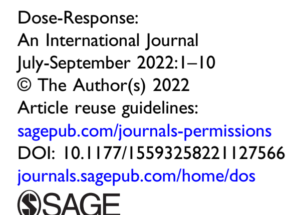
Original Article

Pharmacological Investigation of *Asphodelus tenuifolius* Cav. for its Potential Against Thrombosis in Experimental Models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Abstract

Background: Thrombosis is a major disorder which is an outcome of an imbalance in the hemostatic system that develop undesirable blood clot and hinder blood circulation.

Purpose: The current study was designed to verify the potential of aqueous methanolic crude extract of *Asphodelus tenuifolius* Cav. (At.Cr), used traditionally as remedy in circulatory problems.

Research Design: Antioxidant activity, FTIR, and HPLC analysis were performed. *In-vitro* clot lysis assay was performed on human blood samples, and *in-vivo* acute pulmonary thromboembolism model was developed by administering the mixture of collagen and epinephrine in tail vein of mice. Carrageenan-induced thrombosis and $FeCl_3$-induced carotid arterial thrombosis models were developed in rats.

Results: At.Cr demonstrated significant increase in lysis of human blood clot. Bleeding and clotting times were increased dose-dependently. Lungs histology showed clear alveolar spaces with decreased red blood cells congestion. Reduction in infarcted tail length, augmentation in prothrombin time, and activated partial thromboplastin time with decrease in platelet count were observed. At.Cr also prolonged the arterial occlusion time and reduced the weight of thrombus and TXB_2 levels dose-dependently.

Conclusions: The results demonstrated the antithrombotic and thrombolytic potential of At.Cr due to activation of co-agulation factors through extrinsic and intrinsic pathways.

Keywords
thrombosis, *Asphodelus tenuifolius* Cav., prothrombin time, activated partial thromboplastin time, platelet count

Introduction

Thrombosis is a major disorder which is an outcome of an imbalance in the hemostatic system and results in blood clot formation thus obstructing the blood flow in circulatory system.[1] It is the most common pathology of major cardio-vascular complications: stroke, myocardial ischemia (acute coronary syndrome), and venous thromboembolism (VTE).[2] Venous thrombosis is the collective term used for pulmonary embolism (PE) and deep vein thrombosis (DVT) indicative of blood clots thus obstructing blood flow in the pulmonary arteries and deep veins, respectively. It is considerably the third most common cardiovascular problem at estimated occurrence rate of 1–2 among 1000 persons every year with connected substantial morbidity and mortality. The prevalence of arterial thrombosis is 10 million deaths in a year

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worldwide.[3] An acute massive pulmonary embolism with unstable hemodynamics results in circulatory shock and leads to sudden death.[4] Platelets are vital to maintain the vascular integrity and control of bleeding, but they may induce thrombosis by adhesion to the sites of vascular injury, aggregation in developing hemostatic plugs, or thrombi thus accelerating the cascades of coagulation, leading to thromboembolic complications.[5]

Awareness campaigns, especially the "World Thrombosis Day" initiated globally, brought awareness to the general community, medical professionals, and stakeholders about the burden of thrombosis and promoted wide implementation of available management policies.[6] Thrombogenesis is a critical condition that should be targeted at multiple levels since only anticoagulation seems insufficient. Agents in practice for prevention of cardiovascular events act either on the risk factors of atherosclerosis or directly on thrombosis. The effective therapies can be implemented by knowing the nature of thrombus, that is, mainly antiplatelet agents (aspirin, clopidogrel, and ticlopidine) for arterial thrombi and anticoagulants (warfarin, heparin, apixaban, and dabigatran) for venous thrombosis. The fibrinolytic agents (streptokinase, urokinase, alteplase, and tenecteplase) are used for the lysis of established thrombus.[7] However, associated unwanted interactions, for example, bleeding risk, hemorrhage, narrow therapeutic window, antigenicity, and hypertensive crisis, must be conceded. Antithrombotic agents of natural origin with least side effects and efficient therapy is the objective of remarkable pharmacological efforts.[8]

Asphodelus tenuifolius Cav., belonging to family Asphodelaceae is an annual, wild and stemless weed (herb), known as bhokal by local community, piazi in Urdu, and asphodel or onion-like weed in English.[9] It is broadly distributed in the Mediterranean region, southern Europe, North Africa, India, and Pakistan.[10] *Asphodelus* seeds are considered as diuretic and utilized as condiment in controlling the blood pressure. Traditionally, they are used to treat variety of cardiometabolic problems, for example, hypertension, diabetes, and atherosclerosis. In traditional medicine, the plant is used in rheumatic pain, hemorrhoids, and inflammatory, digestive and circulatory complications.[11] It has been used to manage dyslipidemia, oxidative stress, insulin resistance, and aortic endothelial dysfunction.[12] The present study was conducted to rationalize antithrombotic and thrombolytic activity of *A. tenuifolius*.

Material and Method

Ethical Approval

The study protocols were permitted by the Pharmacy Animal Ethics Committee (PAEC) and Pharmacy Human Ethics Committee (PHEC) under reference No. PAEC/21/32 and 113/2020-/PHEC, respectively, in accordance with the US Public Health Services (PHS) for animal care while the Declaration of Helsinki (DOH) was followed for the ethical principles in the model involving human subjects.

