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Evaluation of Anti-clotting and thrombolytic potential of the aqueous-methanolic extract of *Jasminum sambac*

Gul-E-Mizgan[1], Saba Kousar[2*], Imran Ahmad Khan[1], Perwasha Perwasha[1], Humaira Gul[3], Iqra Aslam[1], Ameen Abbas[2] and Qurat-ul-Ain[1]

[1]Ali-Ul-Murtaza, Department of Rehabilitation Sciences, Muhammad Institute of Medical and Allied Sciences, Multan, Pakistan
[2]Fatima-Tu-Zahra, Department of Life Sciences, Muhammad Institute of Medical and Allied Sciences, Multan, Pakistan
[3]Department of Pharmacology, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad

Abstract: This study was done to investigate the anti-clotting and thrombolytic potential of ariel part of *Jasminum sambac* (L.). Healthy male rabbits were divided into five (5) groups with each group containing six (6) animals. Aqueous-methanolic extract of the plant was prepared and given at different doses of 200, 300 and 600mg/kg to three groups in comparison to negative and positive control groups. The aqueous-methanolic extract showed a dose-dependent increase in the activated partial thromboplastin (APTT) and prothrombin time (PT), bleeding time (BT) and clotting time (CT) ($p<0.05$). Warfarin 2mg/kg was used as the standard. The plant extract also showed significant ($p<0.05$) clot lysis in comparison with standard urokinase. Moreover, it also prolonged the ADP induced platelet adhesion at doses of 200, 300 and 600µg/mL dependently. HPLC analysis showed rutin, quercetin, salicylic acid and ascorbic acid as vital phytoconstituents in aqueous-methanolic extract. Anticoagulant and thrombolytic effect of *Jasminum sambac* justified its therapeutic utility in cardiovascular disorders and this may be due to the presence of salicylic acid, rutin and quercetin in the extract.

Keywords: Medicinal herbs, plant extract, thrombosis, urokinase, warfarin.

INTRODUCTION

There are multiple cardiovascular issues that lead to thrombosis which is a disorder characterized by thrombus formation within the vascular system (Khan, 2022, Manzoor et al., 2022). Thromboembolic illnesses including pulmonary embolism, deep vein thrombosis and stroke are main cause of morbidity and mortality in developed countries (Khan, 2022). Thromboembolic illnesses may be caused due to arteriosclerosis, infection or even due to traumatic injury that may lead to clot formation. It may also be formed when blood flows too slowly through the blood vessels. The use of t-PA has been shown to be effective in dissolving the clot (Hall, 2016). Many different coagulation factors and enzymes are required in sequential cascade reactions in the process of coagulation. Intrinsic and extrinsic paths are in front of the initiation of the Stuart-Prower factor (Xa where "a" stands for activated). After that, the Stuart-Prower factor is used in conversion of prothrombin to its activated form, thrombin that leads to fibrin clot formation by activating the fibrinogen to fibrin (Dickneite *et al.,* 1995, Tortora and Derrickson, 2018). Inspite of activation of fibrinogen to its activated form, thrombin also plays role in activation of the XIII and increase in production of factor V. As a result of which thrombin is increased and platelets are activated leading to the aggregation of platelets and clotting is accelerated (Tortora and Derrickson, 2018).The drugs that are being used nowadays such as anticoagulants, antiplatelet and thrombolytic agents are related to several concomitant effects. The medication systems used traditionally are mostly based on plants and herbs for treatment of diseases and are definitely of great worth for those who cannot afford the costly or expensive medications in this age of modern medicine systems (Ghayur *et al.,* 2005). Amongst different countries where the crude form of medicinal herbs is being exported due to lack of technological support, Pakistan is one of them (Sapra and Pandya, 2022). As the weather conditions of Pakistan are suitable for herbs used in medicine and their growth, local manufacturers are producing the medicine on commercial scale. Besides the use of the pharmacological drugs used in treatment and lowering the risks of cardiovascular diseases (CVDs), medicinal plants are also being used in their treatment purposes. Mostly the focus is on the antioxidant properties of such plants but some herbal materials may also show antithrombotic effects and might reduce the cardiovascular risks. The major risk factor in CVDs and most common diseases in modern world are hypertension and diabetes. Increased levels of insulin (hyperinsulinemia) may also impose great risk of coronary heart disease as it might interfere with the phenomenon of fibrinolysis, either by increasing the low density lipid cholesterol or by stimulation of smooth muscle cells proliferation. (Gilani *et al.,* 2010).

