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Phytochemical Screening and *in vitro* Evaluation of the Thrombolytic Activity of *Chenopodium album* L. Leaves

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ABSTRACT

Background and Objective: *Chenopodium album* L. (Family: Amaranthaceae) is well-known for its nutritive and pharmacological values. Its leaves are used as a vegetable and recommended as a dietary inclusion for persons suffering from heart disease. Thrombus is the major cause behind the occurrence of cardiovascular diseases (CVD) and therefore, the objective of this paper was to evaluate the *in vitro* thrombolytic potential of its leaves. **Materials and Methods:** Leaves of *C. album* were collected from Udaipur, Rajasthan, dried and powdered. It is methanolic (ME-I and ME-II) and aqueous extracts were prepared. Besides, qualitative assessment of phytochemicals and *in vitro* clot lysis potential of its leaves in comparison with the positive control (streptokinase) and negative control (distilled water) was evaluated. Blood lipid fractions such as serum triglycerides (TG), total cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C) and Non-High-Density Lipoprotein Cholesterol (Non-HDL-C) were estimated for finding out their correlation with thrombolytic activity. **Results:** Preliminary qualitative phytochemical analysis has shown the presence of flavanoids, terpenoids, steroids, phenols, tannin, saponin, cardiac glycosides, carbohydrates and amino acids and the absence of phlobatannins. A statistically significant ($p<0.001$) *in vitro* clot lysis activity of 39.70±0.99% was exhibited by methanolic extract of leaves as compared to streptokinase having 55.20±1.50% and distilled water having 3.62±0.30% clot lysis (n = 10). Notably, a moderate positive correlation between thrombolytic activity and total cholesterol, triglycerides and non-high-density lipoprotein-cholesterol was observed. **Conclusion:** The present study has first time demonstrated the significant *in vitro* thrombolytic potential of leaves of *C. album*. However, *in vivo* studies on a larger number of subjects along with the identification and isolation of phytochemicals responsible for thrombolysis are required.

KEYWORDS

Clot lysis, pigweed, rutin, anti-thrombotic, total cholesterol, ascorbic acid

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INTRODUCTION

Thrombus formation is the main culprit behind cardiovascular diseases (CVD) and mortality. The formation of blood clots (thrombus) occurs due to failure of the homeostasis process in the circulatory system which has serious consequences such as vascular blockage. Myocardial and cerebral infarction are often fatal outcomes of atherothrombotic disorders[1]. Lipids and lipoprotein particles also play an important role in the pathology of CVD by influencing inflammatory processes as well as the function of leukocytes and

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vascular and cardiac cells[2]. Blood stack occurs as a result of a series of sequential events, the final step in this process is the formation of thrombin, in which the soluble fibrinogen converts to insoluble fibrin[3]. To dissolve this thrombus, various thrombolytic agents such as tissue plasminogen activator (tPA), urokinase (UK), streptokinase (SK) and others are being used worldwide[4]. Due to their lower cost than t-PA, SK and UK are frequently utilized for thrombolysis, nonetheless, their use is linked to an increased risk of bleeding and allergic reactions[1]. To find a safe and effective thrombolytic agent, several research studies have been conducted worldwide on medicinal herbs and natural resources that have effective anti-thrombotic activity[5]. Plant-based herbal drug formulations have demonstrated that medicinal plants can be a good source of new therapeutic remedies for various clot-related disorders[6].

Chenopodium album L. (Family: Amaranthaceae) is a small, herbaceous plant, known as Lamb's quarter, Pigweed, Bethu sag, Beto, Bathua, Parupukkirai and Pappukura etc. in different languages. It is majorly distributed in South East Asia and found in the wild as well as cultivated throughout India up to an altitude of 4000 ft. Its leafy shoots are consumed as a vegetable[7-9]. In traditional medicine, it is used for the treatment of several human ailments such as asthma, back pain, cardiac disease, diarrhea, intestinal worms, jaundice, skin eruption, sprain, indigestion, seminal weakness, swollen gum, stomach pain, piles and wound etc.[10-12]. Many pharmacological activities have also been reported from the plant such as anticancer, analgesic, antimicrobial, anti-inflammatory, antioxidant, antidiabetic, antiulcer, hepatoprotective, anthelmintic, contraceptive, antinociceptive, anti-diarrheal and immunomodulatory[13,14].

