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Original Article

In Vitro study of Thrombolytic activity from the different parts of Carica papaya plant on COVID-19 patients.

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ABSTRACT

Carica papaya plant has been used for medicinal purpose throughout the world because it have anti-inflammatory, anti-oxidant, antiviral, anti-cancer and wound healing like properties. **Objective:** To evaluate the thrombolytic activity of Carica papaya plant in normal healthy individuals and COVID-19 patients to determine either parts of the plant (roots, seeds and leaves) exhibit more activity in normal persons or in COVID-19 patients. **Methods:** For this study, total 20 blood samples were taken, 10 for normal individuals and 10 for positive COVID-19 patients. We used two different solvents i.e. autoclaved distilled water and concentrated methanol to prepare the 10% root, seed and leaf extracts of Carica papaya plant. For the thrombolytic activity of these plant extracts, samples were arranged as a triplicate for the accuracy of results percentage. **Results:** Our results evaluated that in normal individuals, distilled water root extract and methanolic seed extract exhibits maximum thrombolytic activity. The mean value with distilled water (root extract) and methanol (seed extract) is 36.9% and 32.9% respectively. While in covid-19 patients, distilled water leaf extract and methanolic leaf extract reveal maximum thrombolytic activity. In patients, the mean value with distilled water and methanol (leaf extract) is 21.8% and 23% respectively. **Conclusions:** In our study, we have observed that normal persons showed highly significant results as compared to COVID-19 patients. Because in COVID-19 disease, plasminogen activator inhibitor.
1 (PAI-1), thrombin activatable fibrinolysis inhibitor and tissue plasminogen activator (tPA) factors elevate which results in hypo-fibrinolysis.

INTRODUCTION

Carica papaya is a genus of Linn that is a member of Caricaceae family [1, 2]. According to phytochemical study, flavonoids, anthraquinones, Saponins, phlobatinins, cardiac glycosides anthocyanosides, alkaloids, tannins and phenols are present in the C. papaya plant in which various bioactive compounds also exhibit the thrombolytic action [3, 6]. The Arachidonate 12-lipoxygenase (ALOX 12), also known as the Platelet-type Lipoxygenase, and the Platelet-Activating Factor Receptor (PTAFR) are two genes that shows thrombolytic activity in Carica papaya plant [7, 9]. The seventh human corona virus, severe acute respiratory syndrome corona virus 2 (SARS CoV-2), was found in Wuhan, Hubei Province, China, during a recent pneumonia epidemic in January 2020, which has a natural zoonotic origin [10, 12]. More than one-third of people with COVID-19 who are very sick have dangerously high blood clotting levels [13].

According to recent reports, a hypothesis stated as that, in endothelium of blood vessels, SARS-CoV 2 infection (co-expression and binding of the spike protein with the ACE2) or virus mediated inflammatory response caused internal injury that result in the constriction of vessels and initiate coagulation & blood clotting pathways [14]. The main objective of this research work was to evaluate the therapeutic properties of Carica papaya plant in COVID-19 patients as well as in normal individuals. Plants have been employed for therapeutic reasons against a range of diseases since the dawn of human civilization [15, 16]. So that's why we design this research project to evaluate that how the extract of this medicinal plant will be helpful for the lysis of clot in COVID-19 patients and which percentage will be more effective for suffering patients.

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METHODS

Collection of Plant:

Plant of Carica papaya that belongs to family of Caricaceae was collected from a local nursery of Lahore, Pakistan during the month of July 2021.
The study was permitted on 26-06-2021 (Ref No: IRB-UOL-FAHS/890-VI/2021) by the institutional Ethics Committee and was in accordance with the declaration of World Medical Association (WMA) made at Helsinki (2013).

Preparation of Parts of Plant Extract:

10% aqueous extract of different parts (roots, seeds, and leaves) of Carica Papaya plant were formed with two distinct solvents e.g. autoclave distilled water and concentrated methanol as per the previous reported protocol [17]. But the novel thing is that we use the two different timings of incubation (Overnight & 15 minutes) for the preparation of extract and for the comparison of results.

Collection of samples:

Blood samples were collected from university of Lahore teaching hospital, Lahore. As per inclusion criteria, the total number of samples was 20, in which 10 samples for normal healthy individuals and 10 for COVID-19 patients. The samples of COVID-19 patients were confirmed through PCR technique and 10ml whole blood was drawn from each individual.