Crude Extract Preparation

Whole plant of *Asphodelus tenuifolius* Cav. was collected in the month of March from Cholistan Desert, district Bahawalpur, Punjab, Pakistan. The plant was identified by the taxonomist, deposited in the herbarium, and for future reference, herbarium number was issued (Herbarium no. AT-WP-05-18-173). Plant was washed with fresh water to remove dirt and extraneous matter, dried under the shade, and then ground into coarse powder. 1.5 kg coarse powder was subjected to maceration in aqueous methanol (30:70) with occasional shaking and stirring. Filtrate was subjected to rotary evaporator (Heidolph Laborota 4000 efficient, Germany) under reduced pressure at 50°C.[13,14] The thick viscous paste was dried at 40°C in hot air oven. The crude extract (At.Cr) prepared was labeled after weighing (percent yield, 9.15%) and stored in freezer below 0°C for future use.

Chemicals and Reagents

The chemicals utilized were of pure quality and analytical grade; such as, carrageenan, aspirin, collagen (Sigma-Aldrich, USA), streptokinase (SK, Biotech GmbH, Germany), epinephrine (Ameer Pharma, Pakistan), formalin (Riedel-de Haen, Germany), xylazine (My Lab Pharmaceuticals, Pakistan), and ketamine (Global Pharmaceuticals, Pakistan).

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) of At.Cr was performed by directly placing the extract on the crystal plate center of FTIR spectroscope (Bruker™, Alpha Platinum, Germany), and measured in a range of 500–4000 cm^{-1} with a resolution of 4 cm^{-1}. Estimated scan time for spectral acquisition was 25 seconds.[15]

Chromatographic Analysis

A specific and easy procedure for HPLC was adopted with slight modifications for the simultaneous assessment of flavonoids and phenolic constituents in At.Cr. Briefly, crude extract was characterized using HPLC on Shim-Pack CLC-ODS-2 (C-18 column, Shimadzu, Japan; maintained at 23–25°C) equipped with a UV–visible detector (SPD-10 AV, Shimadzu, Japan) and an HPLC pump (LC-10AT Shimadzu, Japan). At the flow rate of 1 ml/m, absorbance was identified at 280 nm. Quantitative analysis and identification of components was conducted by comparing with standards. The CSW32-Chromatography Station Data Apex Ltd. 2001 software was used for data acquisition.[16]

Antioxidant Activity by DPPH

Antioxidant potential of At.Cr was determined using the DPPH radical scavenging method. 0.2 mM methanolic solution of DPPH (1 ml) was mixed with At.Cr (0.5 mg/ml) at different concentrations. Reference standard, ascorbic acid was also prepared in various concentrations. 1 ml of methanol is added to DPPH solution and used as control. Absorbance was measured at 517 nm after keeping all solutions in dark for 30 minutes.[17] Radical scavenging activity was calculated by following formula

$$\text{Percent scavenging activity} = (\text{Ay} - \text{Ax}/\text{Ay}) \times 100$$

where Ay = the absorbance of control and Ax = the absorbance of crude extract.

Experimental Animals

Wistar albino rats (200–300 g) and Swiss albino mice (20–30 g) of either sex were housed under optimal conditions of temperature (23 ± 2°C), humidity (55 ± 5%), and 12 h/12 h light and dark period in the animal house of Pharmacology Research Laboratory, the Islamia University of Bahawalpur (IUB), Pakistan. Animals were provided with standard pellet diet with tap water *ad libitum* and allowed to acclimatize for at least one week prior to experimental work.

Acute Toxicity Assay

Acute toxicity studies were performed according to the OECD guidelines 425 (Oct, 2008). Mice were divided into four groups (n=5). Animals were fasted 12 h before dosing. The control group received normal saline (10 ml/kg; p.o.). At.Cr was administered to three groups at increasing doses of 1, 3, and 5 g/kg (p.o.) to investigate the acute toxic effects. Mice were observed for the initial 6 h and then once daily for 14 days. The mortality was noted after 14 days and behavioral changes were closely observed such as hyperactivity, tremors, ataxia, salivation, convulsions, diarrhea, lethargy, sleep, coma, and somatomotor activity.[18]

In-vitro *Clot Lysis/Thrombolysis Assay*

Stock solution of streptokinase (SK 30,000 IU/100µl) was prepared. Human blood (5 ml) was drawn from subjects without history of anticoagulants and oral contraceptives. 500 µl of blood was added to each of previously weighed Eppendorf tubes and incubated for 45 minutes (37°C). Serum was removed after clot formation. Weight of clot was determined, 100 µl of At.Cr (1, 3, 5, and 10 mg/ml) was added in tubes and incubated for 90 minutes (37°C). Tubes were again weighed after removal of fluid.[19] Percent clot lysis was calculated, and experiment was repeated with six volunteers

$$\text{Weight of clot} = \text{weight of clot containing tube} - \text{weight of tube alone}$$

$$\%\,\text{Clot lysis} = (\text{Weight of the lysed clot} / \text{Weight of clot before lysis}) \times 100$$

Collagen and Epinephrine-Induced Thrombosis in Mice

Mice were divided into different groups (n=6). Control and intoxicated groups were administered distilled water (10 ml/kg; p.o.). Treatment groups received At.Cr, at the doses of 100, 300, and 500 mg/kg, respectively. The standard control group received aspirin (ASA, 5 mg/kg). All the doses were given orally for seven days. Pulmonary thrombosis was induced by intravenous injection of thrombogenic solution (collagen, 80 mg/kg + epinephrine, 1 mg/kg) in the tail vein. Animal behavior was observed for 15 minutes, and a number of dead and paralyzed animals were noted. The percent protection of At.Cr against pulmonary thrombosis was calculated with the following formula

$$\%\,\text{Protection} = [1 - (\text{dead} + \text{paralyzed})/\text{Total}] \times 100$$

