International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

ISSN:2347-6567

IJAMSCR |Volume 8 | Issue 2 | Apr - Jun - 2020
www.ijamscr.com

Research article *Medical research*

Potency of Tecoma Stans flowers on Atherothrombosis *(In Vitro)*

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ABSTRACT

Tecoma stans and Phytol (PHY) are evident in different test systems because of their positive biological results. This research tests anti-atherothrombosis activity of the Tecoma stans (EETS) and Phytol (PHY) ethanolic extract. The EETS and/or PHY clotlysis activity was investigated by taking Streptokinase (SK) as a normal drug used for clotlysis. The findings indicate that the EETS and PHY showed concentration-dependent clotlysis activity in coagulated human bloodThe EETS and PHY's maximum clotlysis was observed at 62.42 ± 1.14 percent and 55.04 ± 0.24 percent, respectively with 80 µg and 150 µg per pipeline. The EETS co-treated with PHY showed greater potential for clotlysis than the EETS and PHY. The vehicle displayed marginal clotlysis efficiency (3.49 ± 2.51 percent) while the regular SK (100 I.U.) demonstrated clotlysis activity at 76.54 ±2.23 percent. The EETS and/or PHY demonstrated in vitro clotlysis behavior in human coagulated blood.

Keywords: *Tecoma stans*, Streptokinase, Antioxidant, Atherothrombosis, Phytol.

INTRODUCTION

Atherothrombosis is characterized by disturbance of the atherosclerotic lesion with superimposed thrombus formation, which is the main cause of acute coronary syndrome (ACS) and cardiovascular death worldwide [1]. The causes, current therapies and challenges [1] were outlined in an excellent review by Viles-Gonzalez et al. Streptokinase (SK) is a widely used thrombolytic drug among the clotlysis agents [2]. Nonetheless, SK is obvious to cause some side effects, including nausea, vomiting, low blood pressure and allergic reactions, so its use during pregnancy should be limited. Urokinase, isolated from human urine, the other clot lysis product, is found to correlate with tumor malignancy at an elevated expressional level [3].

Tecoma stans leaves contain tecomin alkaloids, and tecostamine when given intravenously is a potent hypoglycaemic agent. The responsible for Diabetic Activity is anthranilic acid. There are

clear roots of diuretics and vermifugees. Tecoma is not a poisonous herb, because it is used for diabetes care in Latin America and for cattle and goats feeding in Mexico, respectively. *Tecoma stans* 'preliminary phytochemical screening of ethanol l extract showed the presence of flavaniods, phenols, alkaloids, tannins, steroids, triterpenes, anthraqunones, and saponins, and so on [4]. Phytol (PHY) is an abundantly present in nature, diterpenoid derived from chlorophyll. Both of these compounds are evident to have many essential biological activities, including antioxidant, anti-inflammatory, and defensive organ activities [5]. However, their operation against arterothrombosis has yet to be tested. This research therefore targeted the evaluation of EETS and/or PHY anti-atherothrombosis (in vitro) in human clotted blood.

MATERIALS AND METHODS

Collection and extraction of plant

The *Tecoma stans* flowers were collected at Tamil Nadu in Rasipuram [Namakkal Dt] in the month of May 2019. The section Pharmacognosy had deposited a specimen of plant herbarium. Dr. G.V.S.Murthy, Joint Director of the Indian Botanical Survey, Southern Circle, TNAU Campus, Coimbatore has described the herb from the descriptions available in the literature. Around 10–12 days the floral petals were dried up in the morning. After full drying in a mechanical grinder the flower petals were pulverized to a coars 40-mesh powder. Sohxlet extraction of the powdered material was performed at 50–55 ° C with ethanol during 18 h. The extract was subsequently concentrated under vacuum, and dry air.

Reagents and chemicals

In Sigma Aldrich, USA we have purchased PHY and other reagents and chemicals used in this research.