Procedure of thrombolytic activity:

The obtained sample of each COVID-19 patient and normal individuals were divided and added as 0.5mlinto eppendroffs for replicates. The activity was done through 10% autoclaved distilled water and concentrated 10%methanolic extract of leaf, root and seed of Carica papaya on the replicates of blood samples, in which streptokinase (1.5MIU) use as a positive control. (As per manufacturer's instruction, streptokinase solution was prepared by adding normal saline) [5].

Calculation:

Percentage of clot lysis = (weight of released clot / clot weight) × 100 = (WR / WC) × 100 [17].

Data analysis:

Nonrandom sampling technique was used for the collection of samples and data was analyzed by applying independent sample T test through statistical package for social sciences (SPSS) software.

RESULTS

Carica papaya plant proved to be very beneficial to health by reducing the risk of heart diseases, diabetes, cancer and also lower blood pressure and improve wound healing. Different components of Carica papaya plant such as root, seed and leaves exhibits thrombolytic, wound healing and antiplatelet properties [18].

Thrombus formation in blood vessels obstructs blood flow via the circulatory system, resulting in hypertension, heart attack, anoxia, and other complications [19]. In COVID-19 patient blood clotting occur due to internal injury of endothelium blood vessels [14].

We used multiple solvents for the thrombolytic activity but concentrated methanol and autoclaved distilled water gave significant results, therefore we further precede our experiment with these two solvents. Concentrated methanol and distilled water extracts of roots, seeds and leaves of Carica papaya filtered by muslin cloth as well as whatmann filter paper 1. These extracts were screened against clot.
We calculated the values of clot lysis as mean and expressed them as percentage. The statistical values of mean and standard deviation of all clot lysis results were evaluated by independent sample t-test. Following are the Tables (1 and 2) that show the comparison between normal individuals and covid-19 patients by using solvents (distilled water and methanol) based on independent sample t- test. P-value <0.05 was considered as statistically significant.

	Normal		Covid-19		P-Value
	Mean	SD	Mean	SD	
Papaya leaf	36	9.14	21.8	12.41	0.009
PapayaRoots	36.9	13.48	20.8	8.904	0.006
Papaya Seeds	36.5	14.23	21.4	11.39	0.01
Streptokinase	41.9	20.38	27.9	21.97	0.15

Table No. 1: Comparison between normal individuals and covid-19 patients using distilled water (solvent).

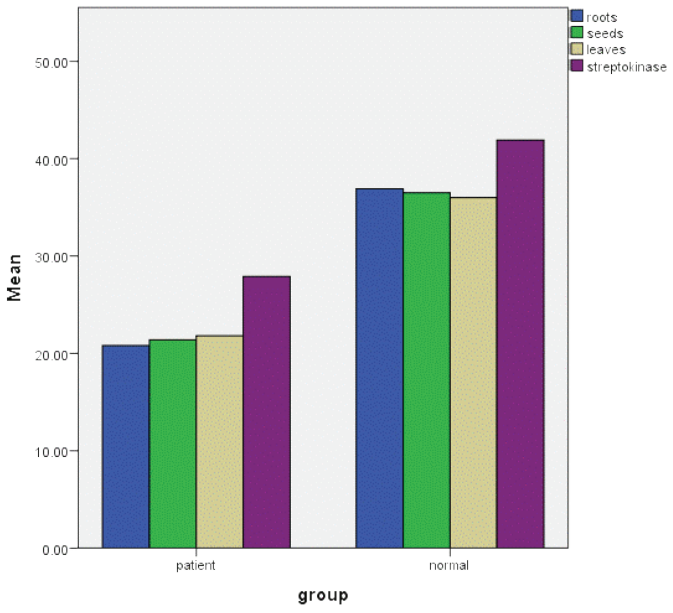

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Figure 1: This graph shows the comparison between clot lysis% of normal healthy individuals and covid-19 patients by using aqueous preparation (distilled water) of Carica papaya parts extract.

When we added 100ul of Carica papaya aqueous preparation (distilled water) of roots, seeds and leaves extract in replicates of different blood clotted eppendroffs of normal individuals, it showed 36.9%, 36.5% and 36.00% clot lysis respectively. Addition of 100ul streptokinase to pre-weighed blood clots of 10 normal individuals showed 41.9% lysis. Among all three extracts, distilled water root extract exhibit maximum thrombolytic activity in normal individuals. The order of thrombolytic activity of various parts of papaya in normal individuals was: Root > seed >leaf .Whereas, addition of 100ul roots, seeds and leaves extract to 10 clotted blood eppendroffs of COVID-19 patients showed 20.8%, 21.4% and 21.8 clot lysis respectively. Streptokinase as a positive control showed 27.9% clot lysis. In COVID-19 patients, distilled water leaves extract exhibits maximum thrombolytic activity. P-value of papaya roots, seeds and leaves (<0.05) was highly significant.

