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In vitro Clot Dissolving Activity of Carbomer Based Gel Containing Ethanolic Extract *Calotropis gigantea* Leaves

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ABSTRACT

Blood clotting is an important and vital process that takes places in humans, animals and birds. At the same time blood clots sometimes considered an issue when developed in the circulatory system due to failure of hemostasis causes vascular blockage and leads to serious consequences in thrombolytic diseases such as acute myocardial or cerebral infarction which may cause death. Certain substances like Alteplase, anistreplase, streptokinase, urokinase, tissue plasminogen activator, Heparin and Aspirin are used as clot dissolving agents. Plants have also been proved to perform such activity. *Calotropis gigantea* L. is a traditional medicinal plant with several pharmacological properties belongs to the family Apocyanaceae of *Asclepiadaceous* was investigated for its efficacy for blood clot dissolving activity under *in vitro* conditions when incorporated into carbomer based gels. The ethanolic extract of the leaves of *C. gigantea* was prepare by soxhlation which upon analysis reported to contain alkaloids, glycosides, flavonoids, tannins, terpenoids and saponins as usual components as were reported in earlier investigations. There were 3 gels with ethanolic leaf extract *C. gigantea* with 1 mg, 0.5 mg and 0.25 mg per gram of 1% Carbopol-940 gel containing methyl paraben, glycerol and water. The two gels containing sterile distilled water and 0.1 mg per gram gel streptokinase enzyme served as –ve and positive controls respectively. The *in vitro* thrombolytic of clot dissolving activity war performed on goat blood collected from slaughter houses. From the results the percentage clot dissolving activity of gels containing 1 mg, 0.5 mg and 0.25 mg *C. gigantea* extract per gram were reported as 34%, 27% and 14% respectively considered significant when compared to the activity of standard streptokinase in the gel. The activity of these experimental gels describes the possibility of development of new safe, and non reactive topical anti-clot gel products using herbal or plant components like *C. gigantea* extracts.

Keywords: *Calotropis gigantea* L, Streptokinase, Thrombolytic activity, Carbopol gel, Biopharmaceutical.

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INTRODUCTION

Referring to the article published in 1879, blood clotting is an important and vital process that takes places in humans, animals and birds[1]. Maintenance of normal blood flow requires equilibrium between procoagulant and anticoagulant factors within the blood circulatory system. But when procoagulant activity preponderates, results into the formation of blood clots where tissue damage becomes a factor in the process in general[2]. Blood clotting is a complex interaction of various mechanisms, where activation of the coagulation, fibrinolytic systems, disruption of the vascular endothelium, and the generalized activation of cellular mechanisms leads to clotting on the surface of monocytes and platelets in circulation[3]. Blood clots sometimes considered an issue when developed in the circulatory system due to failure of hemostasis causes vascular blockage and leads to serious consequences in thrombolytic diseases such as acute myocardial or cerebral infarction which may cause death [4]. Hereditary factors, primary or acquired, play a role in the development of thrombosis[3]. The breakdown of blood clots is referred to as thrombolysis or clot busting in pharmacological terms and that can be done by stimulating secondary fibrinolysis by plasmin through infusion of analogs of tissue plasminogen activator the protein that normally activates plasmin. Certain substances are used as Clot dissolving agents available in the market like Alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator. Where moderately efficient Heparin and Aspirin are also available accelerating the lysis and prevent the reoccurrence of thrombus but are safe[5]. As per some literatures, anticoagulants that are being used presently shows not only the negative and life-threatening side effects but runs high in cost from therapeutic point of view, thus researchers and investigators are now finds their interest in exploring the natural resources in order to develop alternate novel agent that could perform similar

