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Evaluation of *Sida cordifolia* L. for its potential against thrombosis in experimental models

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Abstract: Thrombosis, the formation of blood clots due to platelet aggregation, vascular injury or hypercoagulability, leads to cardiovascular pathologies including myocardial or cerebral infarction. Antiplatelet and thrombolytic agents have promising effects in ameliorating thromboembolism and dissolving blood clots. However, the associated limitations generate the need to explore agents from natural origin. The aim of the study was to explore the potential of aqueous methanolic extract (Sc.Cr) of an indigenous plant, *Sida cordifolia* L., traditionally used for cardiovascular complaints. Sc.Cr was evaluated by clot lysis assay, acute pulmonary embolism, carrageenan-induced tail vein thrombosis and ferric chloride-induced carotid arterial thrombosis models. Hemostasis parameters were increased in a dose-dependent manner. Histological studies showed restoration with clear alveolar spaces and less red blood cell congestion. Significant reduction in infarcted length of thrombus, escalation in coagulation parameters with a profound decrease in platelet count (PC) were observed. Arterial occlusion time was increased with a reduction in weight of thrombus dose-dependently with significant augmentation in PT and APTT. Sc.Cr was also analyzed for phytochemical constituents and antioxidant potential. The results demonstrated the antithrombotic and thrombolytic potential of Sc.Cr using in vitro and in vivo experimental models.

Keywords: Thrombosis, antithrombotic, platelet, coagulation parameters, *Sida cordifolia* L.

INTRODUCTION

The development or occurrence of thrombus or blood clot in circulation due to attachment and aggregation of platelets, vascular damage, intrinsic or extrinsic coagulation systems and formation of fibrin mesh is characterized as thrombosis (Li *et al.*, 2019). Usually, thrombosis is a very intricate and multifaceted pathologic phenomenon triggering several diseases including atherosclerotic plaques, deep vein thrombosis and myocardial ischemia leading to sudden death. Additionally, thrombosis is a major reason causing mortality and morbidity thus intensely affecting human life (Cui *et al.*, 2018). The fibrinolytic system consisting of plasminogen activator inhibitor, tissue plasminogen activator and euglobulin, contributes to the physiology and pathophysiology of several processes including atherosclerosis, hypertriglyceridemia and vascular complications. Pathogenesis of thromboembolic complications is also attributed to reduced activity of this system thus resulting in the emergence of thrombosis and arterial complications. Platelet aggregation is an essential phenomenon to form hemostatic plug during vascular injury. Collagen provides strength in platelet adhesion to the sub-endothelium thus initiating procoagulant activity. Therefore, a promising approach to prevent thrombosis is inhibition of an interaction between platelets and collagen (Kwon *et al.*, 2019).

Antiplatelet agents primarily aspirin, have a promising effect in ameliorating venous thromboembolism and thrombolytics such as tissue plasminogen activator, streptokinase and urokinase in already established clots in the vessels (Puurunen *et al.*, 2017). However, associated limitations including hemorrhagic complaints, severe anaphylaxis and gastrointestinal bleeding and immunogenicity induced by streptokinase have developed the need to explore compounds from natural origin having less immunogenicity and better therapeutic efficacy with minimum side effects (Rahman *et al.*, 2019).

Sida cordifolia Linn. belonging to the family Malvaceae, commonly known as "Bala" or "Beejband", is extensively used in Ayurveda. It is also used as a traditional entity in Brazil, China, Pakistan and India for a wide range of ailments including hypoglycemia, asthma, cardiovascular complaints, arrhythmia, neuritis, neuralgia, frequency, diabetes, dysentery, diarrhea, hemorrhoids and anorexia, epilepsy, rheumatism, sciatica, fatigue, gonorrhea, impotence, cystitis, leucorrhea, urinary chronic fever. The seeds are smooth in appearance and grayish-black in color. *Sida cordifolia* reportedly has various pharmacological actions including laxative, anti-inflammatory, antipyretic, analgesic, antiviral, antifungal, antimicrobial and antioxidant activities (Arshad *et al.*, 2020). The scientific study on the effects of the plant against thrombosis has not yet been reported. The present study was investigated to evaluate the antithrombotic and thrombolytic potential of *Sida cordifolia* L. in experimental models.