Table No. 2: Comparison between normal individuals and covid-19 patients by using methanol (solvent).

	Normal		Covid-19		P-Value
	Mean	SD	Mean	SD	
Papaya leaf	29.7	13.96	23	12.49	0.273
PapayaRoots	31.4	12.28	20.4	7.96	0.02
Papaya Seeds	32.9	15.1	18.4	7.29	0.01
Streptokinase	38.2	14.55	27.6	11.83	0.09

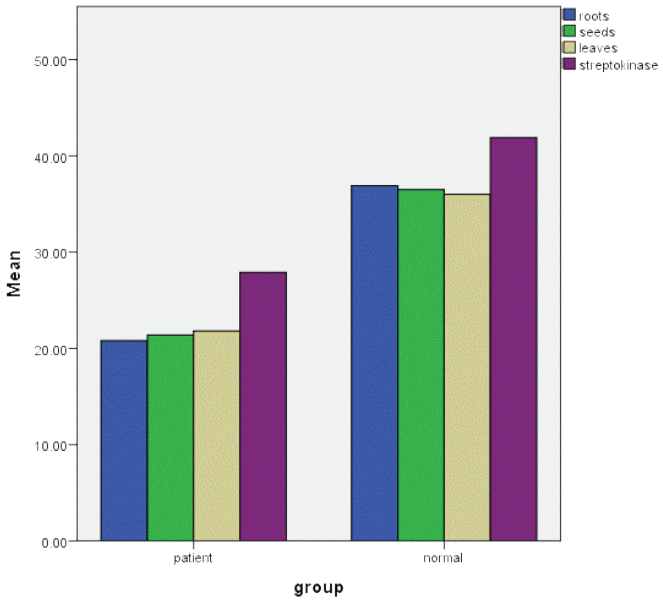

Figure 2: This graph shows the comparison between clot lysis% of normal healthy individuals and covid-19 patients by using methanolic extract of Carica papaya parts.

Addition of 100ul methanolic roots seeds and leaves extract to samples of normal persons showed 31.4%, 32.9% and 29.7% clot lysis respectively with 38.2% lysis of streptokinase. In normal persons, methanolic seed extract showed maximum thrombolytic activity. Similarly, by treating the blood sample of COVID-19 patients with 100ul methanolic roots, seed and leaves extracts, 20.4%, 18.4% and 23.00% clot lysis respectively observed. Mean value of positive control (streptokinase) was 27.6%. In COVID-19 patients, methanolic leaf extract showed maximum thrombolytic activity. P-value of papaya roots and seeds (<0.05) was significant.

DISCUSSION

For the first time, COVID-19 blood samples were taken to evaluate the thrombolytic activity through the different parts of Carica papaya extract. Fresh different parts (roots, seeds and leaves) of the plant were collected and washed properly. After washing, different parts were shaded dry for 15 days. Kalaiyarasi and Mubeen reported in-vitro study of thrombolytic activity in 2014 by using aqueous preparation of different parts of Carica papaya plant extract. They collected blood samples from 10 normal healthy volunteers and prepared extract with distilled water. In their study, they performed experiment with fresh extract to evaluate thrombolytic activity [5]. In another previous study, Sarker MM and his colleagues reported in 2021, that Carica papaya leaves inhibit thrombus formation in dengue patients [20].

In present study, we determined thrombolytic activity in healthy individuals and COVID-19 patients. For this purpose, the powdered form of roots seeds and leaves of Carica papaya were taken and extracts were prepared. These extracts were made by using two different solvents i.e. autoclaved distilled water and concentrated methanol. We used two different protocols (overnight incubation and fresh extract) to analyze the thrombolytic activity of Carica papaya parts. We used streptokinase as a positive control because it is a standard thrombolytic agent as in 2017, Tabassum; Chandi et.al also used streptokinase as a control for in vitro thrombolysis study [6]. In our study, we performed experiments with different solvents that were acetone, n-hexane, chloroform, methanol and distilled water as Vimal Singh and his colleagues conducted a report in 2017, which showed the preparation of papaya leaf extract with these solvents (hexane, acetone and distilled water) to studying the effect of extract on shelf life of platelets. Highest concentration of hexane showed better results in reducing platelet storage lesions [21]. In another study, Yusha'u M and his colleagues reported the preparation of leaf extract with chloroform to evaluate that Carica papaya used for the production of medicine to treat urinary tract infections (UTIs) [22]. On normal healthy volunteer, acetone showed 0.8% clot lysis with 8% leaf extract because it cannot properly penetrate in blood clot. Chloroform showed 10.0% clot lysis with 9% leaf

