

Exploring the Hemostatic and Hematological Impact of Butea monosperma Bark Ethanolic Extract in a Rodent Model

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Research Article

Keywords: Astringent properties, Butea monosperma, Haemostatic activity, ethanolic bark extract, Tannin

Posted Date: November 6th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7934816/v1>

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Additional Declarations: No competing interests reported.

Abstract

Objective

With the global wound care market valued at approximately \$20 billion in 2023, is projected to reach \$30 billion by 2030, due to the increasing incidences of chronic wounds, ulcers, diabetic ulcers, and the rising volume of surgical procedures worldwide. While synthetic hemostatic agents are widely used, they suffer from limitations such as high costs, cytotoxicity, poor biocompatibility, and environmental concerns, thus there is a rising demand for cost-effective, natural alternatives. So, as an alternative, plant with rich source of tannins and antimicrobial properties may be the best reason to choose **Butea monosperma** (BM) to scientifically validate the hemostatic and wound healing effects benefits. The study employs basic preliminary in vivo bleeding models to evaluate the efficacy of *Butea monosperma* bark extracts as natural hemostatic agents.

Materials and Methods

To study the haemostatic activity of ethanolic bark extract of *Butea monosperma* different doses at a concentration of 100mg/kg, 200mg/kg and 400mg/kg were given orally for 10 days in the albino wistar rats. Hemostatic activity is determined by (i) clotting and bleeding time, (ii) bleeding amount, (iii) bleeding amount, and haematological parameters like RBC, Hb count, and ESR.

Results

From the data it was observed that ethanolic extract of BM, there was a decrease in both the clotting and bleeding time though there was no significant difference seen in the other haematological parameters (RBC, Hb, and ESR) compared to the control group. The decrease in clotting time indicates the qualitative test for measurement of factors involved in the intrinsic pathway of blood clotting whereas a decrease in the bleeding time was assumed to be an increase in the platelet count.

1. Introduction

Herbal remedies are growing in popularity due to the increasing demand for effective yet reliable alternatives to conventional medications, which often have undesirable side effects (Ekor, 2014). Traditional medicine offers numerous natural crude drugs with significant therapeutic potential for treating various diseases and disorders. One such remarkable plant is *Butea monosperma* (Lam.) Taub from the Fabaceae family. Commonly known as 'dhak' or 'palas,' it is also referred to as the "Flame of the Forest" because of its vivid red blossoms. Other regional names include palash, mutthuga, bijasneha, khakara, chichara, Bastard teak, and Bengal kino. Native to India, *Butea monosperma* has long been utilized in Ayurveda as a tonic, anthelmintic, and astringent. Traditionally, it has been employed in treating a wide range of clinical conditions, including dyspepsia, diarrhea, dysentery, ulcers, sore throat,

and even snake bites (Srivastava et al., 2023). Several scientific studies highlight the therapeutic potential of various parts of the plant—bark, seeds, gum, leaves, flowers, and roots—which exhibit diverse pharmacological properties such as anthelmintic, anti-inflammatory, antibacterial, diuretic, analgesic, antitumor, and anti-diabetic activities (Sutariya & Saraf, 2015).

2. Background

This study provides scientific evidence in support of Palash's traditional use in the treatment of blood-related illnesses. Through investigating this medicinal tree's therapeutic potential, the study promotes the incorporation of conventional treatments into contemporary healthcare, providing more effective and less harmful treatment alternatives.

3. Materials & Methods

3.1 Procurement of plant specimens:

The Bark of *Butea monosperma* (Lam.) was collected from nearby area of Durgapur (23.5204° N, 87.3119° E) in different months and all collected were used to prepare the extract. The plant was identified with available literature and authenticated by Botanical Survey of India, Howrah and has preserved with a voucher no. **BCRCP/SB-02**. Collected bark was shed dried for two weeks, finely powdered and kept in desiccator for further study.

3.2 Extraction procedure:

Finely powdered *Butea monosperma* bark where weigh 30 gram each and then placed in 300 ml of methanol at room temperature for 72 hours. The mixture was stirred daily and 72 hours later filtered through Whitman filter paper No.1, and were evaporated to dryness using rotary evaporator at a much-reduced temperature or freeze drying.