Jasminum sambac (L.) ariel part was used in this study. The entire plant is listed to be of great interest in the traditional herbal use for treatment purposes. Its common

**Corresponding author:* e-mail: sabakousar888@gmail.com

name is "*Jasmine*". Moreover, this plant belongs to "Oleaceae" family. It has fragrant flowers and has been one of the oldest cultivated plants by humans. Its native land is subtropical regions from where it has taken to other global regions. The term jasmine is derived from the word "Jessamine" in Arabic and is known as "Yasmine" or "Yasmyn" in Persian language, that means fragrance (Bailey, 1947). Phytochemical studies of *Jasminum sambac* (L.) showed the presence of proteins, glycosides, carbohydrates, flavonoids, resins, terpenes, essential oils, salicylic acid and many other useful compounds. The pharmaceutical effects of the plant include antimicrobial, antipyretic, antioxidant, anti-diabetic, anti-inflammatory activities along with central nervous system (CNS), peripheral nervous system (PNS), cardiovascular system (CVS), anti-obesity and analgesic effects (Al-Snafi, 2018). The plant is used traditionally in the treatment of multiple diseases along with anti-septic, sedative and tonic (uterine) effects (Mourya *et al.,* 2017). The roots of *Jasminum sambac* (L.) are very beneficial for the treatment of wounds and snake bite (Sapra and Pandya, 2022). Flowers and leaves possess decongestant and anti-pyretic properties. Moreover, due to their lovely fragrance, the essential oils of *Jasminum sambac* (L.) are used in the production of perfumes. Inspite of multiple medicinal uses, not enough information about this plant has been published. Although the entire parts of the plant are described for their beneficial use in folk medicines, still much more needs to be reported about the plant. The leaves and flowers of this plant are also used to treat breast cancer. In brewed form it helps prevent breast cancer and stops the uterine bleeding. Moreover, this plant also possesses anti-fungal properties (Mourya *et al.*, 2017). The climatic conditions of Pakistan are very favorable for the growth of different types of medicinal plants and some local manufacturers are also producing different herbal medicines on a commercial scale for export to the foreign countries and the yearly income of these products is comparable to any international pharmaceutical company. Moreover, due to lack of the proper techniques and multiple other insignificant approaches, scientific investigations in detail haven't been done on traditional practices. Although, different researches on different medicinal herbs would impose a great impact by decreasing the cost of medicines in many countries where the major cost is spend on import taxes. Very limited amount of work has been done on the plants in the field of medicine, therefore its insignificance is much more is yet to be done. Therefore, we decided to do some screening of *Jasminum sambac*. It might prove beneficial enough to replace or at least show good efficacy comparable to other costly medicines.

MATERIALS AND METHODS

Preparation of plant material

The ariel part of fresh plant was taken, washed thoroughly and was subjected to shade drying for 7 days. The foreign pollutants were removed from the dried plant. Afterwards the plant was ground to coarse powders with the help of special herbal grinder and (250 g) was soaked in (1 L) aqueous-methanolic mixture (70:30 v/v) in amber colored air tight jars for nine (9) days (Alamgeer *et al.*, 2013). Episodically the gentle shaking was done. Then crude extract from the amber colored air tight jars was collected as crude extract pool in one container. Stock solutions were prepared for the dilutions and doses and were kept in refrigerators at low temperature of 4◦C.

The estimated 10% yield of extract was taken using the formula:

% Yield = (Weight after evaporation ÷ Dry weight of leaves) ×100

Ethical approval

The Ethical Committee of Muhammad Institute of Medical and Allied Sciences allowed the use of animals for the conduct of the study under the reference number 2021/IRB/13/PT/04, according to the rules of Institute of Laboratory Animal Resources, Commission of Life Sciences, National research Council (Murray *et al.*, 1999).