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extract, N-hexane 10.7% lysis with 10% leaf extract, methanol 18.8% lysis with 10% leaf extract and distilled water gave 24.4% clot lysis with 10% leaves extract. Concentrated methanol and autoclaved distilled water revealed better clot lysis. We also performed experiment on one sample with overnight incubation and fresh extract prepared by these two solvents (distilled water and methanol) to compare the difference. After overnight incubation, distilled water showed 21.6% clot lysis and methanol showed 14.8% lysis and after fresh extract distilled water showed 25% lysis and methanol showed 18.8% lysis. So we preceded further samples with these two solvents. Concentrated methanol used for the first time to prepare the extracts of different parts of Carica papaya (roots, leaves and seeds) in Pakistan. COVID-19 disease is associated with elevated levels of plasminogen activator inhibitor 1 (PAI-1), thrombin activatable fibrinolysis inhibitor (TAFI) and tissue plasminogen activator (tPA) resulting in hypo-fibrinolysis and fibrin persistence [23]. We realized that the extracts of Carica papaya parts can be used as an anti-thrombotic agent for natural therapeutic medicine.

CONCLUSION

We analyzed that the thrombolytic activity of Carica papaya plant in normal individuals give significant results as compared to COVID-19 patients. Because in all COVID-19 patients, very high thrombin generation occurred and impaired fibrinolysis was observed. So, according to our assumptions that the bioactive compound (Alkaloids) which act as a thrombolytic agent in Carica papaya plant could be isolated and further processed as a therapeutic agent in pharmaceutical industry after the clinical trials. Our novel research work would be helpful for the better achievement in natural therapeutic medicines.

REFERENCES

[1] Wemambu, II, Ajose DJ, Eni CC. Antibacterial effect of Carica papaya root extract on some selected pathogens from clinical isolates. Acta Sci Microbiol. 2018;1(7):6-10. https://www.actascientific.com/ASMI/pdf/ASMI-01-0076.pdf

[2] Singh SP, Kumar S, Mathan SV, Tomar MS, Singh RK, Verma PK, Kumar A, Kumar S, Singh RP, Acharya A.Therapeutic application of Carica papaya leaf extract in the management of human diseases. DARU. J. Pharma. Sci. 2020;28(2):735-744. https://doi.org/10.1007/s40199-020-00348-7

[3] Srivastava AK, Singh VK. Carica papaya-a herbal medicine. Inter. J. Res. Stud. Biosci. (IJRSB). 2016;4(11):19-25. https://doi.org/10.20431/2349-0365.0411004

[4] Vij T, Prashar Y. A review on medicinal properties of Carica papaya Linn. Asian. Pac. J. Trop. Dis. 2015;5(1):1-6. https://doi.org/10.1016/S2222-1808(14)60617-4

[5] L K, sultana DM. Invitro Study of Thrombolytic Activity by Using Aqueous Preparation of Different Parts of Carica papaya Plant Extract. IOSR J. Pharm. Biol. Sci. 2014;9(3):34-9. https://doi.org/10.9790/3008-09323439

[6] Tabassum F, Chandi SH, Mou KN, Hasif KI, Ahamed T, Akter M. Invitro thrombolytic activity and phytochemical evaluation of leaf extracts of four medicinal plants of Asteraceae family. J. Pharmacogn. Phytochem. 2017;6(4):1166-9. https://www.researchgate.net/publication/320407833_In-vitro_thrombolytic_activity_and_phytochemical_evaluation_of_leaf_extracts_of_four_medicinal_plants_of_Asteraceae_family

[7] Subenthiran S, Choon TC, Cheong KC, Thayan R, Teck MB, Muniandy PK, Afzan A, Abdullah NR, Ismail Z. Carica papaya leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue haemorrhagic fever. Evid. Based Complement and Alternat Med. 2013;10 (7):61-67. https://doi.org/10.1155/2013/616737