One hour after the last treatment, bleeding time (BT), clotting time (CT), and euglobulin lysis time (ELT) were recorded. After one hour of last administration, tail was cut 5 mm from the tip. When the blood flowed, timings were noted, and the tip was wiped off every 30 seconds until the bleeding was ceased. The total time recorded was calculated as the bleeding time.[20]

Clotting time was determined by placing a drop of blood on a clean glass slide taken from the tip of the tail and stopwatch was started to note the time. A pin was used to pass across the drop of blood once after every 15 seconds. As the threads of fibrin appeared, the stopwatch was stopped and the time recorded was taken as clotting time.[21]

A slightly modified method as reported by Zhou et al was adopted to determine ELT.[20] After one hour of therapy, blood (1 ml) was taken from the retro-orbital venous plexus. Sodium citrate (3.8%) was added in test tube. A blood sample was centrifuged for 10 minutes at 3000 rpm, after which the plasma (0.25 ml) was extracted and 4.5 ml of distilled water and 0.05 ml of 1% acetic acid were added. For 10 minutes, the mixture was refrigerated at 4°C. The mixture was centrifuged at 3000 rpm for 10 minutes to separate the euglobulin fraction. The excess liquid was removed by aspirating the supernatant fluid out of the tube and then placing it upside down on the filter paper to get that absorbed. Then 0.25 ml of borate buffer (61.83 g boric acid, 10 g sodium hydroxide, and distilled water up to 1L, pH=9.0) was added to the test tube, and it was stirred for about 1 minute with a fine glass rod to dissolve. The tube was then immersed in a water bath at 37°C for 2 minutes. To solidify the euglobulin, 0.25 percent (0.25 ml) calcium chloride was added and set aside. The time until the clot lysed was recorded as the euglobulin lysis time.

The lungs after excision and rinsing with normal saline (0.9%) were fixed in formalin (10%) for 24 h. Lung sections

were cut (7 μm) and stained with hematoxylin and eosin stains to observe histological changes underneath microscope (ACCU-Scope, 3000-LED, USA).

Carrageenan-Induced Thrombosis Model

Rats were randomly divided into different groups each group having six animals. Groups I and II (control and intoxicated) were administered normal saline (2 ml/kg). Group III received streptokinase (SK 60,000 IU/kg), groups IV, V, and VI were administered At.Cr (100, 300, and 500 mg/kg; i.p., respectively). Rat tail vein was injected with 1 mg/kg of κ--carrageenan prepared in normal saline, 12 cm from the tip of the tail along with ligation for 10 minutes, and once thrombus was developed, different doses were administered for 14 days. Bleeding time and clotting time were noted. Blood was collected by cardiac puncture for platelet count and plasma was separated to measure prothrombin time (PT) and activated partial thromboplastin time (APTT) using commercially available reagents.

$FeCl_3$-Induced Carotid Arterial Thrombosis Model

Rats were divided into different groups ($n = 6$). Distilled water was given orally to the control and model groups and aspirin (5 mg/kg) to the standard control and the treatment groups received At.Cr (100, 300, and 500 mg/kg, along with intoxication). After 14 days, rats were anesthetized (ketamine xylazine; i.p.) and fixed in supine position to make an incision (3 cm) on the throat to isolate common carotid artery (2 cm) and plastic sheet (3×1.2 cm) was placed below the vessel. The carotid artery surface was wrapped with filter paper (1×1 cm) saturated with $FeCl_3$ solution (40%), temperature was monitored with a thermometer, and thrombosis occlusion time (OT) was recorded. Segment of carotid artery (0.6 cm) was removed and weighed.[22] Rate of inhibition was calculated as follows

$$\text{Rate of inhibition}\,(\%) = (\mathrm{C} - \mathrm{C_1})/\mathrm{C} \times 100$$

where C = wet weight of thrombus in the model group and C_1 = wet weight of thrombus in treated group.

Estimation of Coagulation Parameters and Thromboxane B_2 Level

described earlier.[23] Rats were divided into various groups and treated in the same way as in the $FeCl_3$-induced carotid arterial model. 90 minutes after surgery, maintaining the sterile conditions, abdominal aorta was excised, and 3 ml of blood was collected in EDTA tubes. 1 ml blood was separated to determine the platelet count. The blood samples were centrifuged (865Xg) at 4°C for 15 minutes. The plasma was separated and stored at −20°C for the estimation of PT and APTT. Clotting time

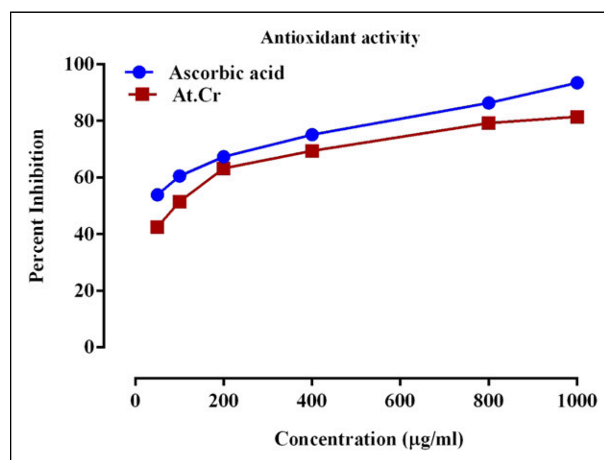

Figure 1. Scavenging activity of At.Cr on DPPH radicals.

was recorded using coagulation analyzer (HumaClot Junior, Human Diagnostic, Germany). Plasma level of thromboxane B_2 was measured according to the recommended protocol of commercially available ELISA kit (Elabscience, USA).