3.3 Phytochemical Screening:

The methanolic extract (1 g) was completely dissolved in 100 mL of methanol for prepared the stock solution. The obtained stock solution was used for phytochemical screening following the methodology of (Harbone,1973) and (Kokate., A.P. Purohit., S. B. Gokhale., 2009)

3.4 Animals:

Sixteen male rats weighing between 100-170g were taken. They were divided into four groups each having four animals. Rats were acclimatized for three weeks in standard condition (temperature 25–29°C, 12 hr light and 12 hr dark cycle). The rats were feed on standard normal diet and water given *ad libitum*. The animal experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and approved by the Institutional Animal Ethics Committee (IAEC) of Dr. B. C. Roy College of Pharmacy & AHS, under the approval number “BCRCP/IAEC/7/2017”.

3.5 Toxicity study:

Toxicity study was conducted following the OECD 425 guideline; a single dose of 2000 mg/kg of ethanolic extract of *Butea monosperma* (EEBM) was given orally to the animal and were observed at 30 min, 1 hour, 2-hour, 4-hour, 24-hour, 48-hour, 7th day and 14th day to evaluate any potential toxic effects. During this period, key parameters such as body weight, behavioral changes, signs of distress, and mortality were carefully monitored. Dose of EEBM were fixed based on LD₅₀ value as below-

1) Lower dose, 1/15th of LD (mg/kg)

2) Intermediate dose, 1/10th of LD (mg/kg)

3) Higher dose, 1/5th of LD (mg/kg)

3.6 Experimental design:

All the animals divided into four groups each containing 6 animals. Group I is the control group. Extract treatment –Group II, III & IV were assigned as treated group with the extract at a concentration of 100mg/kg, 200mg/kg and 400mg/kg orally for 10 days. Bleeding time and clotting time was recorded on 10th day. Blood sample were collected from the retro-orbital plexus of controlled and treated group for hematological study.

3.7 Bleeding Time Determination:

Bleeding time was assessed using a modified Duke method. The tail tips of the rats were incised (4–5 mm) with sharp scissors, and a stopwatch was used to record the time. The wound was blotted with filter paper every 15 seconds.(Russeau et al., 2024)

3.8 Bleeding Amount Determination:

The amount of bleeding was quantified following the method by Cipil. The tail tips of the rats were cut (4–5 mm) with scissors and a stop watch was started immediately. Blood drops were collected using pre-weighed blotting paper. After the last drop of blood appeared, the blotting paper was re-weighed using an electronic balance. The difference in weight between the dry and wet blotting paper was taken as the amount of blood lost.(Cipil et al., 2009, Yalcinkaya FR.,2011)

3.9 Clotting Time Determination:

Clotting time was measured using the Lee-White method. Blood (0.8 ml) was collected directly from the heart of each rat, and 0.2 ml of blood was placed into four glass test tubes pre-warmed to 37°C. The

tubes were placed in a 37°C water bath and tilted every 40 seconds until the blood stopped flowing when the tubes were tilted at a 90° angle. The time taken for clotting was recorded.(Ayodele et al., 2019)

3.10 Determination of Hematological Parameters:

3.10.1 RBC Count: Blood was drawn to the 0.5 mark in an RBC pipette, then diluted to the 101 marks with diluting fluid. The mixture was shaken for 3 minutes, and the chamber was charged. RBCs were counted using a 40X objective in 80 small squares. The RBC count was calculated as (Math et al., 2016) :

$$RBC\ count = \frac{No.\ of\ cells\ counted * dilution\ factor * dilution\ factor}{Area\ counted}$$

$$RBC\ count = \frac{No.\ of\ cells\ counted * 200 * 10}{\text{\textcolor{red}{1ex1}}/\text{\textcolor{red}{1ex5}}}$$