[8] Liu H, Hu M, Wang Q, Cheng L, Zhang Z. Role of Papain-like Cysteine Proteases in plant development. Front plant sci. 2018;9 (17):1-10.https://doi.org/10.1016/j.plantsci.2017.11.001

[9] Baig MU, Bodle J. Thrombolytic Therapy. StatPearls. Treasure Island (FL): StatPearls Publishing. 2021 https://www.ncbi.nlm.nih.gov/books/NBK557411/

[10] Ciotti M, Ciccozzi M, Terrinoni A, Jiang WC, Wang CB, Bernardini S. The COVID-19 pandemic. Crit. Rev. Clin. Lab. Sci. 2020;57(6):365-88. https://doi.org/10.1080/10408363.2020.1783198

[11] Morens DM, Breman JG, Calisher CH, Doherty PC, Hahn BH, Keusch GT, Kramer LD, LeDuc JW, Monath TP. The origin of COVID-19 and why it matters. Am J Trop Med Hyg. 2020;103(3):955-9. https://doi.org/10.4269/ajtmh.20-0849

[12] Alanagreh La, Alzoughool F, Atoum M. The human coronavirus disease COVID-19: its origin, characteristics, and insights into potential drugs and its mechanisms. Pathogens. 2020;9(5):331.https://doi.org/10.3390/pathogens9050331

[13] Lemke G, Silverman GJ. Blood clots and TAM receptor signalling in COVID-19 pathogenesis. Nat. Rev. Immunol. 2020;20(7):395-6.https://doi.org/10.1038/s41577-020-0354-x

[14] Biswas S, Thakur V, Kaur P, Khan A, Kulshrestha S, Kumar P. Blood clots in COVID-19 patients: Simplifying the curious mystery. Med Hypotheses. 2021;146(110371): 1-4. https://doi.org/10.1016/j.mehy.2020.110371

[15] Ullah N. Medicinal plants of Pakistan: challenges and opportunities. Inter. J. Complement & Altern. Medi. 2017;6(4): 1-3. https://doi.org/10.15406/ijcam.2017.06.00193

DOI: https://doi.org/10.54393/pbmj.v4i2.107

[16] Rasool HB. Medicinal plants (importance and uses). Pharmaceut Anal Acta. 2012;3(10):2153-435. https://doi.org/10.4172/2153-2435.1000e139

[17] Boddupally SR, Sushma K. Evaluation Of Thrombolytic Activity In Various Parts Of Papaya Plant. World J. Pharm. Res. 2018;7(15):663-667. https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/1533025985.pdf

[18] Rahman A. Health benefits, chemistry and mechanism of Carica papaya a crowning glory. Adv. Nat. Sci. 2013;6(3):26-37. doi:10.3968/3732

[19] Jain S, Dangi CBS, Kaur M, Singh H, Peter J, Kosta S. Plant as anticogulant/antithrombotic agent. World J. Pharm. Res. 2014; 3(3):4573-83. https://www.researchgate.net/publication/262070850_Plant_As_Anticogulantantithrombotic_Agent

[20] Sarker MMR, Khan F, Mohamed IN. Dengue Fever: Therapeutic Potential of Carica papaya L. Leaves. Front Pharma. 2021;12(33):1-18. https://doi.org/10.3389/fphar.2021.610912

[21] Singh V, Goyal I, Saini A, Chandra R. Studying the Effect of Carica papaya Leaf Extract on the Shelf Life of Platelets. Int. J. Sci. Res. 2017;6(5):2138-2146. https://www.researchgate.net/publication/322498708_Studying_the_Effect_of_Carica_papaya_Leaf_Extract_on_the_Shelf_Life_of_Platelet

[22] Yusha'u M, Onuorah FC, Murtala Y. In vitro sensitivity pattern of some urinary tract isolates to Carica papaya extracts. Bayero J. Pure. Appl. Sci. 2009;2(2):75-8. https://doi.org/10.4314/bajopas.v2i2.63786

[23] Nougier C, Benoit R, Simon M, Desmurs-Clavel H, Marcotte G, Argaud L, David JS, Bonnet A, Negrier C, Dargaud Y. Hypofibrinolytic state and high thrombin generation may play a major role in SARS-COV2 associated thrombosis. J. Thromb Haemost. 2020;18(9):2215-9. https://doi.org/10.1111/jth.15016