Qualitative phytochemical screening and in vitro thrombolytic activity of Capparis decidua Edgew. Fruit

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Abstract

Abnormal thrombosis plays an important role in development of ischemic heart disease and stroke. Synthetic thrombolytic agents are effective but possess some adverse effects. Therefore, the need for development of comparatively safer anti-thrombotic drugs from natural resources such as plants arises. Capparis decidua Edgew. (Family – Capparaceae) is a densely branching, xerophytic shrub and grows abundantly in arid and semiarid regions. Its fruits are major ingredient of the popular 'Panchkuta' vegetable of Rajasthan and also used to prepare pickle. Caper fruits are recommended for treatment of several diseases in traditional medicine including cardiac ailments and shown antioxidant and hypolipidemic potential in scientific studies and therefore, fruits of C. decidua were evaluated for their in vitro thrombolytic potential for the first time. Preliminary qualitative phytochemical screening of fruits of C. decidua; purchased from local market of Udaipur has shown the presence of flavonoids, terpenoids, phenol, phlobatimin, amino acids and carbohydrates and absence of saponin, tannins, steroids and cardiac glycosides. A significant percent clot lysis activity of 23.16±1.26 and 32.39±2.10 was exhibited by methanolic extracts (ME-I and ME-II) of fruits of C. decidua respectively as compared to the positive control streptokinase and negative control as distilled water. However, characterization of bioactive anti-thrombotic molecules as well as large scale, clinical studies is warranted to establish in vivo thrombolytic efficacy of its fruits. Thrombolytic potential of Caper berries as observed in the present study could be useful to recommend its consumption as a dietary health supplement in order to prevent from thrombotic cardiovascular diseases.

Keywords: Cardiovascular Diseases; Clot Lysis; Platelet Aggregation; Stachydrine; Streptokinase

1. Introduction

Thrombus is a blood clot, which forms as a natural physiological response to injury of the blood vessels. However, abnormal formation of thrombus without any intravascular damage can cause problems of heart attack, stroke, etc. Abnormal thrombus formation leads to interferences in blood circulation and partial or complete block of blood supply to any body part may result in catastrophic consequences. Ischemia is one of the most common examples of thrombus abnormality. Altered thrombus formation is one of the major causes of cardiovascular diseases (CVD). Fibrin, thrombin and platelets play an important role in formation of thrombus. Platelet aggregation inhibition, fibrinolysis and plasminogen activator activities are required for lysis of thrombus [1]. There are some well-known thrombolytic agents such as Streptokinase (SK), Urokinase and recombinant tissue Plasminogen activator etc. but having safety issues [2]. In this context, plants are always considered safe and effective with comparative fewer side effects.

Capparis decidua Edgew.; member of family – Capparaceae is a densely branching, xerophytic, climbing shrub. It grows mainly in arid and semi-arid regions and widely distributed in tropical and subtropical regions of Chad, Egypt, Ethiopia,
Iran, Jordan, Mauritania, Nigeria, Arabia, India, Pakistan, etc. It is known by different names such as *Karira*, *Kariramu*, *Sengam*, *Karil*, *Kair*, *Bare Caper* etc. in various languages [3-5]. Immature fruits of Caper plant are edible and mainly consumed as pickle and vegetable. It is one of the main ingredients of traditional popular Rajasthani cuisine ‘Panchkuta’ which is a mixture of five dried plant species and especially prepared during traditional ‘Basoda festival’ in Rajasthan, India [6]. Various parts of *C. decidua* are used for treatment of several diseases such as asthma, body pain, body fracture, cardiac trouble, cough, cholera, digestive disorders, dysentery, earache, eczema, intermittent fever, migraine, muscular injury, toothache, typhoid, rheumatism in both codified and non-codified systems of medicine [7-9]. The plant also possess immense pharmacological potential as demonstrated in various animal and clinical studies like antioxidant, anti-diabetic, anti-inflammatory, analgesic, anticancer, hepatoprotective, hypolipidemic, anti-atherosclerotic, antihypertensive, antiinfective, antimicrobial, anti-nociceptive, antirheumatic, anticonvulsant activities etc. [10].