Experimental design

The study was conducted on male rabbits (weighing 1.5 to 2 kg) that are commercially available. They were kept in stainless steel cages under temperature (22±2°C) at the animal house of Muhammad Institute of Medical and Allied Sciences, Multan in sporadic 12h light/dark cycle and provided a good commercially available diet.

Chemicals

Urokinase was purchased from Highnoon Laboratories Ltd. Pakistan. The warfarin vials were purchased from Mehran Traders Ltd. Pakistan. Prothrombin time and activated partial thromboplastin time reagents were purchased from Javaid Pharmaceuticals Ltd. Pakistan. Methanol was purchased from Merck, Germany. Ferric chloride ($FeCl_3$) was purchased from BDH Laboratory Pvt. Ltd. England.

Preliminary phytochemical screening

This was done for the endorsement of many phytochemical constituents (alkaloids, saponins, anthraquinones, flavonoids, tannins, glycosides) in the hydroalcoholic extract of *Jasminum sambac* (L.) by means of standard procedure (Evans, 2009).

HPLC analysis

Phytochemical compounds were parted via the Agilent HPLC (Santa Clara, CA, USA) sequence 1100 system provided by autosampler, UV/vis detector, quaternary pump, 5µm, 250mm × 4.6mm i.d. and C18 reversed-phase column (Thermo Electron Corporation, Waltham, MA, USA) connect to HP Chem Station software (Fernández-Ponce *et al.*, 2012). An (acetic acid- water, 2:98, v/v) and B (water-acetic acid, 2:98, v/v) were the solvents used to form the portable point (methanol). The

subsequent elution setups were used: 0-2 min, 5 percent B isocratic; 2-7 min, 5-25 percent B linear gradient; 7-11 min, 25 percent B isocratic; 11-19 min, 25-32 percent B linear gradient; 19-27 min, 32 percent B isocratic; 27-28 min, 32-40 percent B linear gradient; 28-38 min, 40 percent B isocratic. The solvents used for creation of the portable point, also the column's cleaning and renovating processes (38-50 min, linear gradient 40-100 percent B; 50-60 min, 100 percent B isocratic; 60-70 min, linear gradient 100-5 percent B; and 5 min, 5 percent B (isocratic). The noticeable flavonoids in *J. sambac*, rutin, and quercetin 3-d-glucoside were observed and they had the subsequent standardization curve:

$$A = 54{,}252\ C - 100.12 \quad (2)$$
$$A = 87{,}077\ C - 130.11 \quad (3)$$

Where A is the range exhibited in mAu and C is the concentration indicated in g/mL. The correspondence coefficient (R2) was 0.9999 for both standardization curves. All the trials were executed in triplicate to have unvarying outcomes. HPLC chromatograms of the extract achieved for two variants studied as presented in table 1.

Preparation of dosage

Doses of *Jasminum sambac* (L.) extract were prepared as 600, 300 and 200mg was contained in 1mL each. Warfarin (5mg) tablets were used to prepare (2mg) solution (active drug/mL).

Acute oral toxicity testing

Acute oral toxicity examination was executed following OECD423 (Toxicity–Up, 2001). Total twenty four rabbits were weighing 1 to 1.5kg were selected for randomization in five (5) groups having six (6) animals in each group. Each group was given *Jasminum sambac* (L.) extract orally at dose of 2500mg/kg. The rabbits were individually observed after dosing through the first 30 min and then occasionally through the first 24h, with extraordinary consideration given through first 4h and then daily afterwards, for two weeks.

In-vitro experiments on human blood

Anti-coagulant Activity

Blood samples (3 mL from each) were withdrawn from healthy human volunteers (n=25) by using no contraceptives and analgesics. After that each blood sample was divided into five separate test tubes. 0.2mL of 20%, 10%, and 5% dilutions of aqueous-methanolic extract and warfarin (250 IU/mg) as a positive control, and distilled water as negative control were mixed in these five (5) test tubes then subjected to incubation at 37°C. Then clotting time (CT) was measured with help of a stopwatch (ul Ain *et al.*, 2018).