functions without any harm [6,7]. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs, due to the presence of different chemicals used as phytomedicine, derived from different parts of the plants[8]. In recent studies, *Campomanesia xanthocarpa*, *Alpinia conchigera, Lannea grandis* Engl. *Aglaonema hookerianum* Schott. *Tridax procumbens* L. *Nigella sativa, Capsicum frutescens, Brassica oleracea, Calotropis gigantea* like plants have been proved to perform such activity[9-12]. *Calotropis gigantea* L. is a traditional medicinal plant has reported to have analgesic, antipyretic activity, antimicrobial, anti-diarrheal and cytotoxic, properties belongs to the family Apocyanaceae of *Asclepiadaceous* thrives habitat of Asian countries[13]. *C. gigantea* L. has been considered to cure cardiovascular diseases, skin diseases, use as anticancer, antifertility and antidote for snakebites in traditional system of medicine[14-17]. This plant has also been investigated for thrombolytic activity[12]. Thus current preliminary study aimed at investigation efficacy of carbomer based gel prepared from ethanolic extracts of *C. gigantia* leaves blood clot dissolving activity under *in vitro* conditions.

MATERIALS AND METHODS

Sample collection and processing

The leaves of *C. gigantea* were collected from local area around the Indore City and were authenticated with use of herbarium available in the college, literatures and images from web resources. The collected leaves were washed thoroughly with tap water, cleaned and dried at room temperature followed by grinding them into fine powder. These finely grounded leaf powders were subjected to phytochemical extraction.

Phytochemical extraction and analysis

The fine powdered leaves of *C. gigantea* were first defatted with petroleum ether at ambient temperature for 24 hours and then the defatted marc was further subjected to phytochemical extraction using Soxhlet extractor in 50% ethanol as solvent. The menstruum so obtained after extraction was allowed to concentrate in a boiling water bath to evaporate the solvent. The percentage yield of extraction and preliminary phytochemical analysis was done[18-20].

Preparation of carbopol based gel

Stock of phytochemical extract was prepared in sterile distilled water with a concentration of 100 mg/ml. In present study the Carbopol gel were prepared with suitable modifications [21-23]. The composition of the gel base used in present work is depicted in table 1. The stock concentration of 100 mg/ml crud phytochemical extract was prepared in sterile distilled water homogenized and degassed in ultrasonic bath which was used to be added in gel to make varied final concentrations of extract in per gram of gel.

Table 1 Composition of Cabopol-940 gel base

S.N.	Composition	Amount
1	Water	100 ml
2	Carbopol -940	1 gm
3	Methyl paraben	200 mg
4	Glycerol	1 ml
5	Phyto extract stock	As required

In vitro clot dissolving activity

The *in vitro* thrombolytic activity of prepared gels was done with some modifications[5, 24, 25]. The blood samples were collected from the slaughter houses where goat blood was used instead of human blood because of similarities in clotting properties. The blood samples were collected in 1.5 ml microfuge and incubated at 37°C for 2 hours to allow the clot formation followed by removal of serum carefully and weight of the clot was measured. Now, to each of the pre-weighed clot in microfuge, 100 µl of prepared carbopol based gel containing extracts were added with the help of micropipette (from HiMedia) touching the surface of blood clot. A blank gel without any extract and a gel containing streptokinase were used as negative and positive control respectively. The set of experiment was incubated at 37 °C for 2 hours followed by observation *in vitro* thrombolytic activity. The liquid released after incubation was drained and remaining clot was washed with sterile distilled water and dried well to remove moisture followed by weighing the clot again to determine the percentage thrombolytic activity of gels compared to standard using following formulae;

$$\% \text{ Thrombolysis} = \frac{\text{Weight of clot after incubation}}{\text{Weight of clot before incubation}} \times 100$$

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

The results of phytochemical extraction and analysis for the presence of phytoconstituents are more or less coincides with earlier investigations[26-28]. In present study the yield of extraction with hydro ethanolic solvent was 18.1%. While this ethanolic extract of leave of *C. gigantea* was reported to contain alkaloids, glycosides, flavonoids, tannins, terpenoids and saponins as usual components as were reported in earlier investigations[29].

In vitro clot dissolving activity

The *in vitro* clot dissolving activity of carbopol based gels shows promising results in development of biopharmaceutical purpose for therapeutic substances. There were 3 different concentrations of extracts in gels were used in present study whose percentage thrombolytic activity are depicted in table 2 compared to the standard streptokinase.