Statistical Analysis

GraphPad Prism software was employed for the statistical analysis of the observed results. One-way analysis of variance (ANOVA) with Bonferroni's post hoc test was used for data analysis, values were expressed as mean ± SEM, and $P < .05$ was considered statistically significant.

Results

Antioxidant Activity by DPPH

At.Cr showed 81.43% radical scavenging activity by DPPH at 1000 μg/ml concentration (Figure 1).

Fourier Transform Infrared Spectroscopy

The FTIR spectroscopy of the crude extract of *Asphodelus tenuifolius* Cav. (At.Cr) was performed to identify the biologically important functional groups which are responsible for thrombolytic potential. The FTIR spectrum showed prominent absorption bands at 3274, 2922, 2852, 2359, 1742, and 1602 and 1339, 1210, and 1037 cm^{-1} as major peaks (Figure 2A). The spectrum demonstrated absorption signals for various wave number ranges, which were identified as phytoconstituents in the crude extract (At.Cr), that is, carbohydrates and alcohols at 3016–3274, 2852–2922, and 998–1037 cm^{-1} (O–H, N–H, and C–O), fatty acids at 1602–1742 cm^{-1}(N–H, C=O), phenyl groups at 1339 cm^{-1} (O–H), phenol or tertiary alcohol (O–H and N–H), and acid or ester at 1150–1210 cm^{-1}. The FTIR spectrum of At.Cr provided evidence for the presence of various

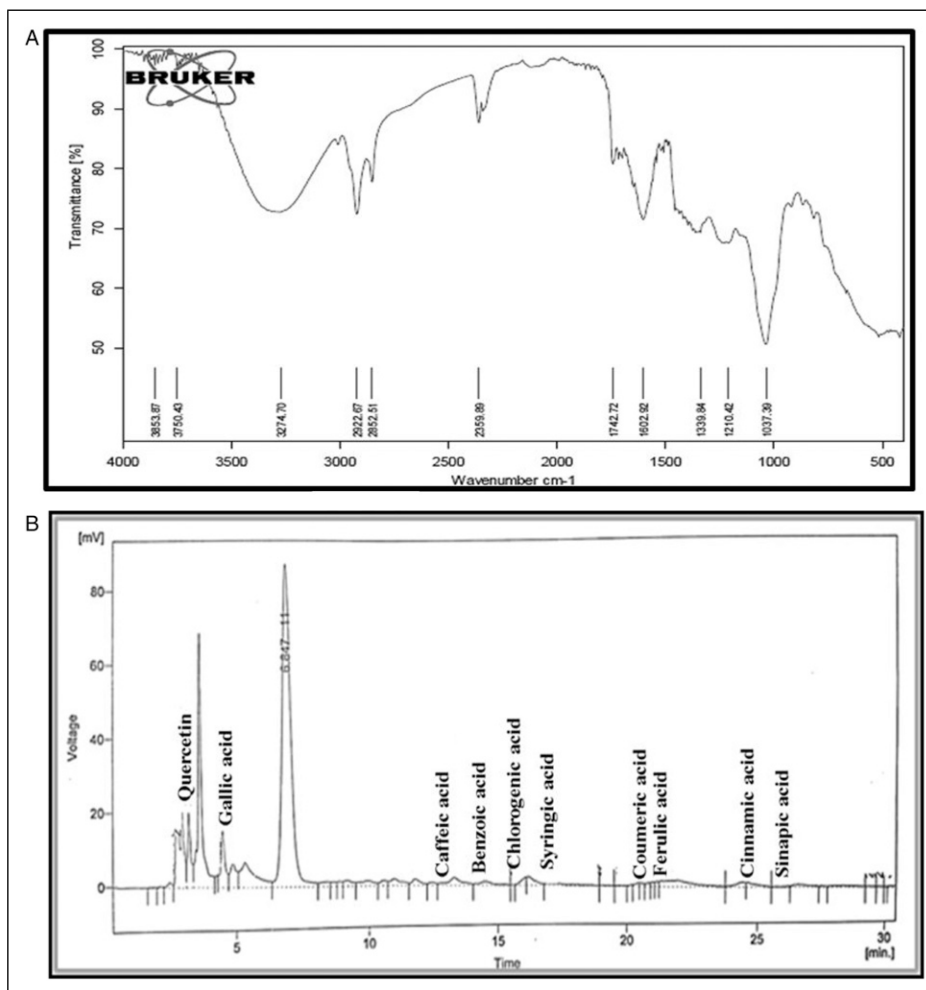

Figure 2. Phytochemical screening of At.Cr: **(A)** FTIR spectrum and **(B)** HPLC chromatogram.

biologically important functional groups, that is, hydroxyl and carboxylic acids and alkenes which were reported earlier to impart their effect in antithrombotic activity.[24]

Chromatographic Analysis

The HPLC chromatogram of aqueous methanolic crude extract of *Asphodelus tenuifolius* Cav. (At.Cr) revealed the occurrence of quercetin (Rt.3.14 m), gallic acid (4.44), caffeic acid (12.40), ferulic acid (21.91), syringic acid (16.98), sinapic acid (26.22), m-coumaric acid (20.43), 4-hydroxy,3-methoxy benzoic acid (14.49), cinnamic acid (24.64), and chlorogenic acid (15.64) (Figure 2B).