Determination of activated partial thromboplastin time and prothrombin time

Blood samples (3mL from each) were taken from healthy human volunteers (n=25), taking no contraceptives, and analgesics, were transferred into sodium-citrate-

Table 1: Phytochemical analysis of hydroalcoholic extract of *Jasminum sambac* (L)

Tests	Observations	Results
Alkaloid	No ppt	-
Saponins	1cm froth	+
Tannins	No light purple	-
Anthraquinones	No pink color	-
Coumarins	Yellow flouresence	+
Phenols	Light purple	+
Flavonoid	Light yellow color	++

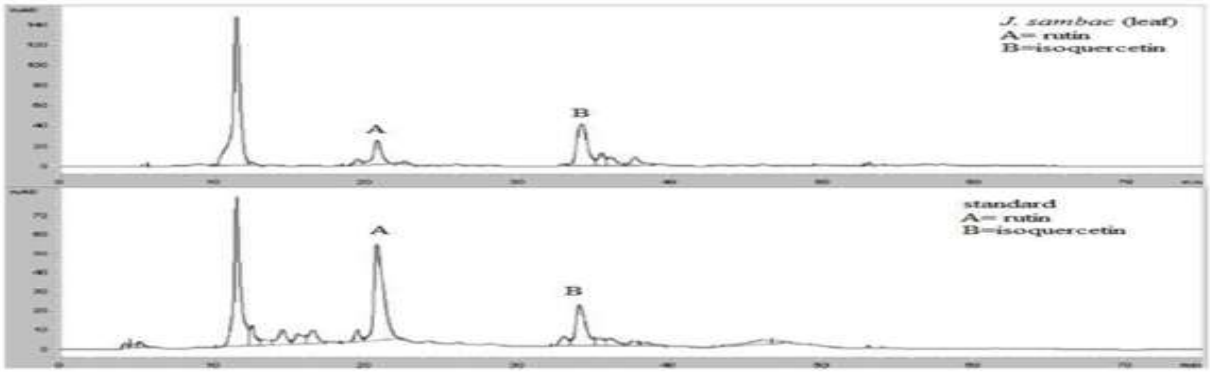

Fig 1: HPLC chromatogram of hydroalcoholic leaf extract of Jasminum sambac (L.) showing rutin (A) and isoquercetin (B) similar to standard.

containing tubes and centrifuged for 5 min at 3000 rpm. Plasma was transferred to distinctive eppendorf tubes of every member of the group by micropipettes. Different dilutions 200mg/kg. 300mg/kg and 600mg/kg of aqueous-methanolic extract (100µL) were mixed with the same amount of plasma in distinctive eppendorf tubes of each member of the group. The 100µL of warfarin (80 IU/mg) was used as a positive control and distilled water was used as a negative control. To determine the prothrombin time (PT) sample was subjected to incubation for 5 min at 37°C. Then prothrombin time reagent (200µL) was mixed in each eppendorf tube being tested, and time was measured as PT with help of a stopwatch. To determine activated partial thromboplastin time (APTT), an activated partial thromboplastin time reagent (100µL) mixed with plasma was tested. The mixture was incubated for 1 min, then calcium chloride solution (100µL) was mixed and hatched for 15 sec and clotting time was measured as APTT with the help of a stopwatch (Asif and Rasool, 2016).

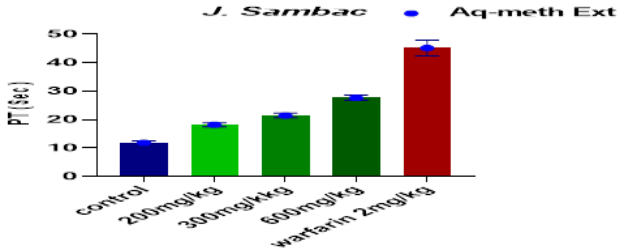

Fig. 2: Effect of *Jasminum sambac* (L.) Extract on Prothrombin Time (PT).