Clot lysis test (*In Vitro*)

In this analysis the EETS at 5 to 80 µg, PHY at 25 to 150 µg and the combinations of EETS and PHY were used. Clot lysis experiments were carried out, as previously stated by Prasad et al[6]. Briefly, 3 mL of venous blood from healthy volunteers (n=5 male and female actually not using oral contraceptive or anticoagulant therapy) and administered in: Vehicle (VEH: 0.05 percent Zwischen 80 dissolved in 0.9 percent NaCl solution); Normal (SK: Streptokinase) and various EETS and/or PHY concentrations of pre-weighted sterile microcentrifuge (alpine / ependorf) tubes (0.5 percent).

After the formation of the clot, the serum was removed completely without disrupting the clot and each tube with clot was weighed again to determine the weight of the clot (clot weight = weight of clot containing tube – weight of the tube itself). 100 µL of test sample / control was applied separately to each micro-centrifuge tube containing pre-weighed clot. 100 µL of SK (100 I.U.) and 100 µL of VEH, respectively, were used as positive and negative power. Then all the tubes were incubated for 90 min at 37 ° C, and examined for clot lysis. After incubation, fluid released was removed, and after discarding the lysed weight, tubes were weighed again to determine the difference in weight. The difference in weight obtained was expressed as a percentage of clotlysis, taken before and after clot lysis. The experiment was performed in triplicate.

STATISTICAL ANALYSIS

To this end Graph Pad Prism 6.0 (Data Pad Inc., San Diego, CA) has been used. For multiple comparisons between the study groups, a one-way variance analysis (ANOVA) followed by the Tukey post hoc test was applied. The measured values are expressed as mean ± standard deviation (SD), and are considered statistically significant with $p<0.05$, $p<00.01$ and $p<0.001$.

RESULTS

Table 1 shows that EETS was reconstituted with the vehicle, exercising clot lysis operation in a concentration-dependent manner, where maximum clot lysis was seen with the 80 µg / tube. The EETS lysed the clot at 10 to 80 µg (EC50: 47.21 ±1.35; CI: 34.25 to 68.21; R2: 0.84) significantly relative to the vehicle category ($p<0.05$, $p < 0.01$, $p < 0.001$).

PHY also demonstrated concentration-dependent activity in the clot lysis. PHY exercised strong clotlysis at all concentrations ($p < 0,05$, $p < 0,01$, $p < 0,001$) (EC50: 121,54 ± 1,63; CI: 95,31 to 157,25,78; R2: 0,97). EETS demonstrated greater clot lysis efficiency at 40 and 80 µg than PHY 100 and 150 µg [Table 1], respectively.

At all concentrations EETS co-treated with PHY increased the effects of clot lysis as opposed to the EETS and PHY individual classes. EETS combined with PHY showed clotlysis activity at the maximum concentration of 72.51 ± 0.48 percent. In the vehicle group, the co-treated group conducted strong clot lysis activity at all concentrations. SK, at I.U. 100 Clotlysis was developed 76.54 ±2.23 percent [Table 1].

Table: 1 Clotlysis ability of of EETS

Drug Treatment	Concentration	Percentage of clot lysis
VEH (0.05% Tween 80 + 0.9% NaCl solution)	100 µL	3.49 ± 2.51
Streptokinase (100 I.U)	100 I.U.	76.54 ± 2.23***
EETS	5 µg	6.25 ± 2.35
	10 µg	13.51 ± 1.32*
	20 µg	25.54 ± 1.36**
	40 µg	45.25 ± 2.14***
	80 µg	55.04 ± 0.24***
PHY	25 µg	7.51 ± 4.10*
	50 µg	14.84 ± 2.68*
	75 µg	29.44 ± 2.30**
	100 µg	40.72 ± 3.11***
	150 µg	55.04 ± 0.24***
EETS (µg/tube) + PHY (µg/tube)	5 + 25 µg	13.09 ± 1.09*
	10 + 50 µg	23.75 ± 2.11**
	20 + 75 µg	32.70 ± 2.79**
	40 ± 100 µg	56.44 ± 3.69***
	80 ± 150 µg	72.51 ± 0.48***

Values are mean ± SD (n= 5); *p< 0.05, **p< 0.01 and ***p< 0.001 compared to the VEH (0.05% Tween 80 dissolved in 0.9% NaCl solution); two-way ANOVA (with non-parametric test) followed by Tukey's test; EETS: Ethanolic Extract of *Tecoma stans*; PHY: Phytol.