There are many plant species including fruits and vegetables which have demonstrated antithrombotic, antiplatelet and anticoagulant potential in scientific studies and can be utilized to control risk factors that are responsible for development of cardiovascular diseases [11-13]. Moreover, plants as dietary supplement in the form of nutraceuticals are in demand due to their safety and cost effectiveness. In view of nutritional and pharmacological potential of *C. decidua* as well as its recommendation for cardiac troubles in traditional medicine, its fruits were evaluated for *in vitro* thrombolytic potential for the first time.

2. Material and methods

2.1. Plant material

Fruits of *C. decidua* were purchased from Sainani dry fruits shop at Surajpol, Udaipur, Rajasthan and authenticated at Raw Material, Herbarium and Museum, New Delhi (RHMD), NISCAIR, India (Ref. No.- NISCAIR/RHMD/Consult/2020/3761-62). Fruits were washed under running tap water; air-dried under the shade at room temperature and powdered.

2.1.1. Preparation of Plant extracts

Following extracts were prepared from the powdered plant material to carry out phytochemical analysis and evaluation of thrombolytic potential:

- Methanolic Extract - I (ME-I)
  Fifty ml methanol was used to soak 5 g dried powder of fruits of *C. decidua* for 24 hours at room temperature with occasional stirring and filtered with Whatman’s filter paper no. 1. This process was repeated for three times with 50ml of methanol and the pooled filtrate was evaporated on boiling water bath at 40°C. Final extract yield of 20% was stored in sterile glass petri plate at 4°C in refrigerator. This extract was named as ME-I and used for qualitative phytochemical analysis (wherever required) as well as for preliminary *in vitro* clot lysis assessment as described earlier [14].

- Methanolic Extract - II (ME-II)
  One hundred gram dried fruit powder of *C. decidua* was soaked in 500 ml methanol for eight days with occasional stirring and then filtered with Whatman’s filter paper no. 1. The filtrate was evaporated on boiling water bath at 40°C and a yield of 23.74% was obtained. The concentrated extract was stored in sterile glass petri plate at 4°C in refrigerator and named as ME-II. This extract was used for evaluation of *in vitro* clot lysis activity.

- Aqueous extract
  Twenty ml distilled water was used to soak 400mg dried powder of *C. decidua* fruits and it was boiled for 20 minutes. After boiling, it was filtered with Whatman’s filter paper no. 1 and used for preliminary qualitative phytochemical analysis and always prepared fresh.

2.2. Qualitative phytochemical screening

Aqueous/ME-I or dried fruit powder of *C. decidua* were used for qualitative phytochemical screening of amino acids, carbohydrates, terpenoids, steroids, cardiac glycosides, phlobatannins, flavonoids, phenols, tannins, and saponins as required [14-16].
2.3. Evaluation of *in vitro* thrombolytic activity [17]

2.3.1. Ethical approval

Institutional ethical approval was sought out to perform the study (Ref.PMU/PMCH/IEC/2019, dated 26.12.2019). Written informed consent was also taken from all the study participants before collecting the blood samples.

2.3.2. Collection of blood samples

*In vitro* thrombolytic activity of ME-I and ME-II was evaluated in blood samples of ten healthy volunteers each in aseptic conditions. Persons who were receiving medication for any disease, or taking oral contraceptives or anticoagulant therapy were not included in the study.

2.3.3. Plant extract preparation

Fresh plant extracts were always prepared for conducting *in vitro* evaluation of thrombolytic activity. For this purpose, 10 mg/ml concentration of both ME-I and ME-II was prepared by suspending 100 mg crude extract in 10 ml sterile distilled water after shaking vigorously on vortex mixture and keeping it for overnight. Filtration of the extract was carried out next day using syringe filter having 0.22µ pore size for removal of any microbial contamination and 100 µl (1 mg/ml) of these extracts was used for the final evaluation of clot lysis activity.