Researcher has recommended many medicinal herbs and spices for treatment of heart disease[15] as well as the therapeutic use of many vegetables as a dietary inclusion for persons recovering from heart disease and *C. album* is one of them. In view of all this, in the present study, for the first time, leaves extract of *Chenopodium album* was evaluated to find out it's *in vitro* thrombolytic potential and further, its correlation with various blood lipid fractions such as serum total cholesterol (TC), triglycerides (TG), Low-Density Lipoprotein Cholesterol (LDL-C) and Non-High Density Lipoprotein Cholesterol (Non-HDL-C) was also assessed.

MATERIALS AND METHODS

Collection and preparation of plant material: Leaves of *C. album* were collected from an open farm at Shobhagpura, Udaipur, Rajasthan and identified with the help of flora of Rajasthan[7]. A voucher specimen of the plant was preserved at Herbarium, Department of Botany, Govt. Meera Girls' College, Udaipur, Rajasthan, India and authenticated from Botanical Survey of India (BSI), AZRC situated at Jodhpur District in Rajasthan (BSI/AZRC/I.12012/Tech./2020-21-(Pl.Id.)/424, dated 08/02/2021, Sl. No. 2). Leaves of the plant were dried on the room temperature in shade and powdered. The study was executed during the period between August, 2020 to October, 2022.

Extraction details

Preparation of methanolic extracts[16]: Methanolic extract-I (ME-I) was prepared for preliminary qualitative phytochemical analysis by soaking 5 g dried powder in 50 mL methanol at room temperature and filtered after 24 hrs. This was repeated three times with 50 mL methanol. Methanolic extract-II (ME-II) was prepared for evaluation of *in vitro* thrombolysis by soaking 100 g dried powder in 500 mL of methanol for 8 days with occasional stirring and filtering. The filtrate was evaporated at a temperature of 40°C in a boiling water bath and then stored in the refrigerator.

Preparation of aqueous extract: Preliminary qualitative assessment of certain phytochemicals was done using freshly prepared aqueous extract by soaking 400 mg dried powder in 20 mL of distilled water, boiling for 20 min and then filtering.

Phytochemical analysis: The standard methodology was employed to detect the presence or absence of amino acids, carbohydrates, steroids, terpenoids, phenols, cardiac glycosides, flavanoids, phlobatannins, tannins and saponins in the leaves of *C. album*[16-18].

Evaluation of *in vitro* thrombolytic activity: Approval from the institutional ethical committee was obtained (Ref.PMU/PMCH/IEC/2019, dated 26.12.2019) and after informed consent, *in vitro* thrombolytic activity of ME-II was evaluated in the blood samples (10 mL each) of ten healthy volunteers. Healthy individuals who were not taking any drugs were included in the study. The experiment was carried out in triplicate. The methodology demonstrated by Prasad *et al.*[19] was used to assess the *in vitro* clot lysis potential of leaves of *C. album*. Streptokinase (SK) was used as a positive control by dissolving lyophilized SK of 15,00,000 IU in 5 mL of sterile distilled water (STPase manufactured by Cadila Pharmaceuticals, Ahmedabad, India) out of which 100 µL (30,000 IU) was used for the test. Distilled water was used as a negative control. Initially, 20 sterile micro-centrifuge tubes were weighed. Then after, 500 µL of the blood was added to each tube and the tubes were kept in an incubator for 45 min at 37°C temperature. After the clot formation, serum was removed after centrifugation at 2000 rpm for 10 min and the weight of the clot was obtained by subtracting the weight of the tube from the weight of the tube with the clot. Then after, 100 µL of ME-II, sterile distilled water and SK were added to the tubes and kept at 37°C for 90 min. The impact of clot lysis was observed by removing the fluid and re-weighing of tubes to find out the weight of the clot after lysis. Percent clot lysis was determined as:

$$\frac{\text{Weight of clot after lysis}}{\text{Weight of clot}} \times 100$$

Estimation of blood lipids: Lipid fractions such as serum TC[20] and TG[21] were measured using standard enzymatic kits (Reckon Diagnostics P. Ltd., Baroda, India). Low-Density Lipoprotein Cholesterol (LDL-C) was calculated by the Friedwald *et al.*[22] formula as follows:

$$\text{VLDL-C} = \text{Triglycerides}/5$$

$$\text{LDL-C} = \text{Total cholesterol} - (\text{HDL-C} + \text{VLDL-C})$$

Non-High Density Lipoprotein-Cholesterol (Non-HDL-C) was calculated as follows[23]:

$$\text{Non-HDL-C} = \text{Total cholesterol} - \text{HDL-C}$$

Statistical analysis: Results of *in vitro* thrombolytic activity were expressed as Mean±Standard Error of the Mean (SEM) for three replicates. Student's paired t-test was used to check statistical comparisons by Microsoft Excel software (2010). Results were considered to be significant when the p-value was <0.01. The correlation between the blood lipids and clot lysis activity was assessed using Microsoft Excel (2010) at a significance level of 0.05 ($p<0.05$).

RESULTS

Qualitative phytochemical analysis: The preliminary phytochemical analysis has shown the presence of carbohydrates, amino acids, flavanoids, phenols, tannin, terpenoids, steroids, saponins and cardiac glycosides and the absence of phlobatannin in the leaves of *C. album* as shown in Table 1.

***In vitro* thrombolytic activity:** The significant ($p<0.001$) *in vitro* clot lysis (39.70±0.99%) of ME-II as compared to SK (55.20±1.50%) and distilled water (3.62±0.30%) was observed as shown in Table 2.

Correlation analysis between blood lipids and thrombolytic activity: A moderate positive correlation was observed between thrombolytic activity of *C. album* leaves and serum TC (Fig. 1), TG (Fig. 2) and non-HDL-C (Fig. 3) and a negative correlation was observed with LDL-C (Fig. 4). However, the correlation results were statistically not significant.

Table 1: Qualitative preliminary phytochemical analysis of leaves of *Chenopodium album*

Phytochemical	*Chenopodium album* leaves
Carbohydrate	+
Amino acid	+
Saponin	+
Flavanoid	+
Phenol	+
Tannin	+
Phlobatannin	-
Terpenoid	+
Cardiac glycoside	+
Steroid	+

+: Present and -: Absent

Table 2: *In vitro* percent clot lysis activity of methanolic extract of *C. album* leaves (ME-II)

Plant extract (ME-II)/control (100 µL)	Percentage of clot lysis (Mean±SE)
Methanolic extract (1 mg mL^{-1})	$39.70\pm0.99^{a,b}$
Distilled water	$3.62\pm0.30^{b,c}$
Streptokinase (30000 IU)	$55.20\pm1.50^{a,c}$

p-values: [a]p<0.001 ME-II as compared with streptokinase, [b]p<0.001 ME-II as compared with distilled water and [c]p<0.001 distilled water as compared with streptokinase

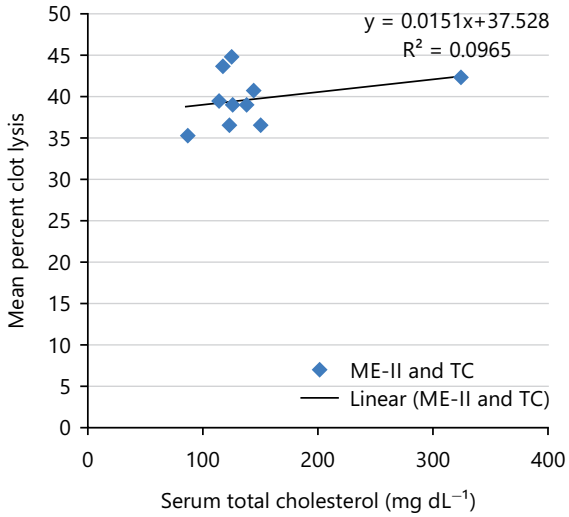

Fig. 1: Correlation between total cholesterol (TC) and *in vitro* thrombolytic activity of *C. album* leaves (r = 0.3106 and p = 0.38^{ns})