Acute Toxicity Assay

At.Cr showed safety up to 5 g/kg dose without toxicity signs and no mortality was observed till 14th day.

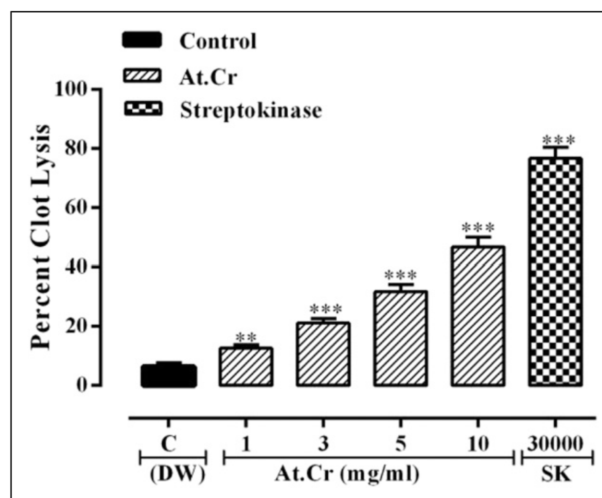

Figure 3. Effects of At.Cr and SK on in-vitro clot lysis in human blood. Mean ± SEM, n=6. (*) $P<(0.05)$, (**) $P<(0.01)$, and (***) $P<(0.001)$ vs. control group.

Table 1. Effects of At.Cr and ASA on bleeding time (BT), clotting time (CT), and euglobulin lysis time (ELT) and percent survival in collagen and epinephrine-induced thrombosis in Wistar albino rats

Treatment groups	Bleeding time (m)	Clotting time (m)	Euglobulin lysis time (m)	% protection
Control (D/W, 10 ml/kg; p.o.)	3.0 ± 0.20	3.35 ± 0.18	281.9 ± 6.60	—
Intoxicated (C, 80 mg/kg + E, 1 mg/kg; i.v.)	1.80 ± 0.14	2.06 ± 0.10	—	0
Aspirin (5 mg/kg) + intoxication	10 ± 0.38***	10.83 ± 0.30***	75.86 ± 3.59***	100
At.Cr (100 mg/kg) + intoxication	4.1 ± 0.20*	5.07 ± 0.26*	252.6 ± 5.64**	33.3
At.Cr (300 mg/kg) + intoxication	5.9 ± 0.20**	6.15 ± 0.35***	141.0 ± 4.85***	50
At.Cr (500 mg/kg) + intoxication	7.2 ± 0.21***	9.01 ± 0.19***	105.6 ± 4.05***	83.3

Mean ± SEM, n=6. (*) $P<(.05)$, (**) $P<(.01)$, and (***) $P<(.001)$ vs. control group (C: collagen, E: epinephrine, ASA: aspirin).

In-vitro *Clot Lysis/Thrombolysis Assay*

Figure 3 presents the thrombolytic activity of At.Cr with highly significant and dose reliant (1, 3, 5 and 10 mg/ml) increase in lysis of human blood clot. SK (30,000IU) as standard control drug showed more promising lysis of clot in comparison to the control (distilled water) group (Figure 3).

Collagen- and Epinephrine-Induced Thrombosis

At.Cr, at the doses of 100, 300, and 500 mg/kg, elevated the bleeding time (BT) and clotting time (CT) in dose-dependent manner after 7 days of p.o. treatment in comparison to the control group. ASA, the standard drug at 5 mg/kg dose showed maximum increase in BT and CT in comparison to the control. At.Cr (100, 300, and 500 mg/kg) showed significant and dose-dependent decrease in euglobulin lysis time (ELT) and aspirin at the dose of 5 mg/kg showed maximum decrease in ELT (Table 1). At.Cr showed percent protection from acute pulmonary thromboembolism (APT) at the doses of 100, 300, and 500 mg/kg; 33.3, 50, and 83.3%, respectively. ASA, the standard drug showed highest percent survival (100%) at the dose of 5 mg/kg (Table 1).

The histological changes were observed in the lung tissues. The microscopic observation of lung slides of the control group showed the presence of clear alveolar–capillary membrane without congestion while the collagen epinephrine intoxicated lung tissues exhibited thrombi and hemorrhages along with the red blood cells and congestion in the surrounding tissues due to the formation of fibrin mesh. The treated groups presented decreased red blood cells congestion and pulmonary thrombi at the graded doses of At.Cr. The aspirin-treated group showed clear red blood cell separation from the alveolar spaces and epithelial membrane (Figure 4).

Carrageenan-Induced Thrombosis Model

The overall efficiency of extrinsic clotting pathway can be measured by PT to evaluate the coagulation factors (V, VII, and X). However, APTT test is used for the coagulation factors VIII, IX, XI, and XII and Von Willebrand's factor in the intrinsic clotting pathway. At.Cr at 100, 300, and 500 mg/kg in carrageenan (1 mg/kg)-induced tail vein thrombosis showed significant ($P < .001$) reduction in tail infarction dose-dependently after 14 days of intraperitoneal administration in comparison to the intoxicated group. SK (60000 IU/kg) as standard drug showed highly significant response in reducing thrombus length of tail as compared to the intoxicated group. At.Cr showed significant and dose-dependent (100, 300, and 500 mg/kg) increase in BT and CT of carrageenan-induced tail thrombosis model in comparison with the intoxicated group. Streptokinase as the standard control group prolonged the BT and CT in highly significant ($P < 0.001$) manner as compared to the intoxicated group (Figure 5).