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MATERIALS AND METHODS

Preparation of crude extract

The seeds of *Sida cordifolia* L. were obtained locally from Bahawalpur (Punjab), Pakistan and verified by the taxonomist. Seeds, after washing, drying and grinding into a coarse powder (2kg) were subjected to maceration in aqueous methanol (30:70) with occasional shaking and stirring for 3 days thrice. The filtrate was subjected to a rotary evaporator under reduced pressure at 50ºC and then at 40ºC in a hot air oven. The thick pasty extract; Sc. Cr (9.5% yield) was kept in storage below 0°C for future use.

Ethical approval

The study procedures were approved by the Pharmacy Animal Ethics Committee and Pharmacy Human Ethics Committee under reference No.PAEC/21/32 and 113/2020-/PHEC, respectively.

Experimental animals

Wistar albino rats (200-300g) and Swiss albino mice (18-30g) of any gender were kept in the animal room of Pharmacology Research Laboratory (IUB). Animals were kept in cages of dimensions 48 ×35 × 18cm^3 under optimal conditions of temperature (25±2°C), humidity (56±5%), 12h/12h day and night period, provided with standard feed with tap water, and allowed to acclimatize for at least 1week prior to experiments.

Chemicals

Chemicals consumed were of analytical grade in this study. Carrageenan and aspirin were procured from Sigma-Aldrich (USA), collagen (Sochim International Spa), streptokinase (SK, Biotech GmbH, Germany), epinephrine (Ameer Pharma Pvt. Ltd.), formalin (Riedel-de Haen, Germany), xylazine (My Lab Pharmaceutical, Pvt. Ltd. Pakistan) and ketamine from Global Pharmaceuticals, Pakistan.

Preliminary phytochemical screening

Sc.Cr was subjected to phytochemical screening for primary and secondary constituents like carbohydrates, alkaloids, flavonoids, phenols glycosides, saponins, tannins and terpenes.

Antioxidant activity by DPPH assay

DPPH assay was utilized to determine antioxidant potential (Jabeen *et al.*, 2021). Methanolic solution (0.2mM DPPH) was mixed with Sc.Cr (0.5mg/ml) and standard antioxidant ascorbic acid at different concentrations. Control was prepared by the addition of 1ml of methanol in DPPH. Solutions were shaken vigorously, placed in the dark for 30 min and at 517nm change in absorbance was measured. Antioxidant activity was calculated by the following formula:

Percent scavenging effects = (Ax- Ay/Ax) x 100
(Ax = absorbance of the control and Ay = absorbance of crude extract)

HPLC analysis for identification of constituents

Stock solutions for reference standards were prepared and ethanol was used for dilution to make up the final volume (50µg/ml). As a solvent for polyphenols ethanol at a concentration of 10mg/ml was prepared. Before analysis, fresh samples were prepared and placed at 4°C. The rate of flow was 1mL/min and absorbance was measured at 280 nm. Quantitative analysis and identifications were done to make a comparison with standard solutions (Imtiaz *et al.*, 2019).

Acute toxicity study

Four groups each of five mice were fasted 12h before dosing. Control was given normal saline (10ml/kg) while study groups received Sc.Cr (1, 3 and 5g/kg; orally). Mice were not given food and water for 4h. Animals were observed closely for the initial 6h and for 48h for mortality and then for 14days. Behavioral effects were noted observed for ataxia, convulsions, hyperactivity, salivation, diarrhea, convulsions, lethargy, sleep, lacrimation and coma (Ugwah-Oguejiofor *et al.*, 2019).