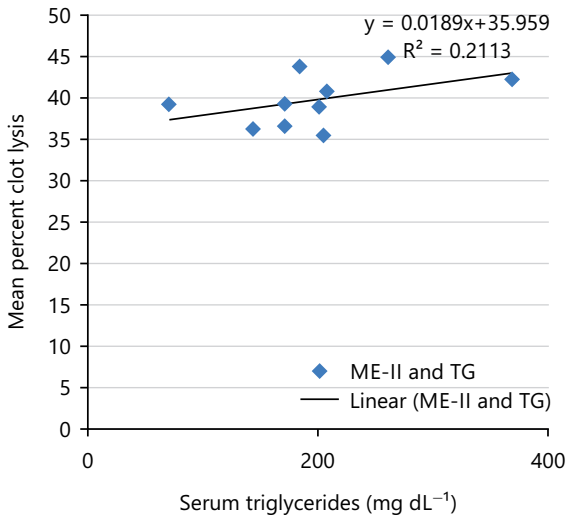

Fig. 2: Correlation analysis between serum triglycerides (TG) and *in vitro* thrombolytic activity of *C. album* leaves (r = 0.4596, p = 0.18^{ns})

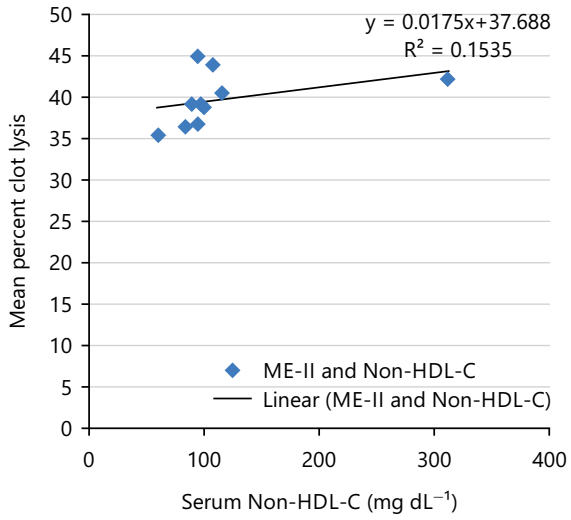

Fig. 3: Correlation analysis between serum Non-HDL-C and mean percent clot lysis of *C. album* leaves (r = 0.3917, p = 0.26^{ns})

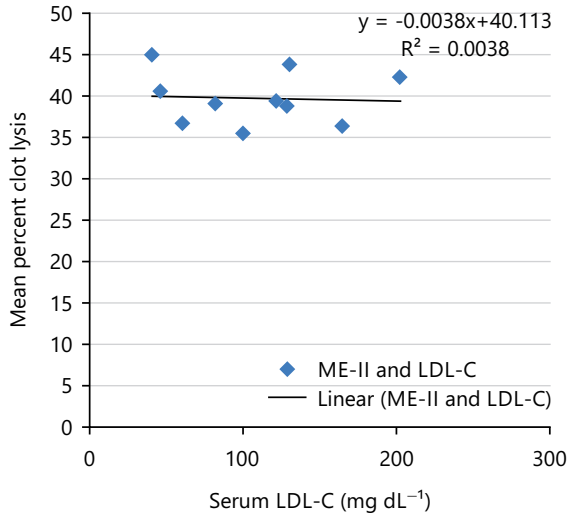

Fig. 4: Correlation analysis between mean percent clot lysis of *C. album* leaves and serum LDL-C (r = -0.0618, p = 0.86^{ns})

DISCUSSION

In the present study, leaves of *C. album* have also shown statistically significant *in vitro* thrombolytic potential of 39.70±0.99% as compared with both positive and negative control (Table 2). In addition to this, the presence of therapeutic secondary metabolites such as cardiac glycosides, flavonoids, phenols, steroids, terpenoids, saponins and tannins in its leaves, is an important finding (Table 1).

For the prevention and treatment of various chronic diseases use of natural foods or nutraceuticals is considered to be very beneficial. In traditional medicine, several food plants are recommended to treat various ailments but their systematic scientific validation studies are the need of the hour[24]. In this regard, the current scientific investigation on an edible plant *C. album* is noteworthy.