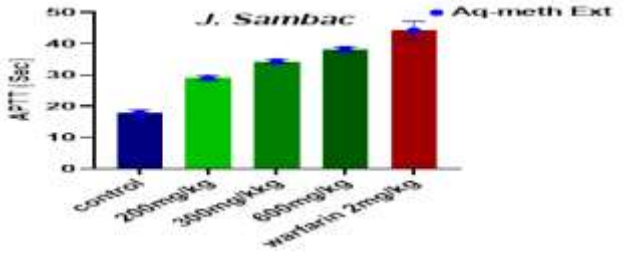

Fig. 3: Effect of *Jasminum sambac* (L.) Extract on Activated Partial Thromboplastin Time (APTT).

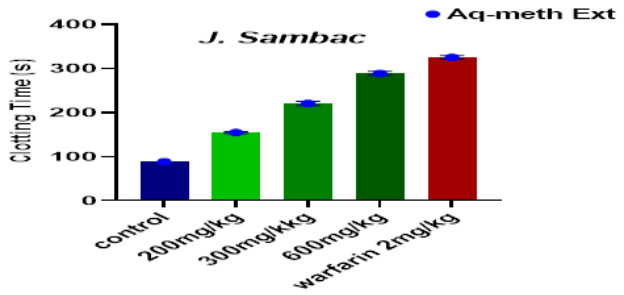

Fig. 4: Effect of *Jasminum sambac* (L.) on Clotting Time (CT).

Thrombolytic activity

Blood samples (2.5mL from each) were collected from healthy human volunteers (n=25), taking no contraceptives and analgesics, transferred into five (5) different already weight eppendorf tubes of each member. Time was allowed for thrombus formation, after 45 min serum was removed from eppendorf tubes. Clots in eppendorf tubes were weighed. After that 600mg/kg, 300mg/kg, 200mg/kg dilutions of aqueous-methanolic extract (100µL) were added in three distinctive eppendorf tubes of each member. Then 100µL Urokinase (50000 IU) was added in the 4th eppendorf tube as positive and distilled water was added in the fifth (5th) eppendorf tube as a negative control. The time allowed for thrombolytic activity. After 90 min, the liquid was removed and the remaining clots in eppendorf tubes were weighed over again. The alteration between before and after clot lysis was taken as % clot lysis (Bannish *et al.*, 2017, ul Ain *et al.*, 2018).

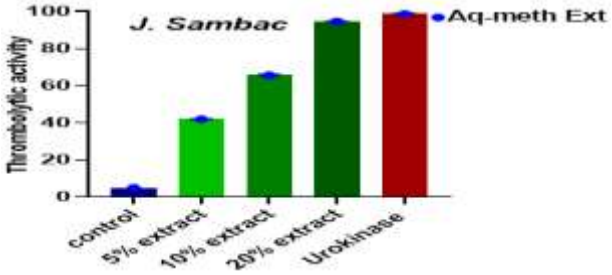

Fig. 5: Effect of Extract on Thrombolytic Activity

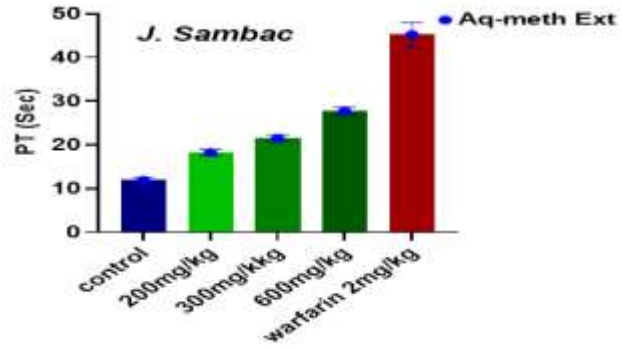

Fig. 6: Effect of *Jasminum sambac* (L.) on intrinsic pathways (prothrombin time). Statistical significance was done considering $p<0.05$ when compared with control.