Clot lysis or thrombolysis assay

A stock solution of SK (30,000IU/100µl) was prepared. 5ml human blood was drawn without a history of anticoagulants and oral contraceptives. 500µl of blood was added to ten eppendorf tubes (already weighed) for incubation for 45min (37ºC). Serum was removed completely when the clot was formed. To determine the weight of the clot, tubes were again weighed, labeled with the addition of 100µl of Sc.Cr (1, 3, 5 and 10mg/ml) in tubes to incubate for 90min (37ºC). Tubes were again weighed by removing the fluid. Percent clot lysis was calculated (Uddin *et al.*, 2020).
Clot weight = weight of clot containing tube - the weight of tube alone
% Clot lysis = (Weight of the lysed clot /Weight of clot before lysis) ×100

Collagen, epinephrine-induced pulmonary thrombosis in mice

Mice were divided into groups, with each of six animals administered distilled water; 10ml/kg (control and intoxicated group), three groups received Sc.Cr (100, 300 and 500 mg/kg) and standard control group received aspirin (ASA, 5mg/kg). All the doses were given orally for 7 days. Pulmonary thrombosis was induced by intravenous injection of collagen (80mg/kg) and epinephrine (1mg/kg) solution in the tail vein. Animal behavior was observed for 15min and the number of dead and paralyzed animals was noted. The percent of protection of Sc.Cr against pulmonary thrombosis was calculated with the following formula:

% Protection = [1-(dead + paralyzed) / Total] x 100

Hemostasis parameters and euglobulin lysis time (ELT) were recorded (Zhou *et al.*, 2014). The lungs were

excised, rinsed with normal saline (0.9%), and fixed in 10% formalin for at least 24h. Lung sections were cut (7μm) and stained with hematoxylin and eosin stains to observe histological variations under a microscope (ACCU-Scope, 3000-LED, USA).

Carrageenan-induced thrombosis model

Rats were divided into six groups, with each group consisting of six animals. Groups I and II as control and κ-carrageenan-induced intoxicated group were given normal saline (2ml/kg). Group III received SK 60,000IU/kg, groups IV, V and VI were given Sc. Cr (100, 300, 500 mg/kg; i.p, respectively). Rat tail vein was injected with κ-carrageenan (1mg/kg) prepared in normal saline 12 cm from the tip of the tail along with ligation for 10 min and once thrombus was developed, all the doses were administered for 14 days. BT and CT were measured. Blood was collected for platelet count and plasma separated to estimate activated partial thromboplastin time (APTT) and prothrombin time (PT) using commercially available reagents (Weiner Lab. Argentina).

Common carotid artery thrombosis model or $FeCl_3$ induced carotid arterial thrombosis

Rats were randomly divided into groups (n=6). Distilled water was given orally to the control and model groups, aspirin (5mg/kg) to the standard control and the treatment groups received Sc.Cr (100,300 and 500mg/kg, along with intoxication). After 14 days, rats were anesthetized (ketamine xylazine; i.p), fixed in a supine position to make an incision (3cm) on the throat to isolate the common carotid artery (2cm) and a plastic sheet (3x1.2cm) was placed under the vessel. The carotid artery surface was wrapped with filter paper (1x1cm) saturated with $FeCl_3$ solution (40%), the temperature was monitored with thermometer and thrombosis occlusion time (OT) was recorded. A segment of the carotid artery (0.6cm) was removed and weighed. The rate of inhibition was calculated.

Rate of inhibition (%) = $(C-C_1)$ / Cx100

(C=wet weight of thrombus in the model group, C_1=wet weight of thrombus in treated group)

STATISTICAL ANALYSIS

The data were analyzed by one-way ANOVA following Bonferroni's test and values were presented as Mean ± SEM. GraphPad Prism version 6 was used for the interpretation of results.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical analysis revealed the presence of various metabolic constituents like carbohydrates, alkaloids, flavonoids, glycosides, saponins, tannins, phenols and terpenes.

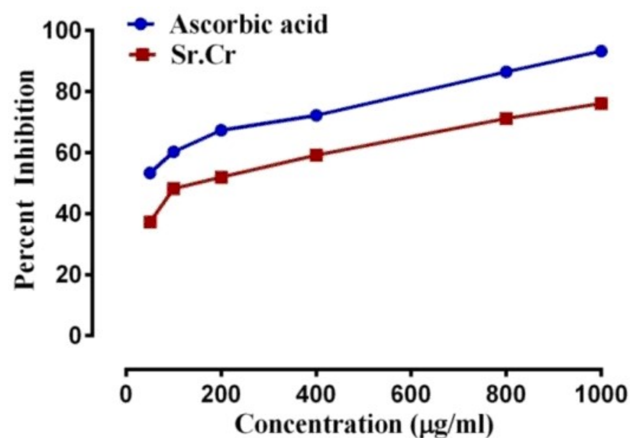

Fig. 1: Scavenging effects of Sc.Cr and ascorbic acid on DPPH radical.