DISCUSSION

Atherosclerosis can begin early in life, and progress through adult life asymptomatically. This is expressed clinically as coronary artery disease, stroke, acute ischaemic attack and peripheral artery disease [1]. In addition to drug therapy, dietary supplements are the primary driving factor for many diseases, including atherosclerosis [7]. In many countries the EETS is a dietary supplement [8]. On the other hand, PHY, an essential oil, is a side chlorophyll chain, and is a compound consumed daily, particularly by the ruminant animals. [9, 18]. T.stans and its comprehensive research in various test systems for cardioprotective effects [10, 11].

PHY, on the other hand, has antioxidant, anti-inflammatory, lipid reducing and defensive effects on the organ [9]. The atherosclerotic plaque is vulnerable to disturbance, resulting in local platelet activation and aggregation, which is a significant impact of thrombus formation [12] In this research, we have reported that the EETS and PHY have induced clot lysis at all stages. Herbal remedies, are widely used today in all medical settings. It is due to their effectiveness and affordability and reduces the likelihood of allergies, allergic reactions or cross-reactivity with other medications and supplements [13]. The herbal combination product's therapeutic effectiveness has been scientifically proven to the highest standards [14]. In our research, the combined EETS and PHY concentrations have been shown to demonstrate clot lysis capability better than the individual EETS and PHY concentrations used in each group. It may be because of their synergistic clot influence.

A variety of spices have been found in a recent study, such as pepper, ginger, garlic, onion, to work against athosclerosis [15]. Wu et al. propose that

andrographolide (a bitter diterpene lactone) enhances atherosclerosis by suppressing pathways for the production of pro-inflammation and Reactive Oxygen Species (ROS)[16] The scavenging and anti-inflammatory effects of ROS are both evident in EETS and PHY. In another study, pseudolaric acid B (diterpene acid) was found to attenuate the progression and inflammation of atherosclerosis by suppressing mice activation by peroxisome proliferator-activated receptor gamma (PPARÿ)-mediated nuclear factor kappa B (NF-µB) [17]. Also obvious are EETS and PHY to move along these paths [4, 18, 20].

In addition, artery inflammation, lipid deposition in the arterial wall, and the development and growth of atherosclerotic plaques accompanied by ischemia are all characterized by atherosclerosis. Traditional anti-athero adjunct sclerotic therapy focuses solely on enhancing the profile of blood lipids and does not target specific stages of plaque progression. Cholesterol preservation goes until plaque formation. Targeted at the latter pathway could be advantageous to avoid further atherogenic progression. Thanks to their strong tolerability and suitability for long-lasting care, herbal formulations can be used in this regard [21].

CONCLUSION

Clotlysis effect of EETS and PHY exerted concentration-dependently on human blood clot. Combination of EETS and PHY exhibited better clotlysis outcome at entire concentrations. Synergistic action might be produced by EETS and PHY. Both EETS and PHY could be an effective ailment for treating atherombosis and other cardiovascular diseases linked to it.

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How to cite this article: Dr.R.Manivannan, Mr.S.Kameshwaran, Mr.M.Sarbudeen, Mr.G.Sureshkumar, Mr.N.Elavarasan, Ms.S.Meena, Mrs.S.Harshini. Potency of Tecoma Stans flowers on Atherothrombosis (In Vitro). Int J of Allied Med Sci and Clin Res 2020; 8(2): 150-154.
Source of Support: Nil. **Conflict of Interest:** None declared.