$$RBC\ count = No.\ of\ cells\ counted * 10000/mm^3$$

1. **3.10.2 ESR Determination:** ESR was determined using the Westergren method. Anticoagulated blood was placed in a Westergren pipette and allowed to stand undisturbed in a vertical position. The initial level of the red cell column was recorded at 0 hours, and the distance moved by the column after 1 and 2 hours was noted as the ESR in mm/hr (Higuchi & Watanabe, 2023) .
2. **3.10.3 Hemoglobin Count:** Add N/10 HCl into the tube upto mark 2g %. Mix the EDTA sample by gentle inversion and fill the pipette with 0.02ml blood. Wipe the external surface of the pipette to remove any excess blood. Add the blood into the tube containing HCl. Wash out the contents of the pipette by drawing in and blowing out the acid two to three times. Mix the blood with the acid thoroughly. Allow to stand undisturbed for 10min. Place the hemoglobinometer tube in the comparator and add distilled water to the solution drop by drop stirring with the glass rod till its color matches with that of the comparator glass. While matching the color, the glass rod must be removed from the solution and held vertically in the tube. Remove the stirrer and take the reading directly by noting the height of the diluted acid hematin and express in g% (Hoffman R., Benz EJ., Silberstein LE., Heslop H., Weitz J., Salama ME., Abutalib SA., 2022,).

3.10.4 Morphological Evaluation of the Bark Extract of *Butea monosperma*:

Blood samples were collected in two types of tubes: a dry tube and a tube containing sodium citrate (0.109 M). Serum and plasma were then separated by centrifuging the dry tubes and sodium citrate tubes at 3500 rpm, respectively. The effect of the aqueous extract on whole blood, plasma, and serum was assessed. For this, 100 µl of aqueous extract was added to 1 ml of the respective blood sample (whole blood, plasma, or serum). The contents of each tube were observed macroscopically before and after the addition of the aqueous extract. Microscopic preparations were made by mixing 10 µl of the aqueous extract with 50 µl of fresh whole blood or plasma (Leite et al., 2023) .

3.10.5 Determination of Astringent Properties:

Two tubes were used: one containing 1 ml of stem-bark extract (test) and the other containing 1 ml of distilled water (control). Both tubes were mixed with 4 ml of milk, homogenized, and allowed to stand for 3 minutes. They were then centrifuged for 1 minute at 3000 rpm. The presence or absence of pellets was observed.(Arokoyo et al., 2015)

3.10.6 Statistics

- 1. The results are expressed as means ± standard deviation (SD). Statistical significance between groups was assessed using one-way analysis of variance (ANOVA) followed by the unpaired Student's t-test. A *p*-value of < 0.01 and < 0.0001 was considered statistically significant

5. Results

5.1 Phytochemical screening

Preliminary phytochemical screening show that the extract has the traces of major phytoconstituent like tannins which is responsible for modulates hemostatic pathway promoting coagulation (Table 1).

Test	Ethanol extract
Steroids	Yes
Flavonoids	Yes
Alkaloids	No
Carbohydrate	Yes
Phytosterols	Yes
Saponins	No
Tannins	Yes
Proteins and amino acids	Yes
Table-1: Phytochemical screening	

4.2 Toxicity study:

The findings demonstrated that over the 14-day monitoring period, the animal showed no symptoms of poisoning, discomfort, or death. Body weight and behaviour did not significantly change, suggesting that the 2000 mg/kg dose under test was well tolerated and LD50 of the extract is above 2000 mg/kg.

4.3 Haemostatic parameter:

The effect of EEBM on haemostatic parameters was evaluated by measuring bleeding and clotting time in different treatment groups. As shown in the Fig. 2, a significant and dose-dependent reduction in bleeding time was observed in all extract-treated groups compared to the control. The most substantial reduction was observed at the highest dose (400 mg/kg). Similarly, clotting time was significantly shortened in the extract-treated groups, as observed in Fig. 3. This dose-dependent reduction in both parameters suggests an enhancement in haemostatic function, potentially through promotion of platelet aggregation or activation of coagulation pathways.

Values are expressed as mean ± SD (n = 6).

****p < 0.01 vs. control group (unpaired t-test).**

Values are expressed as mean ± SD (n = 6).