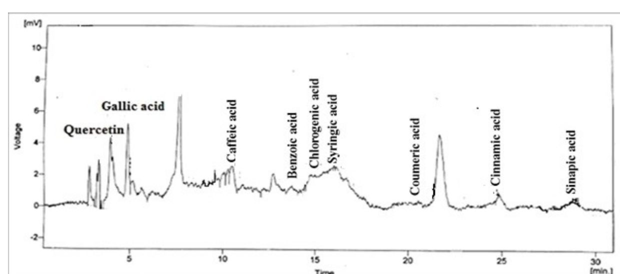

Fig. 2: HPLC chromatogram of crude extract of Sc.Cr

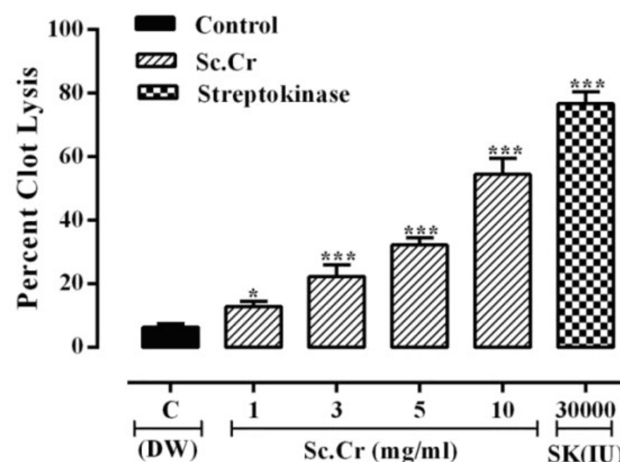

Fig. 3: Effects of Sc.Cr and SK on *in-vitro* clot lysis in human blood. Mean ± SEM, n=6. (*) P<(0.05), (**)P<(0.01) and (***)P<(0.001) vs. control group.

Antioxidant activity by DPPH assay

Sc.Cr neutralized the DPPH giving maximum inhibition of 76.11% at 1000μg/ml concentration (fig. 1).

HPLC analysis for identification of constituents

The HPLC analysis of Sc.Cr revealed quercetin (Rt.3.26 min), gallic acid (4.80), caffeic acid (12.70), syringic acid (16.70), sinapic acid (26.14), m-coumaric acid (20.57), 4-hydroxy,3-methoxy benzoic acid (14.43), cinnamic acid (24.86) and chlorogenic acid (15.64) (fig. 2).

Table 1: Effects of Sc.Cr and ASA on bleeding time (BT), clotting time (CT) and euglobulin lysis time (ELT) in collagen, epinephrine-induced thrombosis model in Wistar albino mice.

Treatment Groups	Bleeding Time (min)	Clotting Time (min)	Euglobulin Lysis Time (min)
Control (D/W,10ml/kg; p.o)	2.69±0.16	3.27 ±0.18	273.5 ± 14.44
Intoxicated (C, 80mg/kg+ E, 1mg/kg; i.v)	1.90±10	2.62±0.13	-
Aspirin (5mg/kg) + Intoxication	8.83±0.54***	9.70±0.47***	79.77 ± 3.31***
Sc.Cr (100mg/kg) + Intoxication	3.37±0.19ns	4.54±0.14*	211.0 ±14.34**
Sc.Cr (300mg/kg) + Intoxication	4.55±0.27**	6.20±0.31***	146.4 ± 6.30***
Sc.Cr (500mg/kg) + Intoxication	6.45±0.29***	7.60±0.28***	101.2±6.35***

Mean ± SEM, n=6. (*)P<(0.05), (**)P<(0.01) and (***)P<(0.001) vs. control group. (C: Collagen, E: Epinephrine)

Table 2: Effects of Sc.Cr and ASA on OT and weight of thrombus in $FeCl_3$-induced carotid arterial thrombosis in Wistar albino rats.