2.3.4. Preparation of Streptokinase as standard drug

Lyophilized SK of 15, 00,000 IU (available commercially as STPase manufactured by Cadila Pharmaceuticals, Ahmedabad, India) was purchased; dissolved in five ml of aseptic distilled water and mixed thoroughly. One hundred micro liters (30,000 IU) of this was used for the test purpose as a positive control.

2.3.5. Clot preparation and evaluation of *in vitro* clot lysis activity

Fasting blood samples (10 ml) were taken from healthy volunteers as per the study protocol and 500 µl was poured in pre-weighed sterile micro-centrifuge tubes. Clot was prepared after incubating the tubes at 37ºC for 45 min. Then, to remove the serum, tubes were centrifuged at 2000 rpm for 10 min and after removal, again weighed to determine the weight of clot (Clot weight = weight of micro-centrifuge tube having clot – weight of micro-centrifuge tube). One hundred µl of plant extracts, either ME-I or ME-II; 100 µl of sterile distilled water (negative control) and 100 µl of SK (positive control) were added to micro-centrifuge tubes. After incubating the tubes at 37ºC for 90 min, fluid released after clot lysis was taken out carefully from the tube with the help of micropipette and tubes were again weighed to determine the weight of clot after lysis by subtracting weight of micro-centrifuge tube having clot from the weight of micro-centrifuge tube after clot lysis. Percent clot lysis was determined as: weight of clot after lysis / weight of clot × 100.

2.4. Statistical analysis

Results are expressed as Mean ± standard error of the mean (SEM) for three replicates and statistical comparisons were performed with Student's Paired t-test using Microsoft Excel (2010). Differences between means were considered to be significant when p value was < 0.01.

3. Results

A preliminary qualitative phytochemical analysis of fruits of *C. decidua*; purchased from Udaipur, Rajasthan has shown presence of carbohydrate, amino acids, terpenoid, flavonoid, phenols and phlobatanin. However, saponin, tannins, steroids and cardiac glycosides were not found to be present in the fruit sample (Table 1).

Dried fruits of *C. decidua* have shown to possess significant *in vitro* clot lysis potential in the present study as compared to distilled water as a negative control and SK as positive control for the first time (Table 2). Preliminary evaluation of *in vitro* thrombolytic activity of ME-I having the final concentration of 1 mg/ml demonstrated 23.16±1.26% *in vitro* clot lysis activity as compared to highest clot lysis activity (51.60±0.77%) of 100 µl SK (30,000 IU). The second methanolic extract (ME-II) also demonstrated a significant (p<0.0001) *in vitro* clot lysis activity of 32.39±2.10% as compared to positive control SK which exhibited 54.45±0.79% clot lysis activity in a concentration of 1 mg/ml. Distilled water as negative control has shown negligible clot lysis activity of 4.08±0.35% and 4.00±0.44%; depicting no role of water in dissolving the clot. Interestingly, a comparison of efficacy of both the methanolic extracts on thrombolysis was also done which was statistically insignificant (p=0.01) indicating that there was no effect of using two different techniques for preparing methanolic extracts on the thrombolytic efficacy of plant.
Table 1 Qualitative phytochemical analysis of fruits of *Capparis decidua*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemical Test</th>
<th><em>C. decidua</em> fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saponin</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavanoid</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Phenol</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Phlobatanin</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Terpenoid</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Cardiac glycoside</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Amino acid</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Tannin</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Steroid</td>
<td>-</td>
</tr>
</tbody>
</table>

* + Present, - Absent

Table 2 *In vitro* percent clot lysis activity of methanolic extracts (ME-I and ME-II) of *Capparis decidua* fruits (n=10)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant extract</th>
<th>Percent clot lysis (Mean ±SE)</th>
<th>Plant extract (I)</th>
<th>Streptokinase (II)</th>
<th>Distilled water (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ME-I</td>
<td>23.16±1.26</td>
<td>51.60±0.77</td>
<td>4.08±0.35</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ME-II</td>
<td>32.39±2.10</td>
<td>54.45±0.79</td>
<td>4.00±0.44</td>
<td></td>
</tr>
</tbody>
</table>