*****p < 0.0001 vs. control group (unpaired t-test).**

Figure 3 : Effect of bark extract in clotting time in groups

4.4 Hematological parameters:

Bleeding amount in treated groups appear to very significantly reduced due the effect of the extract (Fig. 4). EEBM showed no significant alteration in hematological parameters in Table 3. Hemoglobin levels and RBC counts remained within normal ranges across all groups. A slight dose-dependent decrease in ESR was observed, suggesting a potential mild anti-inflammatory effect. WBC counts were not recorded.

Values are expressed as mean ± SD (n = 6).

*****p < 0.0001 vs. control group (unpaired t-test).**

Figure 4. Effect of bark extract in Bleeding Amount

GROUP	BLEEDING AMOUNT (gm)	HAEMOGLOBIN CONC. (gm/dl)	RBC (X10 ¹² cell/L)	ESR (mm/hr)
Control	11.835 ± 0.027	12.2 ± 0.23	247 ± 3.2	3.66 ± 0.33
100	11.95 ± 0.22	11.95 ± 0.22	229.7 ± 10.7	3.67 ± 0.33
200	12.22 ± 0.21	12.22 ± 0.21	260.3 ± 11.3	3.33 ± 0.33
400	11.96 ± 0.21	11.96 ± 0.21	252.7 ± 7.6	3 ± 0.57

Table-3: Effect of bark extract in Hematological parameters

4.5 Effect on morphological evaluation:

Addition of ethanolic extract in fresh serum and plasma has shown no significant observable changes but there was a tendency of partial clot formation which was not clear.

4.6 Effect on astringent property:

The extract precipitate milk protein.

6. Discussion

Traditionally bark of *Butea monosperma* is used by the indigenous people to stop bleeding in various parts of West Bengal and other regions of India as reported in earlier literature. Various extracts of bark of *Butea monosperma* has been shown wound healing activity. This study has been taken into consideration to evaluate the potential of *Butea monosperma* on the haemostatic mechanism with primary interest on how it affects bleeding and clotting time, as there is scanty information available regarding the haemostatic effects of the bark of BM. Further the study also included to relate the mechanism and safety consideration which associated with the various hematological parameters. In this study it was observed that the ethanolic bark extract of BM exhibit haemostatic activity by decreasing the bleeding and clotting time. In other hematological parameter (RBC, WBC, Hb and ESR) no observable difference from the controlled group was observed. The decrease in clotting time indicates its measure the intrinsic pathway. It is a qualitative test measurement of factors involved in the intrinsic pathway, therefore deficiency in the factors of the intrinsic pathway (I, II, V, VIII, IX, X, XI and XII) will affect the result. Thus the result obtained in this study shows there might be an increase in one or more of the factors involved in the intrinsic pathway which has shown co-relation with the result obtained by Okoli et al., (2007) on haemostatic activities of the leaf extract of *Aspilla africana* which has been reported to arrest bleed and bleeding from fresh wounds by reducing both bleeding and clotting time. (Okoli et al., 2007)

In this present study other parameter like bleeding time was observed to be decreased due to the administration of the ethanolic extract of BM. Bleeding time is a sensitive test of endothelial and platelet function and platelet number, hence it especially depends on it. This activity of the extract in decreasing bleeding time may result from an increase in platelet count but the other hematological parameters excluding clotting time were intact. So, it may be assumption that there may be a possible of non-involvement of blood cells in the haemostatic effect of the extract. So, the reduction in bleeding time obtained may suggest that the extract may be acting on the integrity of the blood vessel to produce the desired effect inhibition of formation of the smooth muscle relaxing prostaglandin (prostacyclin PGI₂) by the vessel wall which is been produced as a normal vascular response to injury. Vessel injury is normally associated with transient vasoconstriction which is followed by the vessel relaxation as per (Moncada et al., 1976), vessel relaxation after constriction is contributed to by smooth muscle relaxing prostaglandin that are released in vessel in response to injury and that the inhibition of this prostaglandin is assumed to be sustain the vasoconstriction. Hence this vasoconstriction has a haemostatic effect when the vessel wall is damaged in either normal or thrombocytopenic animal. (Moncada et al., 1976). Astringent test also shows positive result, which is a measure of the presence of chemical compounds that tends