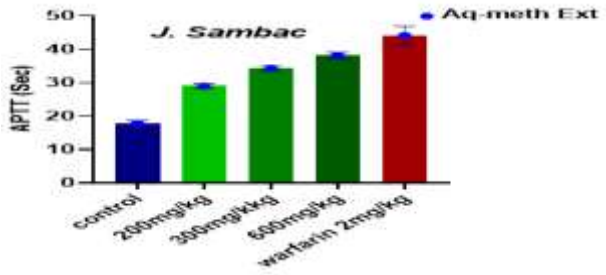

Fig. 7: Effect of *Jasminum sambac* (L.) on the APTT.

$$\%\text{Clot lysis} = \frac{A - B}{A} \times 100$$

A=weight of clot before lysis
B=weight of clot after lysis

In-vivo experiment on rabbit

Determination of Coagulation Parameter

Rabbits were divided into five (5) groups (n=5), and dose 600 mg/kg, 300 mg/kg and 200 mg/kg dilutions of the aqueous-methanolic extract (100mg) were given to rabbits of the 1st, 2nd and 3rd groups. Warfarin (2mg/kg) was injected intravenously to rabbits of the 4th group for seven (7) days as positive control while rabbits of the 5th group treated with distilled water used as negative control. On 7th day, the blood sample was received from the external jugular vein of rabbits of each group (dos Santos et al., 2019). After that PT and APTT tests were performed (Asif and Rasool, 2016). After seven days of treatment to decide the impact, the bleeding time (BT) was measured by prickling the marginal ear vein of each rabbit after an interval of 0, 30, 60, and 90 min, and after each 5sec filter paper was utilized (Nwaehujor Chinaka et al., 2013). Clotting time (CT) was measured by piercing the marginal ear vein of each rabbit of each group with the assistance of capillary tubes by putting it horizontally. The capillary tubes were broken after every 30 sec until the thread was shown as coagulated blood (ul Ain et al., 2018).

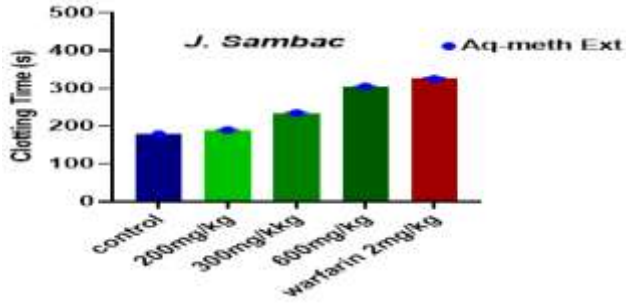

Fig. 8: Effect of *Jasminum sambac* (L.) on the Clotting Time (CT).

Thrombolytic activity

For the thrombolytic activity, the procedure previously done by (Tian et al., 2017) was adopted with slight modification. The rabbits were set in prone lying position then ears were disinfected with the alcohol swabs commercially available. After which 0.2 to 1.1mg/kg of 40% $FeCl_3$ solution was administered to the marginal veins of rabbits' ears intravenously (Majumdar *et al.*, 2016). The animals were kept under consideration after 24h of the thrombus formation. The optimal presence of the $FeCl_3$ was observed on basis of wine colored appearance in the ear vein of rabbits. This was indicating the formation of the thrombus. The rabbits in test groups were given 200, 300 and 600µg/kg of the different concentrations of the extract. The positive control group was given 600µg/kg dose of urokinase (that acts via activation of plasminogen). After 30 min of induction of $FeCl_3$ solution, then the length of the clot region was measured. Then after 24h the percentage of thrombus lysis after administration of thrombolytic agents and test drug was calculated using this formula:

% of thrombus = 100/(Thrombus length in control rabbits × Thrombus length in treated rabbits)

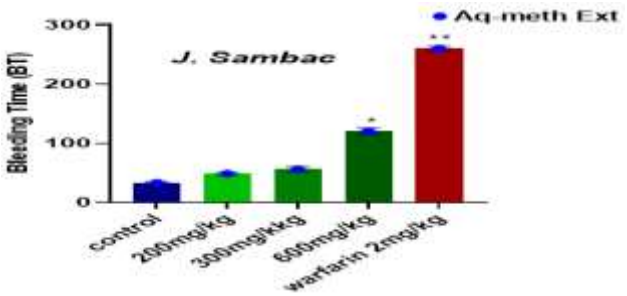

Fig. 9: Effect of *Jasminum sambac* (L.) on the Bleeding Time (BT).