At.Cr-treated groups showed significant escalation in prothrombin time (PT) and activated partial thromboplastin time (APTT) in dose-dependent manner in comparison to the control group. 100 mg/kg dose of At.Cr showed significant increase ($P < 0.05$) in PT while 300 and 500 mg/kg doses of At.Cr showed significant escalation ($P < 0.001$) in PT and APTT in comparison to the control group, suggesting antithrombotic role of At.Cr through extrinsic and intrinsic pathways, respectively. SK (60000 IU/kg), the standard drug presented highly significant ($P < 0.001$) augmentation in PT and APTT as compared to the control group (Figure 6). A profound decrease in platelet count (PC) of carrageenan-induced thrombotic animals was observed at all doses of At.Cr substantially and significantly. SK (60000 IU/kg) showed maximum decrease ($P < 0.001$) in PC in comparison to the control group (Figure 6).

$FeCl_3$-Induced Carotid Arterial Thrombosis Model

At.Cr extended the thrombosis occlusion time (OT) and decreased the weight of thrombus significantly and in dose-dependent (100, 300, and 500 mg/kg, respectively) pattern as compared to the intoxicated group. At.Cr also increased the rate of inhibition such as 18.63, 33, and 43.10% at the doses of 100, 300, and 500 mg/kg, respectively, in comparison to the ferric chloride (40%)–induced intoxicated group. ASA (5 mg/kg), the standard drug showed highly significant ($P < 0.001$) escalation in OT and decreased the weight of thrombus with highest rate of inhibition (56.1%) in comparison to the intoxicated group (Table 2). At.Cr showed significant increase in

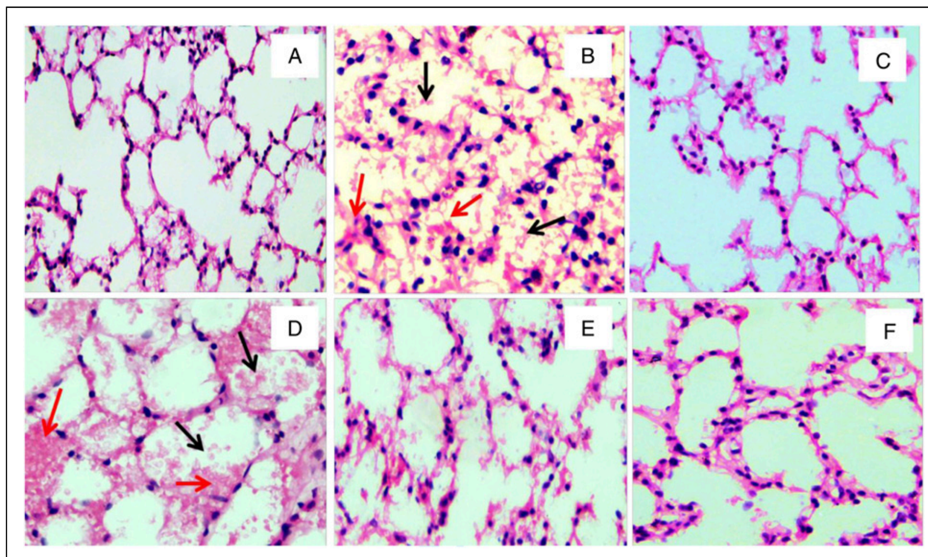

Figure 4. Histological examination of lung tissue: **(A)** Control (10 ml/kg; p.o.), **(B)** intoxicated (C+E, i.v.), **(C)** ASA, 5 mg/kg; p.o. + intoxication, **(D)** At.Cr, 100 mg/kg + intoxication, **(E)** At.Cr, 300 mg/kg + intoxication, and **(F)** At.Cr, 500 mg/kg + intoxication. (↑): Pulmonary thrombi and (↑): red blood cell congestion (ASA: aspirin, C: collagen, and E: epinephrine).

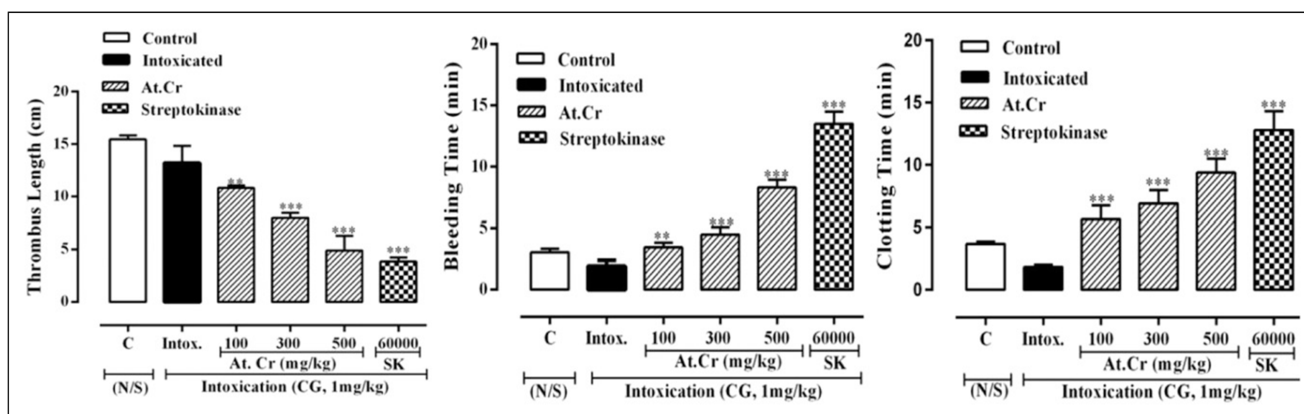

Figure 5. Effects of At.Cr and SK on thrombus length, BT, and CT in carrageenan-induced tail thrombosis. Mean ± SEM, n=6. (*) $P<(0.05)$, (**) $P<(0.01)$, and (***) $P<(0.001)$ vs. intoxicated group (CG: carrageenan).