to make body tissues shrink or constrict. The ethanolic extract of BM showed astringency and this could be due to presence of tannin which agreed with the result as reported earlier by (Padma R. et al., 2013) and (Ashok et al., 2012) that methanolic extract of *Imperata cylindrical* showed the presence of tannins (Padma et al., 2013), and Tannins has astringent activity (Ashok & Upadhyaya, 2012). Phytochemical analysis of the extract indicates the presence of typical plant constituent such as tannin, flavonoid, especially tannin which has been implicated in the haemostatic activity of the plant where they arrest the bleeding from the damage injured vessel by precipitating protein to vascular plug. Ethanolic bark extract of BM effectively decrease bleeding and clotting time which indicate the positive haemostatic effect in the experimental rats. The evaluated haemostatic and hematological parameters provide a useful support of the wound healing activity and traditional use of BM. One limitation of the present study is the absence of a standard hemostatic drug as a positive control. While this limits the ability to benchmark the efficacy of the extract against established agents, the results still provide valuable insight into the extract's intrinsic hemostatic potential. Future studies should include known hemostatic agents such as Vitamin K, Adrenaline, or Tranexamic acid for comparative analysis and formulation-based treatment for wound care.

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Declarations

Ethics approval

All experimental procedures involving animals were approved by the Institutional Animal

Consent to Publish

declaration:

Consent to participate:

Not applicable.

Competing interests:

The authors declare no competing interests

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contribution

S.S.M contributed to generation of the concept, overall experimental work, supervision and drafting the research paper, A.P, R.R contributed significantly in the in vivo experimental A.M, S.B contributed in drafting of manuscript. All authors of this manuscript have read and approved the manuscript for submission.

Acknowledgements

The authors are thankful to the management & Principal, Dr. B. C. Roy College of Pharmacy and Allied Health Sciences (B.C.R.C.P.), Durgapur for providing the laboratory support and the encouragement of this work.