*p value:* a-I v/s II; b- I v/s III; p<0.0001; c- II v/s III; p<0.0001; Values are expressed as Mean ± SEM

4. Discussion

Plants are rich source of various phyto-pharmaceutical compounds. Some plants possess both nutritive and therapeutic efficacy and explored as nutraceuticals. Reduced thrombolysis is one of the major causes behind ischemic heart disease and in this regard, taking nutritive plants in daily diets having thrombolytic efficacy could be a very healthy alternative [14, 18]. In the present study, secondary metabolites such as terpenoid, flavonoid, phenols and phlobatanin were found to be present in the fruits of *C. decidua* that were purchased from Udaipur, India (Table 1). However, absence of tannins and steroids was not consistent as compared with other studies and this difference might be due to environmental, seasonal and maturity variations under which the fruit sample was collected and stored by the seller or divergence in extraction method [19,20]. This further highlights that market samples of Caper fruits should be scientifically certified after both qualitative and quantitative phytochemical analysis.

In addition to the presence of phytochemicals, *C. decidua* fruits were also found to possess thrombolytic efficacy as shown in Table 2. Several plant species have been screened for *in vitro* thrombolytic potential [13,21] and some of the plants have shown similar *in vitro* percent thrombolytic potential for example, 32.58% clot lysis activity by leaves of *Leea indica* [22], 31.61% by leaves of *Senna sophora* and 31.51% fruits of *Solanum torvum* [23], 23.11% by chloroform extract of fruits of *Ficus glomerata* [24], 30.17% and 22.53% clot lysis by bark and seeds of *Tamarindus indica* respectively [25], 32.94% by seeds of *Sesamum indicum* [26], etc.

Many plants and their bioactive compounds having antioxidant and anti-inflammatory potential also exhibit anticoagulant effects [27]. Various parts of *C. decidua* are also reported to be rich in many phyto-constituents such as flavanoids, alkaloids, phenols, terpenoids, carbohydrates, protein, crude fiber and minerals such as Na, Ca, P, Fe, Zn, Cu, Mg, and compounds like capparine, cappariline, capparisine, capparisinine, capparidisine, cadabicine, stachydrine, codonocarpine, isocodonocarpine etc. [10].
Fruits of *C. decidua* are rich in phenolic and flavonoid compounds [28]. A recent study has shown presence of Total Phenol Content of 38.25 ± 1.04 mg gallic acid equivalent/g of methanolic extract of its fruits on dry weight basis and Total Flavonoid Content of 18.58 ± 0.18 mg quercetin equivalent/g on dry weight basis. Besides, flavanoids such as quercetin and rutin have also been isolated from fruits which have shown to possess antioxidant, antiplatelet and antithrombotic potential [19,29-31]. Moreover, fruits of *C. decidua* contain 90 mg/100 g calcium, 120 mg/100 g ascorbic acid and 5.4 mg/100 g beta-carotene [32,33]. Vitamin C has shown to improve conditions of metabolic syndrome mainly because of its antioxidant and anti-inflammatory properties [34] and could also play a significant role in the case of thrombolytic action of Caper fruits. Similarly, carotenoids are reported to possess antioxidant potential and free radical scavenging mechanism is an important mechanism to prevent cardiovascular diseases [35]. Carotene (210 mg/kg) is another constituent of Caper fruits and fruit husk [36] and could be behind the thrombolytic action of fruits of *C. decidua*.

The *in vitro* thrombolytic potential of fruits of *C. decidua* may also be because of the presence of stachydrine. Stachydrine is an alkaloid, isolated from dried fruits of *C. decidua* and exhibited protective role against cerebral ischemia reperfusion injury [37] and cardiac hypertrophy by attenuating the oxidative stress and inhibition of the expressions of phosphorylated IκBα, NF-κB p65, JAK2 and STAT3 in isoproterenol-induced CH rats [38]. In another study, stachydrine has shown to inhibit the deleterious effect of high-glucose on endothelial cells by modulating SIRT1 pathway and thereby, reducing endothelial dysfunction and vascular complications [39].