PT and APTT substantially and dose-dependently at all doses as compared to the intoxicated group. The platelet count (PC) was decreased significantly in all treated groups of At.Cr in dose-dependent pattern as compared to intoxicated group. ASA, the standard drug, at 5 mg/kg of dose showed maximum augmentation in PT and APTT with profound decrease in PC as compared to the intoxicated group (Table 2).

Thromboxane A_2 (TXA_2) exerts platelet activation and promotes thrombus formation. TXB_2 is the stable metabolite of TXA_2.[25] $FeCl_3$-induced endothelial injury on carotid artery surface causes platelet activation and aggregation to form thrombus. At.Cr at 100, 300, and 500 mg/kg decreased the plasma level of TXB_2. Aspirin (5 mg/kg) decreased TXB_2 level ($P < 0.001$) as compared to the control group (Table 2).

Discussion

Asphodelus tenuifolius Cav. has been reported to be used traditionally in wound healing, ulcers, rheumatic pain, and inflammatory disorders.[26] The study was conducted to evaluate the potential of *A. tenuifolius* against thrombosis, as like other antithrombotic plants,[27] it is enriched in polyphenols. Platelets are key element to play physiological function in hemostasis and thrombosis, which can either be constructive or lethal depending on the circumstances.[28] FTIR was performed to assess various types of inorganic and organic substances in At.Cr, and the crude extract affirmed the presence of alcohols, phenols, carboxylic acid, alkanes, and esters. HPLC quantified the quercetin and polyphenolic compounds and their derivatives. Previously, phenolic protein

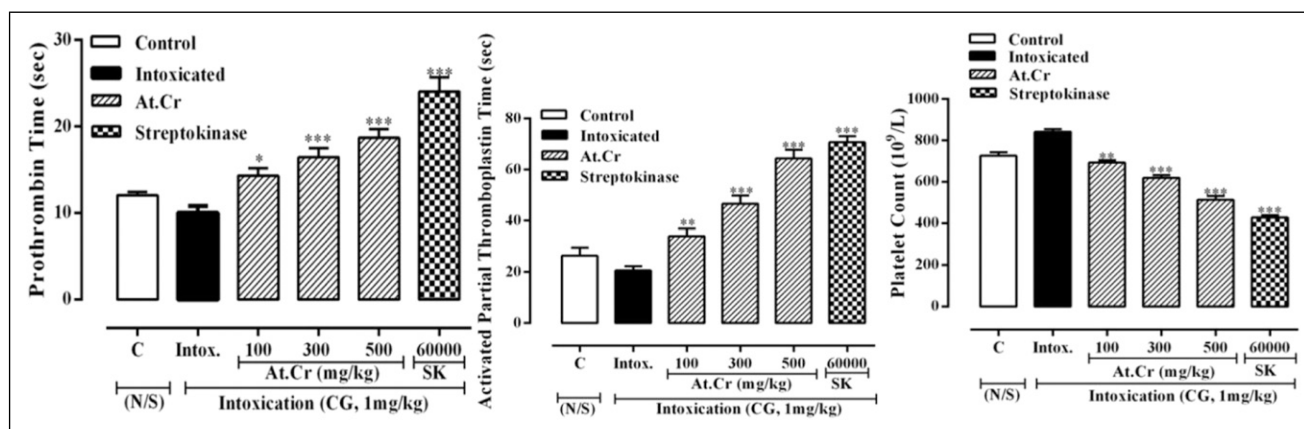

Figure 6. Effects of At.Cr and SK on coagulation parameters in carrageenan-induced tail thrombosis model in Wistar albino rats. Mean ± SEM, n=6. (*) *P*<(0.05), (**) *P*<(0.01), and (***) *P*<(0.001) vs. control group (CG: carrageenan).

Table 2. Effects of At.Cr and ASA on OT, weight of thrombus, PT, APTT, PC, and TXB_2 in $FeCl_3$-induced carotid arterial thrombosis in Wistar albino rats.