Treatment Groups	Occlusion Time (min)	Wt. of Thrombus (mg)	Inhibition Rate (%)
Intoxicated (40%, $FeCl_3$)	7.05±0.33	12.48±0.54	-
Aspirin (5mg/kg; p.o) + Intoxication	27.79±1.01***	5.50±0.23***	55.92
Sc. Cr (100mg/kg; p.o) + Intoxication	12.43±0.62***	11.06±0.27***	11.37
Sc. Cr (300mg/kg; p.o) + Intoxication	17.77±1.09***	9.51±0.22***	23.79
Sc. Cr (500mg/kg; p.o) + Intoxication	22.40±0.52***	6.83±0.31***	45.27

Mean ± SEM, n=6. (*)P<(0.05), (**)P<(0.01) and (***)P<(0.001)as compared to the intoxicated group.

Table 3: Effects of Sc.Cr and ASA on coagulation parameters in $FeCl_3$-induced carotid arterial thrombosis in Wistar albino rats.

Coagulation parameters	Control (DW,5ml/kg, p.o)	Intoxicated (40%, $FeCl_3$)	Aspirin (5mg/kg; p.o) + Intoxication	Sc. Cr (mg/kg; p.o) + Intoxication		
				100	300	500
PT (sec)	13±0.59	12±0.47	28±0.62***	16±0.70**	20±0.93***	24±0.62***
APTT (sec)	20.58±0.58	19.25±0.78	61.83±1.67***	25.08±0.87**	33.83±1.70***	48.0±1.25***
PC (10^9/L)	741.7±1.02	757.2±2.49	482.7±4.69***	739±2.35***	647.2±4.93***	592.5±1.68***

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

Acute toxicity assay

Sc.Cr was safe up to the dose of 5g/kg and no toxicity signs were observed and no mortality was noticed after 48 hrs.

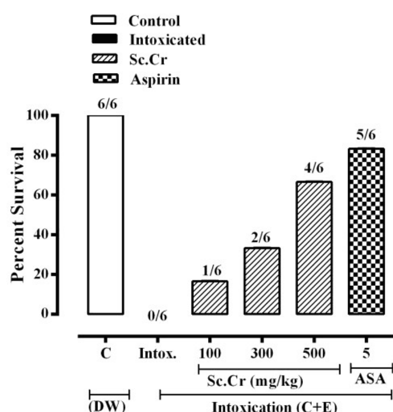

Fig. 4: Effects of Sc.Cr and ASA on the percent survival in collagen, epinephrine-induced thrombosis model after 7days of treatment in Wistar albino mice. C: Collagen, E: Epinephrine.

In vitro clot lysis or thrombolysis assay

Fig. 3 presents *in vitro* clot lysis or thrombolysis assay of Sc.Cr with dose-dependent augmentation in the lysis of blood clot at different doses (1, 3, 5 and 10mg/ml, respectively). SK (30,000IU/100µl) as the standard thrombolytic drug showed maximum clot lysis in comparison to the control.

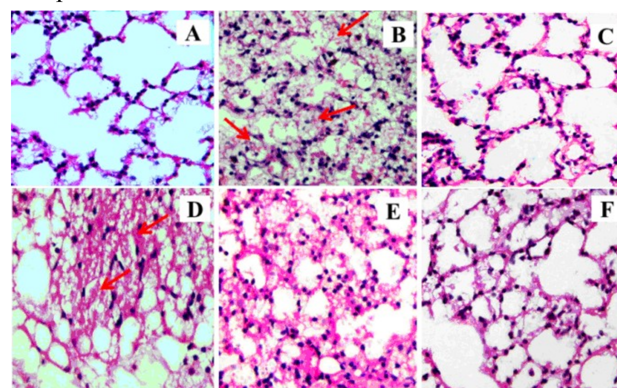

Fig. 5: Histological sections of lung; (A) Control (10ml/kg; p.o) (B) Intoxicated (C+E, i.v) (C) (ASA,

5mg/kg; p.o) (D) Sc.Cr, 100mg/kg (E) Sc.Cr, 300mg/kg (F) Sc.Cr, 500mg/kg) (: Pulmonary thrombi and red blood cell congestion, C: Collagen and E: Epinephrine).