Platelet aggregation inhibition activity is considered helpful in prevention of cardiovascular diseases by maintaining the patency of vessels. In this regard, antiplatelet activity of two sesquiterpene lactones, namely, Germacr-3βol-7,9-dien-6,14-olide-15-oic acid and Germacr-3β-ol-12-ene-6,14-olide-15-oic acid isolated from aerial parts of *C. decidua* is a positive finding [40]. Blood pressure lowering effect of aerial parts of *C. decidua* in the concentration of 3–100 mg/kg was also observed through cardiac depressant and vasodilator effect in an animal study by Shah and Gilani [41].

N-pentacosane, β-sitosterol and β-carotene has also been reported from seeds of *C. decidua* [42] and seeds are also rich in oleic acid (57.2%), palmitic acid (21.1%) and linoleic acid (11.4%) [10]. In some studies, oleic acid has been shown to reduce the risk of cardiovascular diseases [43] and in this regard, presence of oleic acid in seeds of Caper plant keeps significance. Glucocapparin is also present in the seeds of *C. decidua* [44] which has demonstrated anti-hyperglycemic potential [45]. However, it is to be determined that the thrombolytic potential of *C. decidua* fruits is due to the synergistic effect of various phytoconstituents or due to particular bioactive compound.

Abnormal lipid profile and coagulation parameters, high blood sugar, obesity etc. are key factors behind development of CVD [46]. Notably, fruits of *C. decidua* have also shown to possess hypolipidemic potential in streptozotocin induced diabetic rats in a dose of 500 mg/kg body weight. It significantly decreased total cholesterol, triglycerides and LDL-cholesterol levels as well as increased HDL-cholesterol levels [47]. Hypoglycemic activity of its fruit powder has also been demonstrated in alloxan induced diabetic rats [48]. Along with hypolipidemic, hypoglycemic and antioxidant potential, thrombolytic activity of *C. decidua* is an added advantage and therefore, it could be recommended as a dietary nutraceutical as its consumption could be helpful in prevention of CVD associated disorders. However, detailed clinical studies with large number of study subjects are required to validate its thrombolytic potential along with evaluation of the corresponding bioactive molecules responsible for clot lysis potential. Further, demand for its fruits could be fulfilled by large scale cultivation which will be useful for socio-economic development of people dwelling in arid and semi-arid regions.

### 5. Conclusion

Coronary athero-thrombotic heart disease is an impact of several factors among which abnormal thrombosis is one. Due to high cost and associated side effects, synthetic thrombolytic agents are questioned. In this regard, plant based thrombolytic agents provide hope. *C. decidua* is a xerophytic shrub having edible fruits. The plant is recommended in traditional medicine for treatment of various human diseases including cardiac ailments. A significant *in vitro* thrombolytic activity of methanolic extract of *C. decidua* fruits was observed in the present study for the first time. Fruits are rich in phenolic compounds, flavanoids, alkaloids, carotenes, ascorbic acid etc. which might be responsible for its thrombolytic action. It also possesses hypolipidemic, hypoglycemic and antioxidant potential and could be thus, put forward for development of an effective nutraceutical to be included as dietary component for prevention of cardiovascular diseases.
Compliance with ethical standards

Acknowledgments

Authors are thankful to Raw Material, Herbarium and Museum, NISCAIR, New Delhi (RHMD), India for authentication of the plant part.

Disclosure of conflict of interest

Authors hereby declare that there is no conflict of interest in this article.

Statement of ethical approval

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional ethical board of Pacific Medical College & Hospitals, Udaipur (Ref.PMU/PMCH/IEC/2019, dated 26.12.2019).

Statement of informed consent

Written informed consent was obtained from all the study participants before taking the blood samples.

References