Thrombolysis is an important process in dissolving the blood clot and thus, helpful in maintaining the patency of blood vessels[5,25]. Several plants have been screened for *in vitro* thrombolytic potential. For example, 43.94±0.62% clot lysis was shown by chloroform extract of *Centella asiatica*[24], 37.17% by

ethanolic extract of *Ficus palmata*[26], 41.40±2.02 and 20.52±1.51% clot lysis by leaves and flowers of *Moringa oleifera,* respectively[16], 33.17% by roots of *Phyllanthus fraternus*[27] etc. In this context, 39.7% of thrombolysis activity as shown by *C. album* is an important observation of the current study.

The presence of phytochemicals imparts therapeutic potential in plants. For example, cardiac glycosides are used as a treatment for heart problems and have also been used as heart tonics, diuretics and emetics in folk medicine for centuries[28]. Similarly, flavonoids also play an important role in the prevention of CVD, owing to their antiatherogenic, antithrombotic and antioxidant properties[29]. The protective role of phenolic compounds against atherothrombosis is also shown by their antiplatelet, anti-inflammatory and antioxidant activities[30]. Leaves of *C. album* also possess antioxidant[31] and anti-inflammatory activities[32] along with a high amount of total polyphenols and flavanoids (550 mg gallic acid/100 g dry weight and 1880 mg rutin/100 g dry weight), respectively[33]. Phenolic compounds and flavonoids with antioxidant and anti-inflammatory properties have also been shown to possess anticoagulant properties[34]. In this regard, the presence of rutin, cinnamic acid, gallocatechin, catechin, coumaric acid, ferulic acid, sinapic acid, caffeic acid and chlorogenic acid along with other phytochemicals for example, β-carotene, lupeol, β-sitosterol, ascorbic acid, etc.[13,35-37] in *C. album* leaves, could be responsible for its anti-thrombotic potential. However, the quantitative estimation of these potentially heart-beneficial phytochemicals should be carried out along with the isolation of specific anti-thrombotic molecules from its leaves.

Abnormal lipid profile and coagulation parameters, high blood sugar, obesity, etc. are the major reasons behind the development of CVD[38]. The link between cholesterol levels and the occurrence of heart attacks has been shown in scientific studies[2]. Methanolic extract of *C. album* has shown a significant reduction in lipid parameters such as total cholesterol, plasma triglycerides, LDL-C, atherosclerosis Index and suggesting that its consumption may reduce the risk of CVD[39]. Besides the hypolipidemic activity of *C. album*, the moderate positive correlation between TC, TG and Non-HDL-C (Fig. 1-3) and the thrombolytic activity as shown in this study indicates better thrombolytic protection by *C. album* leaves even in the conditions of elevated lipids. Based on this, it could be recommended to be included in the diets of persons suffering from heart disease as also suggested by researcher[15].

The limitation of this study is that it is conducted *in vitro* with a small number of blood samples and with a specific concentration. In the future, a study with a large number of samples and with different concentrations of plant extract could be carried out. Moreover, *in vivo,* studies in doses equivalent to its natural form could be conducted. The plant is eaten in various recipes such as vegetables, parantha, raita, curry, etc. Because of its safety as an edible plant, the present results on thrombolytic activity could be promising for its dietary inclusion for persons who have suffered or are predisposed to CVD.

CONCLUSION

Leaves of *C. album* have been shown to possess 39.70±0.99% *in vitro* clot lysis activity with moderate positive correlation with TC, TG and Non-HDL-C levels. The thrombolytic activity may be due to the presence of the two major phytochemical groups such as phenols and flavonoids. However, *in vivo* studies with a larger number of subjects along with quantitative estimation of bioactive molecules are required to establish the thrombolytic potential of *C. album*.

SIGNIFICANCE STATEMENT

The present study first time reveals the *in vitro* thrombolytic potential of leaves of *Chenopodium album* L. It will motivate the researchers to find out the anti-thrombotic molecules from the plant and help in a recommendation of this edible plant for the prevention of CVD.

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