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Figures

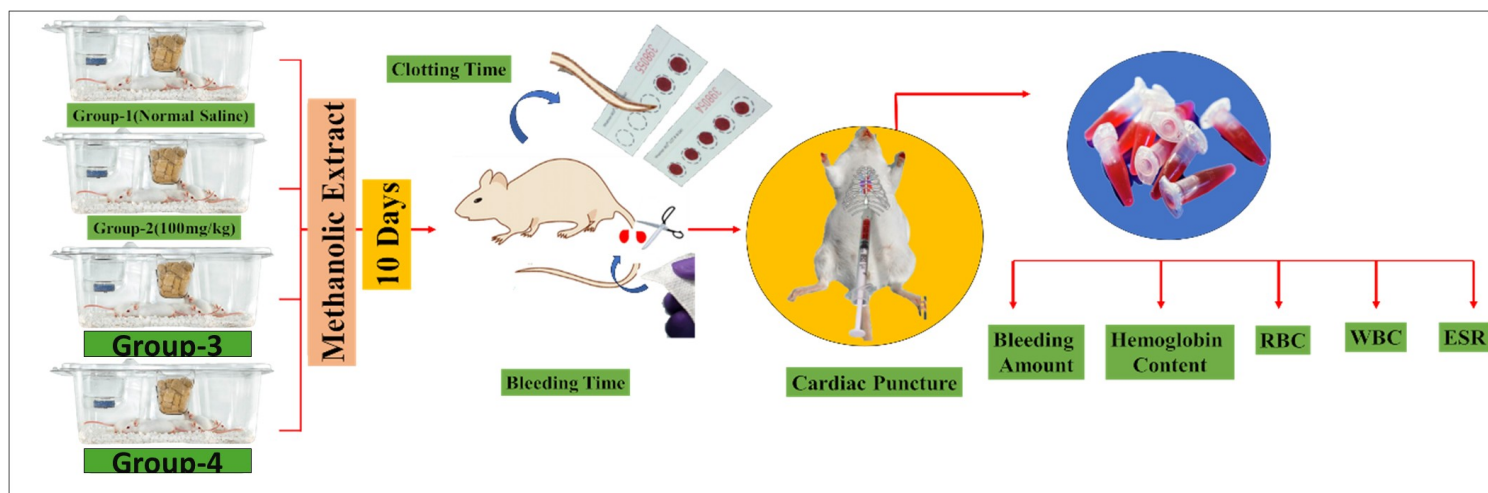


Figure 1

Experimental Design

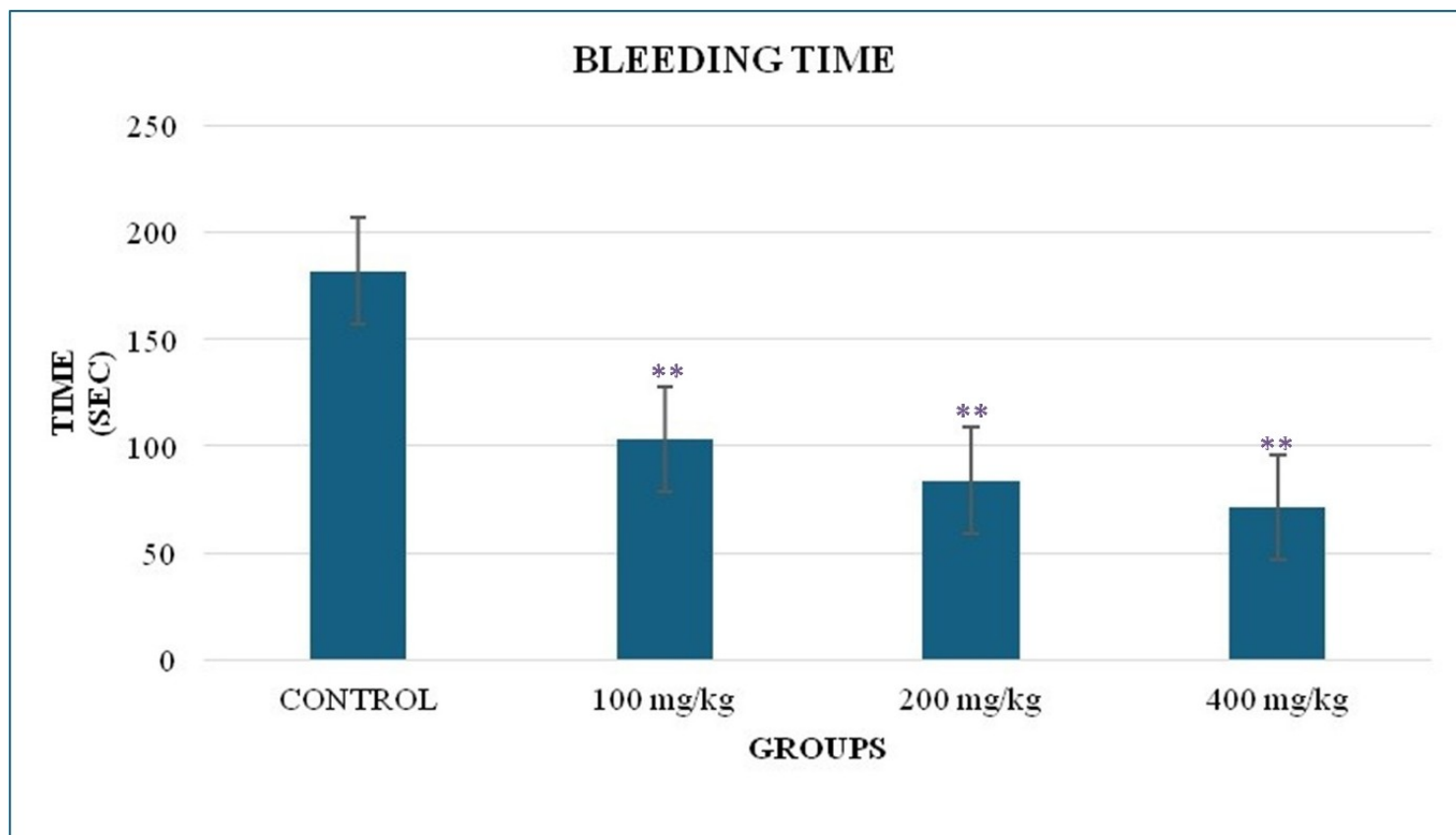


Figure 2

Values are expressed as mean $\pm$ SD (n = 6).