Table 2: *In vitro* clot dissolving activity of carbopol gel containing *C. gigantea* extract compared to gel containing Streptokinase

S.N.	Gels	Designation	Concentration	Percentage Clot Lysis
1	Gel-1	–ve Control with water	NA	1.8%
2	Gel-2	+ve Control with Streptokinase	0.1 mg/g gel	71%
3	Gel-3	Test Gel with extract	1 mg/g gel	34%
4	Gel-4	Test Gel with extract	0.5 mg/g gel	27%
5	Gel-5	Test Gel with extract	0.25 mg/g gel	14%

The early reports on *in vitro* thrombolytic activity of *C. gigantea* extracts encouraged the test the similar effect of ethanolic extracts of *C. gigantea* leave when incorporated in any neutral gels in present study [30-31]. In present study the *in vitro* blood clot dissolving activity of ethanolic leaf extract of *C. gigantea* in carbopol gel at a concentration of 1 mg/g of gel was observed to be 34% which reduces with serial decrease in extract concentration in gels as 27% and 14% with Gel-4 and Gel-5 respectively (Table 2). Strepotkinase at a concentration of 0.1 mg/ml generally produces a thrombolytic activity in range from 80 to 95% [12, 32]. In present study when it was incorporated in gel it showed an activity of 71% at similar concentration in gels that is optimum. The activity of varied concentration of extract with the activity streptokinase in gels, the dissolution of clot by extracts is considerable (Figure 1)

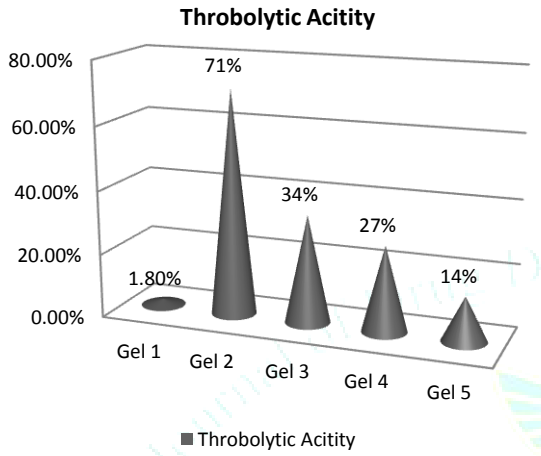

Figure 1: Graphical representation of *in vitro* thrombolytic or blood clot dissolving potential of gels prepares containing streptokinase and ethanolic extract of *C. gigantean*.

Food and Drug Administration (FDA) has approved several pharmaceuticals of plant origin, also several plants have been investigated earlier including *C. gigantea* for anti-coagulant, anti-platelet and fibrinolytic activity where many claims that some herbs and food showing preventive effects on events of coronary blockage and stroke [33 ,34]. Heavy external blood clots due to any tissue tear or rupture or large accidental wound development is also a problem sometimes when causing painful bandage. Incorporation of any pharmacological substance in gel are generally meant for topical applications and here the anti blood clot activity of carbopol based gel containing ethanolic extracts of *C. gigantea* leaves were effectively dissolving blood clot at all 3 different concentration under in vitro experiments. This property of *C. gigantea* leaf extract may be exploited in development of new biopharmaceutical and therapeutic agents upon further extensive investigation related to it are save use on animals and humans.

CONCLUSIONS

From the results of present investigation, it could be concluded that the phytochemical extracts of *C. gigantea* are rich in number of chemical components that posses the biological and pharmacological activities which has been also reported several times in early studies. The clot dissolving activity of extracts works when not only when use as sole component but also when incorporated in gels. Because of the significant inadequacies of the currently available thrombolytic agents of microbial origin sometimes imparting allergy effects where plant derived drugs are considered promising in development of new therapeutic agents. So, upon further extensive studies related to preparation of non reactive polymer based gels containing plant chemicals could be developed into a successful topical biopharmaceutical product with anti-clot activity.

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