Collagen, epinephrine-induced pulmonary thrombosis

Fig. 4 shows the percent protection by acute pulmonary thromboembolism (APT) at 100, 300 and 500mg/kg doses of Sc. Cr; 16.7, 33.3 and 66.7%, respectively. Aspirin, the standard drug at a dose of 5mg/kg gave maximum protection, 83.33%.

Sc.Cr, at 300 and 500mg/kg doses prolonged bleeding time after seven days of p.o treatment in comparison with the control group while 100mg/kg dose showed a non-significant increase. Clotting time showed an increase in a dose-dependent pattern in comparison to the control. Aspirin (5mg/kg) as a standard drug showed a highly significant increase in both the bleeding and clotting times. Sc.Cr (100, 300 and 500mg/kg, respectively) decreased euglobulin lysis time (ELT) significantly and dose-dependently in comparison to control group. Aspirin significantly reduced ELT (Table 1).

Lung tissues were observed for histopathological changes. The control group under the microscope was characterized by a thin alveolar-capillary membrane separating red blood cells from the alveolar space. Intoxicated lung tissues showed the presence of thrombi and hemorrhage due to the development of red blood cells congestion in the surrounding tissues. The endothelium became ulcerated with the formation of fibrin mesh. Polymorph nuclear leukocytes were infiltrated in alveolar and bronchial walls showing injury and thrombosis. The standard drug, aspirin-treated group showed clear capillaries separated from the alveolar spaces. The treatment groups presented clear alveolar spaces without pulmonary emboli and congestion of red blood cells as the graded doses of Sc. Cr were increased (fig. 5).

Carrageenan-Induced Thrombosis Model

Sc.Cr (100, 300 and 500mg/kg) and SK 60000IU/kg, showed a significant reduction in the length of infarction after fourteen days of intraperitoneal administration in carrageenan (1mg/kg) induced tail vein thrombosis. The infarcted tail length was reduced in a dose-dependent manner in comparison to the intoxicated group. SK showed maximum response to reduce the infarction of tail length in comparison to the intoxicated group. Sc.Cr showed a substantial and dose-dependent increase in bleeding time (BT) and clotting time (CT) as compared to the control group. SK 60,000IU/kg prolonged the BT and CT in a highly significant manner (fig. 6).

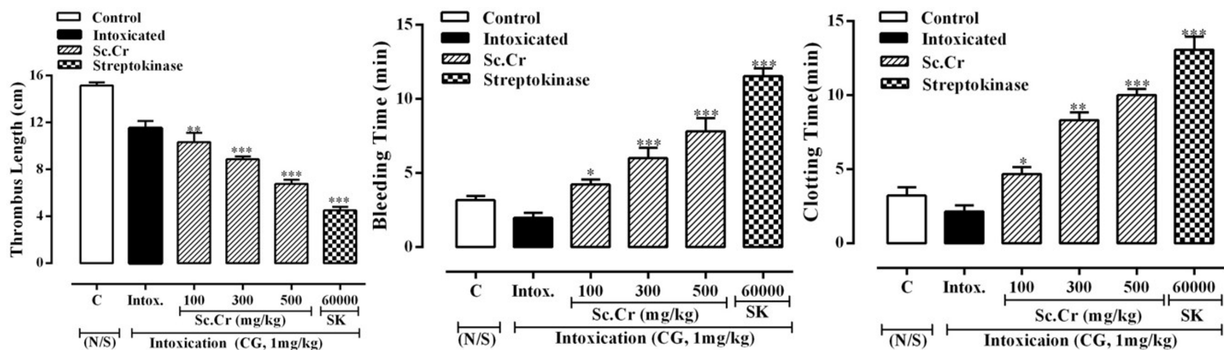

Fig. 6: Effects of Sc.Cr and SK on thrombus length, BT and CT in carrageenan-induced tail thrombosis. Mean ± SEM, n=6. Mean ± SEM, n=6. (*)P<(0.05), (**)P<(0.01) and (***)P<(0.001). Thrombus length vs. intoxicated, BT and CT vs. control. CG: Carrageenan.