** $p < 0.01$ vs. control group (unpaired t-test).

Figure 2 Effect of bark extract in bleeding time

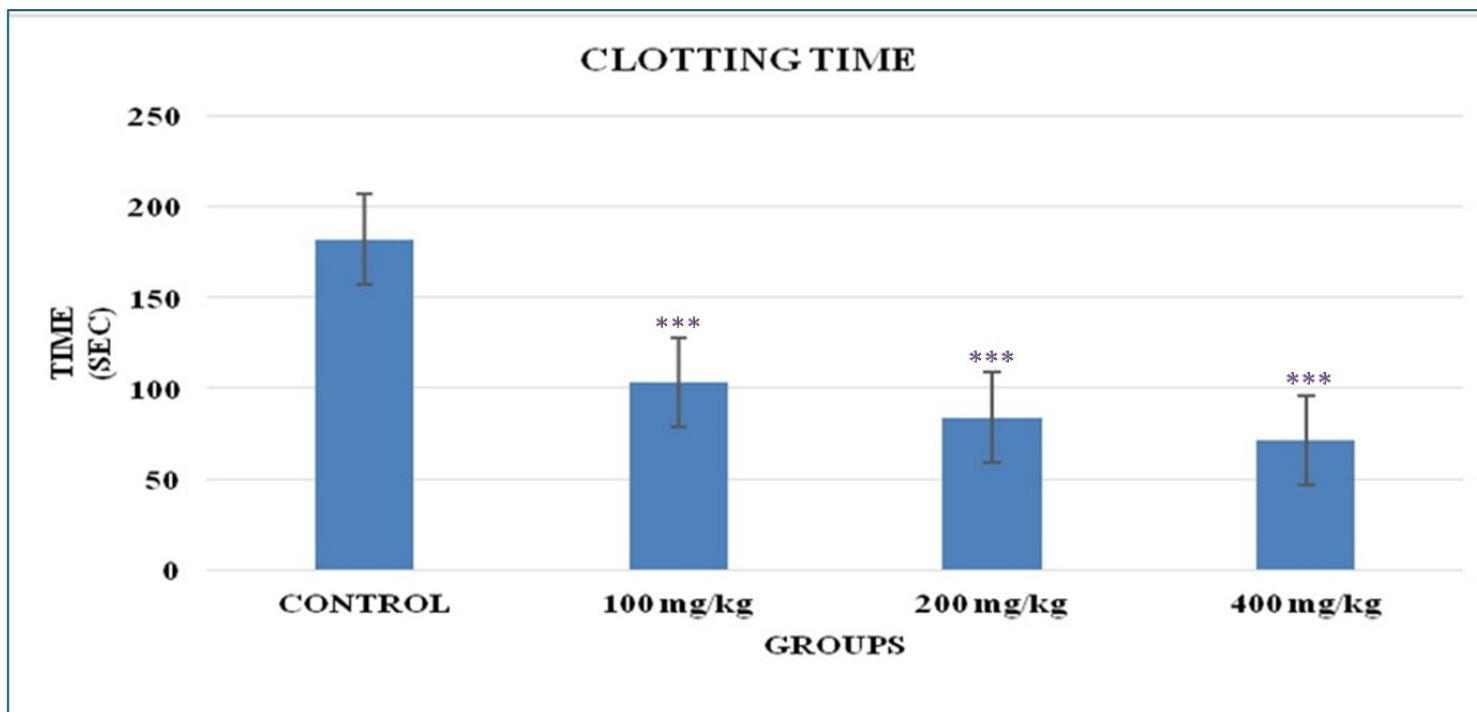


Figure 3

Values are expressed as mean $\pm$ SD (n = 6).

