Extract 50 mg/kg (II)	2.600	ns
	Extract 100 mg/kg (III)	3.500	*
	Extract 200 mg/kg (IV)	4.200	**
Extract 50 mg/kg (II)	Extract 100 mg/kg (III)	0.9000	ns
	Extract 200 mg/kg (IV)	1.600	ns
Extract 100 mg/kg (III)	Extract 200 mg/kg (IV)	0.7000	ns

ns = not significant,

\* =  $p < 0.05$ , \*\* =  $p < 0.01$



## Discussion

Hemorrhage is an extremely common problem and can occur in normal individuals as well as in those with coagulation disorders. It can lead to hemodynamic compromise and death unless treated promptly. General measures, alone, are sometimes not sufficient to restore hemostasis.<sup>16</sup> *Justicia adhatoda* has been used to manage bleeding in traditional medicine for years.<sup>9</sup> This study was conducted to evaluate the hemostatic activity of *Justicia adhatoda* leaf extract. Three different doses of extract used were 50 mg/kg (low dose), 100 mg/kg (medium dose) and 200 mg/kg (high dose).

Mice were chosen as experimental animals because genes and proteins involved in coagulation and hemostasis in mice are very similar to humans.<sup>17</sup> Bleeding time could be done once only in each group to avoid alteration in results due to tail injury variations.<sup>15</sup> The intraperitoneal route was chosen to administer the leaf extract to ensure rapid absorption and quick response, as hemostasis requires urgent intervention.

Mean bleeding time decreased in all the groups, as compared to normal control. A maximum decrease was recorded in the group that received 200 mg/kg leaf extract, with a p-value of  $< 0.01$ . This depicts a graded dose-response relationship between the concentration of extract and the bleeding time. However, further work needs to be done, especially with repeated dosing over a prolonged period of time, to elucidate the mechanism.

No similar study has demonstrated the effect of *Justicia adhatoda* on hemostasis, so far. Therefore, studies conducted on other plants or products with documented effects on hemostasis were used as a reference for comparison to the results of the present study.

The decrease in tail bleeding time was in concordance with the hemostatic effect of Ankaferd Blood Stopper (ABS) on tail tip bleeding time in rats (p-value = 0.001)<sup>18</sup>. Similarly, Antarctic krill chitosan powder decreased bleeding time in mice tail amputation mode (p-value  $< 0.005$ ).<sup>19</sup> Another study demonstrated that extracts from various species of *Lagochilus* caused a significant reduction in bleeding time of mice, in a dose-dependent manner (p-value  $< 0.01$ )<sup>20</sup>.

In contrast, *Justicia adhatoda* did not produce a statistically significant dose-dependent decrease in bleeding time.

The fluidity of blood is maintained by a critical balance between the procoagulant factors and the anticoagulant factors present naturally in the blood. Any vascular or tissue injury disrupts this balance in the favor of hemostasis. Blood coagulation is initiated by vasospasm and adherence of platelets to the damaged vessel wall. The adherent platelets come in contact with exposed collagen, which results in platelet activation and degranulation. Simultaneously, extrinsic or intrinsic coagulation pathway is activated, ultimately leading to the generation of thrombin. Finally, thrombin converts soluble fibrinogen into fibrin meshwork that entraps the red cells and platelets, forming a clot.<sup>21</sup>

It is proposed that *Justicia adhatoda* has produced a hemostatic effect by interfering with one or more steps of the coagulation cascade. It might act at the final common pathway of coagulation, resulting in the acceleration of conversion of fibrinogen to fibrin. However, there are no current studies to support this. Another possible mechanism is by causing vasoconstriction or platelet aggregation. Studies have demonstrated that leaf extract of *Justicia adhatoda* causes enhanced production and release of thromboxane A2 and prostaglandins<sup>22</sup>. It is well established that Prostaglandin G2, prostaglandin H2, and thromboxane A2 cause vasoconstriction and promote platelet aggregation, thereby facilitating the process of hemostasis.<sup>23</sup> Therefore, the hemostatic effect produced by *Justicia adhatoda* leaf extract may be attributed to the release of prostaglandins. However, further work needs to be done in order to scientifically demonstrate this mechanism.

## Conclusion and Recommendations

According to the results of the current study, it is concluded that leaf extract of *Justicia adhatoda* has a hemostatic effect. Nevertheless, further studies should be conducted to assess the time duration for which the hemostatic effect of *Justicia adhatoda* persists and observe the underlying mechanism.

**Disclaimers:** None

**Source(s) of Support:** None



**Conflict of Interest**

There is no conflict of interest.

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