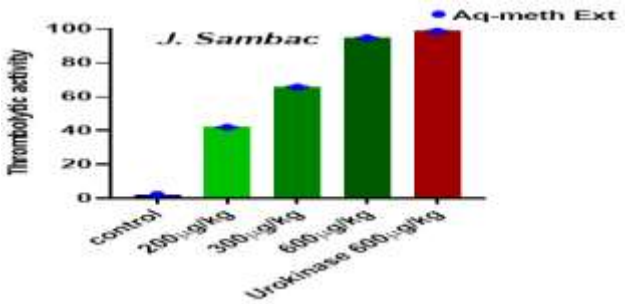

Fig. 10: Effect of *Jasminum sambac* (L.) on Thrombolytic Activity.

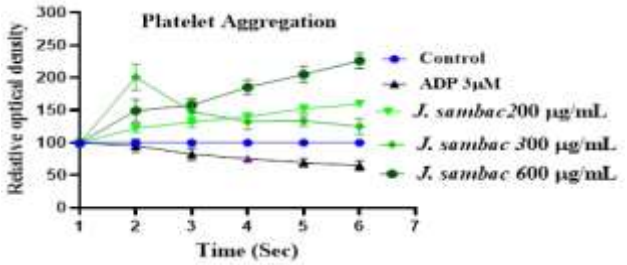

Fig. 11: Effect of Aqueous-methanolic Extract of *Jasminum sambac* (L.) on Platelet Aggregation induced by ADP.

Platelet aggregation assay

Blood was collected from anaesthetized rabbits through the abdominal aorta in sodium citrate tubes. Platelet rich plasma (PRP) was separated by centrifugation of the blood at 800 rpm for 8 min and then platelet poor plasma (PPP) was further obtained by centrifugation at 3000 rpm for 10 min. The number in PRP was adjusted to 3×10^8 platelets/ml.

Then platelet aggregator agent was added to the plasma (0.3mL) i.e. ADP (3µM)/thrombin (0.91 U/ml) (0.03mL). The aggregation was recorded for 5 min using spectrophotometer at 600 nm and then the difference in % transmission was considered as end point. Moreover, the test was conducted within 3h of the blood withdrawal so that they might not become inactivated. The effect of plant extract was expressed as % inhibition (Jagtap *et al.*, 2012).

RESULTS

The results were communicated as mean ± SEM and investigated using one-way analysis variance (ANOVA) followed by Dunnett's t-test, 95% confidence interval $p<0.05$ considered significant. Preliminary Phytochemical screening. The Preliminary phytochemical screening of *Jasminum sambac* (L.) showed the presence of saponins, coumarins, phenols and flavonoids.

HPLC analysis

The analysis of the High Power Liquid Chromatography (HPLC) publicized so many phytoconstituents in changing concentrations, where utmost communal phytoconstituent is isoquercetin.

Acute oral toxicity testing of jasminum sambac (L.) extract

No toxicity profile was observed even at 2500mg/kg.

Effect of aqueous-methanolic extract on the In-vitro studies

Aqueous-methanolic extract of *Jasminum sambac* (L.) showed significant ($p<0.05$) dose-dependent increase in the prothrombin time (PT), activated partial thromboplastin time (APTT), clotting time (CT) as shown in figs. 2, 3 and 4. Moreover, the extract showed significant ($p<0.05$) clot lysis (fig. 5).

The impact of In-vivo studies on different coagulation Parameters and thrombolysis

The Aqueous-methanolic extract of *Jasminum sambac* (L.) significantly showed the increase in prothrombin time (PT), activated partial thromboplastin time (APTT), clotting time (CT) and bleeding time (BT) dose-dependently (fig. 6, 7, 8, 9) and also showed clot lysis significantly ($p<0.05$) (fig. 10).