*** $p < 0.0001$ vs. control group (unpaired t-test).

Figure 3 : Effect of bark extract in clotting time in groups

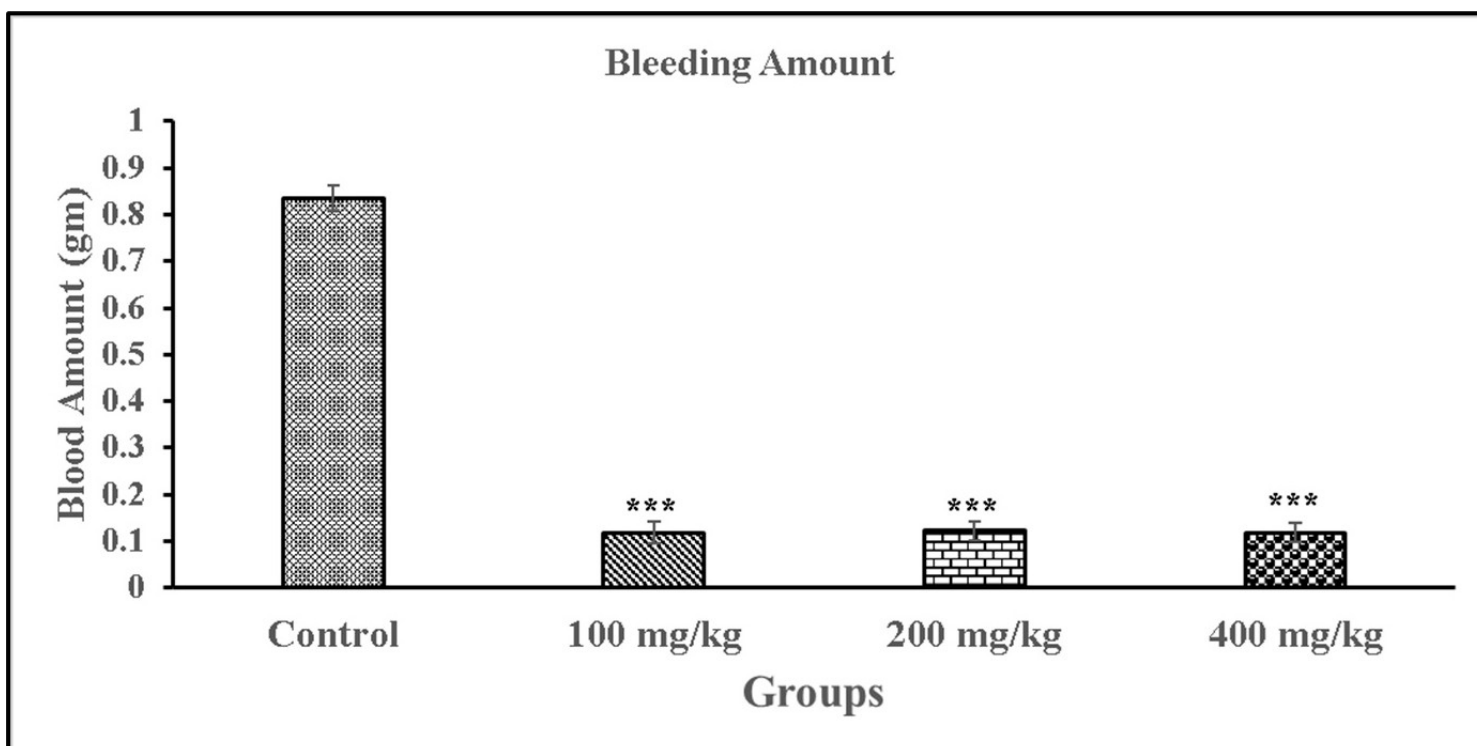


Figure 4

Values are expressed as mean $\pm$ SD (n = 6).

*** $p < 0.0001$ vs. control group (unpaired t-test).

Figure 4. Effect of bark extract in Bleeding Amount