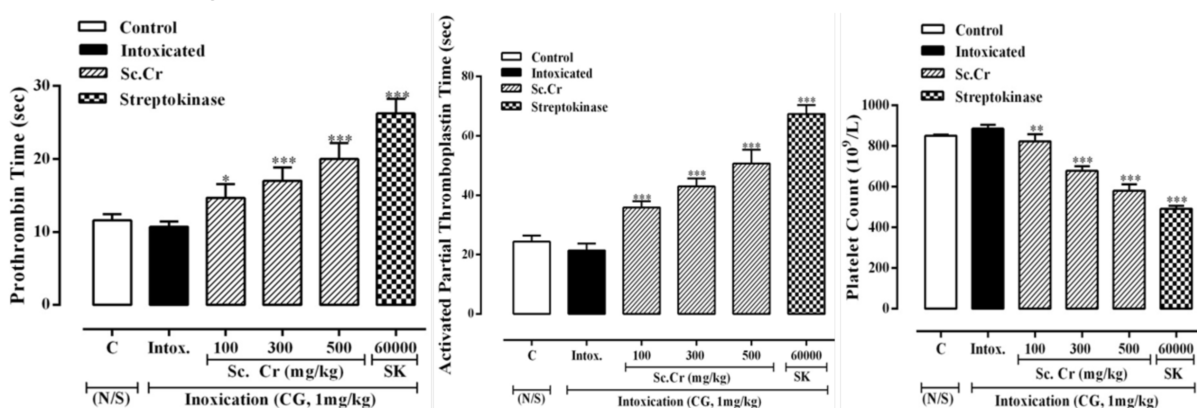

Fig. 7: Effects of Sc.Cr and SK on coagulation parameters in carrageenan-induced tail thrombosis model in Wistar albino rats. Mean ± SEM, n=6. (*)P<(0.05), (**)P<(0.01) and (***)P<(0.001). vs. control group. CG: Carrageenan.

Activated partial thromboplastin time (APTT) and Prothrombin time (PT) were significantly escalated by Sc. Cr in a dose-dependent fashion in comparison to the control group. SK, the standard drug showed maximum augmentation in PT and APTT in comparison to the control group. A profound decrease in platelet count (PC) of carrageenan-induced thrombotic animals was noticed by SK and Sc. Cr (100, 300, 500mg/kg) substantially and in a dose-dependent fashion as compared to the control group (fig. 7).

Common carotid artery thrombosis model or $FeCl_3$ induced carotid arterial thrombosis

Sc.Cr extended the thrombosis occlusion time (OT) and decreased the weight of thrombus significantly and in a dose-dependent (100, 300, 500mg/kg) pattern in comparison to the intoxicated group. Sc. Cr, at 100, 300 and 500mg/kg doses increased the rate of inhibition (11.37, 23.79 and 45.27%, respectively) in comparison to the ferric-chloride (40%) induced intoxicated group. ASA, the standard drug at 5mg/kg dose showed a highly significant rise in OT and decreased the weight of thrombus showing a maximum rate of inhibition (55.92%) in comparison to the intoxicated group (table 2).

Sc.Cr (100, 300 and 500mg/kg) revealed a significant and dose-dependent escalation in PT and APTT after fourteen days of treatment (p.o) in comparison to the intoxicated group. Aspirin, at 5mg/kg dose presented an optimum increase in both PT and APTT. PC was decreased significantly and dose-dependently at all doses of Sc.Cr and ASA, the standard drug as compared to the intoxicated group (table 3).