Parameters	Intoxicated (40%, $FeCl_3$)	Aspirin (5 mg/kg; p.o.) + intoxication	At.Cr (mg/kg; p.o.) + intoxication		
			100	300	500
Occlusion time (OT)	7.06 ± 0.23	30.33 ± 1.50***	13.58 ± 0.67**	18.45 ± 0.45***	24.23 ± 1.61***
Weight of thrombus (mg)	13.2 ± 0.54	5.70 ± 0.23***	10.74 ± 0.27***	8.91 ± 0.22***	7.51 ± 0.31***
Inhibition rate (%)	—	56.81	18.63	33	43.10
Prothrombin time (s)	10.02 ± 0.21	40.15 ± 0.98***	16.67 ± 0.46***	20.77 ± 72***	27.75 ± 1.13***
Activated partial thromboplastin time (s)	19.77 ± 0.90	79.57 ± 2.11***	41.22 ± 0.82***	50.85 ± 1.30***	63.25 ± 0.90***
Platelet count (10^9/L)	683.2 ± 3.32	464.5 ± 4.45***	608.0 ± 4.87***	550.5 ± 4.92***	534.9 ± 7.68***
Thromboxane B^2 (pg/ml)	654.5 ± 12.36	182.7 ± 7.31***	594.8 ± 13.91**	428.0 ± 9.17***	338.3 ± 14.04***

Mean ± SEM, n=6. (*) *P*<(0.05), (**) *P*<(0.01), and (***) *P*<(0.001) vs. intoxicated group.

complex has been revealed to possess anticoagulant effects on blood hemostatic system.[29] Phenolic compounds in plants are the secondary metabolites and one of the most widely occurring groups of phytoconstituents exhibiting antithrombotic, vasodilatory, anti-inflammatory, and cardioprotective activities. Presence of the hydroxyl groups and conjugated ring structures confirmed the presence of various phenolic compounds. A potential function of antioxidants is through scavenging cascade or by alleviating the free radicals involved in oxidative stress through hydrogenation or complexing with oxidizing species.[30] Coumarins are involved in decreasing the extent of coagulation[31] and At.Cr has revealed their presence in HPLC analysis. Formerly, it has also been reported that flavonoids have thrombolytic activity[32] and quercetin also possesses antioxidant effect[33], reducing atherosclerosis which is a major contributor to thrombotic events.[27] Chlorogenic acid has an inhibitory effect on platelet activation and thrombus formation and provides protection to the vascular integrity.[34] Caffeic acid, identified in HPLC analysis of At.Cr has antioxidant property and causes amelioration in thrombus formation.[35] The flavonoids have been reported to possess thrombolytic activity,[32] while quercetin also contributes to the antioxidant effects,[33] thus reducing atherosclerosis which is a major contributor to thrombotic events. The collagen has been reported to cause platelet aggregation by inducing calcium immobilization thus inhibiting the production of hydrogen peroxide and 1,3,4-inositol triphosphate (IP3) formation in human platelets. Quercetin has been reported to inhibit this collagen-induced cascade of platelet aggregation.[36]

At.Cr significantly increased percent clot lysis in human blood dose-dependently which can be attributed due to the presence of flavonoids, alkaloids, and tannins.[27] *A. tenuifolius* inhibited thrombogenic effect of collagen and epinephrine and decrease platelet aggregation substantially and significantly in dose-reliant pattern. At.Cr also showed prolongation of BT and CT dose-dependently. This antiplatelet activity of At.Cr and increase in bleeding time can be contributed to reduction in platelet count and lipid peroxidation, thus triggering platelet aggregation.[37] The formation of blood clots involves both the endogenous and exogenous coagulation system. Clotting time is associated with the endogenous pathway and may prolong or decrease

depending on the content and functionality of various coagulation factors (I, II, V, VIII, IX, X, XI, and XII).[38] At.Cr prolonged the clotting time in dose-dependent fashion as documented in our results.

The terminal process in thrombosis is fibrinolysis.[39] ELT has been studied to measure the plasma fibrinolytic activity and At.Cr reduced the ELT dose-dependently indicating the role of plasmin. Carrageenan, injected in the tail vein of rats, induces thrombosis by damaging the endothelial cells causing hypercoagulability due to inflammation in the blood vessels and by disturbing the homeostasis.[40] Thrombolytic activity of At.Cr was verified by estimating values of standard coagulation parameters (PT and APTT) and the platelet count (PC). $FeCl_3$-induced thrombosis is exploited both for the antiplatelet and anticoagulant agents.[41] $FeCl_3$-induced occlusive thrombosis occurs by reactive oxygen species in the endothelium causing oxidative stress leading to lipid peroxidation and exposure of subendothelial collagen.[42] Both extrinsic and intrinsic pathways activate the blood coagulation factors and thrombin. Vascular endothelial cell injury and necrosis lead to increased generation of TXB_2 thus promoting platelet activation and aggregation causing vasoconstriction and thrombosis[7] as shown in the intoxicated group. However, *A. tenuifolius* showed significant reduction in TXB_2 level at all doses of crude extract. Significant increase in OT with decrease in weight of thrombus dose-dependently demonstrated antithrombotic activity of At.Cr. Moreover, At.Cr prolonged PT and APTT with significant decrease in PC at all the administered doses.

The results of the present study demonstrated that crude extract of Asphodelus tenuifolius Cav. exerts its antithrombotic and thrombolytic effects by increase in the levels of PT and APTT which might be considered due to the relative paucity of platelet count or antilipid peroxidation influence because platelet aggregation is caused due to lipid peroxides.

Which has been substantiated from the current findings in our study. It may also be postulated that A. tenuifolius possess potential against thrombosis due to the contribution of certain flavonoids and polyphenolic constituents which inhibit platelet aggregation by binding to the thromboxane A_2 receptors.

Conclusion

The current study substantiates antithrombotic and thrombolytic activity of At.Cr, demonstrating its potential against thrombosis in experimental models. *A. tenuifolius* may be a suitable choice in prevention and treatment of cardiovascular problems associated with thrombotic events possibly due to the presence of quercetin, caffeic acid, and chlorogenic acid which possess strong antioxidant, anti-inflammatory, and antithrombotic activities thus scientifically validating its traditional use in local community.

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