Effect on platelet aggregation

The Aqueuous-Methanolic extract of *Jasminum sambac* (L.) showed significant platelet aggregation dose dependently induced by ADP (fig.11).

DISCUSSION

Thromboembolic diseases significantly contribute to adverse outcomes. Most of the synthetic pharmaceuticals have long-term downsides, it is crucial to be aware about the use of naturally existing compounds for therapeutic purposes. Pharmaceutically active components are a foundation of many plants that are exploited as therapeutic agents (Banjare and Paul, 2014).

This study was conducted to examine the anticlotting and thrombolytic performance of *Jasminum sambac* (L.) Since, in contrast to other constituents the salicylic acid is also abundant in it. Numerous studies have reported that salicylic acid seems to have a remarkable anticoagulant property (Kalhor and Taghikhani, 2021, Gemcioglu et al., 2020). Salicylic acid is derivative acquired from the metabolism of salicin. Molecularly, it is analogous to aspirin (acetylsalicylic acid). Salicylates are believed to be the compounds of salicylic acid. Salicylic acid is freely accessible as a powder form that melts at 157-159ºC. It is soluble in water, but it becomes notably highly soluble in polar organic solvents. It is used substantially in the cure of numerous ailments. Methylsalicylate is exploited in so many pharmaceutical procedures, consumables and flavorings. Aspirin has a wide range of physiological impacts, but somehow it primarily benefits in analgesia, the suppression of inflammation, and the inhibition of thromboembolism (Ekinci *et al.,* 2011). A natively endogenous flavonoid called isoquercetin can also be found in different parts of various plants, like fruits, shrubs, and veggies (Zhang *et al.,* 2011). The accessibility of isoquercetin is way more than quercetin, and thus the conjugation of several glucose fractions defies augmented hydrophilicity (Paulke *et al.,* 2012). Isoquercetin may also be beneficial for the cure of neuro-inflammatory maladies such multiple sclerosis and Parkinson's disease. Isoquercetin drops blood pressure by suppressing the angiotensin-converting enzyme (Junior *et al.,* 2011). Isoquercetin also has anti-viral properties that have been reported against the influenza virus (Kim *et al.,* 2010). The tumor-exploitive activity of isoquercetin has been reported (Fujii et al., 2013). The rutin has anti-oxidant properties and also shows anti-thrombotic activity (Sharma et al., 2013).

According to proposed mechanism, this activity of plant might be due to the action of salicylic acid on the inhibition of thromboxane A_2 which further leads to the inhibition of platelet aggregation and blood clotting. Moreover, it might be due to action of salicylic acid, rutin and isoquercetin on the different clotting factors. This study showed that aqueous-methanolic extract of *J.sambac* has a significant increment in different coagulation parameters like CT, PT and APTT *in vitro* experiments along with BT *in vivo* experiments, when compare with warfarin as positive control and distilled water as negative control. Flavonoids and other substances present in it as isoquercetin have the ability against platelet aggregation (Okwu and Ezenagu, 2008). In this study, HPLC has shown that salicylic acid, rutin and isoquercetin are also present in aqueous-methanolic extract of *J. sambac*. So it is considered that extract exerts anti-coagulant activity may be due to the presence of salicylic acid, rutin and isoquercetin acting on the mechanisms.

CONCLUSION

Results of this study shown that *Jasminum sambac* (L.) exhibited a significant increase in prothrombin, clotting

time and activated partial thromboplastin time in *in-vivo* activity whereas a significant escalation in clotting time, bleeding time, activated partial thromboplastin time and prothrombin time in a dose-dependent means in rabbits after one week of treatment. The aqueous-methanolic extract of *Jasminum sambac* showed a significant clot lysis in a dose-dependent manner. These may be due to the presence of phytochemical components such as rutin, salicylic acid and isoquercetin in the aerial part of *Jasminum sambac* extract. Hence it can be used for medicinal and prophylactic purposes in the treatment of cardiovascular diseases in future.

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