DISCUSSION

One of the major problems during blood circulation is blood clotting. The clots get lodged into blood vessels, interfering with the blood flow and depriving the localized tissues of oxygen supply causing tissue necrosis (Cano-Espinosa *et al.*, 2020). Agents like aspirin, clopidogrel, streptokinase and warfarin divulge some notable adverse effects resulting in vascular relapses (Gao *et al.*, 2021). Polyphenolic compounds (gallic acid ellagic acid and ferulic acid) and flavonoids (quercetin and kaempferol) produced as secondary constituents in vegetables and fruits have been reported to impart their biological effects as antiplatelet, antioxidant and anti-inflammatory *in vitro* assays (Kyriakidis *et al.*, 2021).

Platelets impart a pivotal part in the pathogenesis of cardiovascular diseases by the acceleration of the coagulation cascades. Collagen causes shape change in platelets during activation, promoting secretion by the storage granules comprising ATP and ADP (Moschona *et al.*, 2017). Induction of acute pulmonary thromboembolism (APT) by collagen and epinephrine mixture exerts a synergistic effect by inducing platelet aggregation. Sc.Cr showed an antithrombotic effect by providing percent protection and increasing hemostasis parameters dose-dependently against APT. Fibrinolysis is linked with thrombosis and impaired fibrinolysis may precipitate thrombotic incidents. ELT was reduced by Sc. Cr dose-dependently in comparison to the control group and aspirin reduced ELT significantly. Carrageenan injected into the rat tail vein causes hypercoagulopathy due to inflammation in the blood vessels and by disturbing homeostasis induces thrombosis (Kala and Khan, 2020). A significant reduction in infarcted length of thrombus was observed by Sc.Cr dose-dependently. The most commonly used detection indices in clinical studies are PT and APTT for evaluation of thrombotic diseases. PT and APTT reflect exogenous and endogenous coagulation pathways respectively (Cui *et al.*, 2018). Sc. Cr escalated both PT and APTT significantly and dose-dependently in all treatment groups as compared to the control. $FeCl_3$ induced occlusive thrombosis by producing reactive oxygen species in the endothelium causing oxidative stress leading to lipid peroxidation and exposure of subendothelial collagen (Kim *et al.*, 2019). Polyphenols and flavonoids inhibit platelet function and flavonoids also cause inhibition of platelet aggregation by binding to the thromboxane A_2 receptors (Faggio *et al.*, 2017). A significant decrease in PC was shown by Sc.Cr in a dose-dependent fashion as compared to the intoxicated group. HPLC analysis of Sc.Cr has revealed the presence of polyphenols (Caffeic acid, gallic acid, benzoic acid, sinapic acid, syringic acid, cinnamic acid and chlorogenic acid) and flavonoids such as quercetin. Quercetin, reportedly caused inhibition of collagen-induced aggregation of platelets by calcium immobilization, inhibiting the production of hydrogen peroxide and formation of 1,3,4 inositol triphosphate in human platelets. Chlorogenic acid has an inhibitory effect on platelet activation, thrombus formation and provides protection to the vascular integrity and cultured endothelial cells against oxidant-induced injury (Choi and Kim, 2017). Caffeic acid quantified in Sc.Cr has antioxidant potential and reportedly causes amelioration in thrombus formation *in-vivo* both in arterial and venous thrombosis (Nam *et al.*, 2020). Platelet function and coagulation are influenced by reactive oxygen species, which cause circulatory problems and polyphenols enhance redox status by inhibiting platelets function (Ed Nignpense *et al.*, 2020). It can be asserted from this study that the antithrombotic and thrombolytic potential of Sc. Cr is attributed to its antioxidant, antiplatelet, antithrombotic and thrombolytic activities due to the presence of polyphenolic compounds and flavonoids. Current study demonstrated that Sc.Cr may be an appropriate choice in preventing and treating cardiovascular ailments associated with platelets in thrombotic incidents. However, further investigations regarding underlying mechanisms need to be explored in future studies.

CONCLUSION

The main findings of the results concluded in this study are the multifunctional role of polyphenolic compounds and flavonoids with their synergistic effect in improving redox status and platelet function attributed to the role of *Sida cordifolia* L. against thrombosis as demonstrated through *in vitro* assay and experimental studies in biological models.

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