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***Research Article** (Open Access)*

IN VITRO THROMBOLYTIC ACTIVITY OF EXTRACT OF PRECLITELLAR, CLITELLAR, AND POST CLITELLAR BODY REGION OF *EUDRILUS EUGENIAE*

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ABSTRACT
The development of thrombosis is the main cause of disease formation in the cardiovascular system. Thrombosis blocks the blood flow some tissue plasminogen activator responsible for the activation of plasmin to facilitate the lysis of intravascular clot lysis. Evaluation of thrombolytic activity has been carried out for Preclitellar, clitellar, and Post clitellar body region extracts of *Eudrilus eugeniae*. This study is carried out for the identification of which body region has the potential for thrombolytic activity. After cleaning of whole earthworm cut into three body regions namely Preclitellar, clitellar, Post clitellar regions, and whole earthworm. These body regions homogenized in to buffer saline and then centrifuged in cold condition. Three different concentrations were made in 20 mM phosphate buffer (pH 7.4) and used as test extracts (10, 20, and 40 mg/ml). The test extracts add to the plasma clot and after 90 minutes of incubation at 37^{o} C; the thrombolytic activity was determined by using standard formula. The present study showed highest thrombolytic activity of clitellar region of earthworm as compared to Preclitellar and Post clitellar regions. The positive results indicated that the component responsible for thrombolytic activity was distributed unevenly all over the body of *Eudrilus eugeniae*.

Keyword: *Eudrilus eugeniae, thrombolytic activity, Plasma clot*

INTRODUCTION

Thrombosis is a pathological condition in coronary heart disease. Formation of thrombus is the characteristic of these diseases in the circulatory system, due to the failure of homeostasis, (Dewan and Abhijit, 2013). The restoration of blood in a normal state plasmin plays an important role. Plasmin is formed under the influence of plasminogen activators (Rozhchenko, 2003). Some of the plasminogen activators like t-PA, u-PA, and s-PA can activate the plasminogen into plasmin, (Ezihe-Ejiofor *et al*., 2013). Some plasminogen activators are available in the market. They broadly categorized into tissue plasminogen activator and other plasminogen activator. These are streptokinase, anistreplase, and urokinase. However, they showed anaphylactic reactions and Hemorrhage, purpuric rashes, thrombocytopenia, and Contradiction with severe hypertension, peptic ulcer disease, and ulcerative colitis (Islam *et al*., 2016). Mihara *et al*., (1991) worked on a Novel Fibrinolytic Enzyme extracted from the earthworm, *Lumbricus rubellus*. Cooper *et al*., (2004) worked on alternative sources of fibrinolytic, thrombolytic, anti-coagulative, antimicrobial, and anticancer molecular.

The medicinal properties of earthworms in various remedies had documented date back to 1340 A.D (Reynolds and Wilma 1972). Nakajima *et al*., (1993) characterized the enzyme from *Lumbricus rubellus* and are important for the lysis of fibrin. The fibrin is the main protein component of blood clots. Sugimoto *et al*., (2001) Work on molecular cloning, sequencing, and

expression of c DNA encoding serine protease with fibrinolytic activity from Earthworms. After studying comprehensive literature related to this topic, observed that there was no work on spotting out the earthworm thrombolytic component. Especially, research work concerning *Eudrilus eugeniae*. Hence the present research focus on the evaluation of the Clitellar region, Pre-clitellar region and Post clitellar region of *Eudrilus eugeniae* for *in vitro* thrombolytic activity.

MATERIALS AND METHODS

Earthworm Culture

In Moolji Jaitha College (Autonomous) vermiculture was established. Healthy and matured earthworms are used for the preparation of earthworm extract. The earthworm *Eudrilus eugeniae* was identified and authenticated by the Zoological Survey of India, Kolkata.

Extraction of Thrombolytic Enzyme

The extract used in the present study was achieved by following the extraction method of Cho *et al*., (2004). Earthworms were washed with tap water, then kept and allowed in annelid saline for 2- 3 hrs to remove the cast from the alimentary canal of the earthworm. The earthworm cut into three pieces *i.e.* Pre clitellar, clitellar and post clitellar regions. Wash these pieces separately, weighed and used them for extract preparation. In the same way after cleaning and weighing the whole earthworm was also used for extract preparation. Three concentrations were prepared for each body region and whole earthworm body at Test I (40 mg/ml), Test II (20 mg/ml) and Test III (10 mg/ml). The three regions of the earthworm and whole earthworm were homogenate in 20 mM phosphate buffer having pH 7.4. Homogenate was centrifuged at 10000 rpm at 4^{o}C and obtained supernatant filter through 0.45µm membrane through a vacuum filter twice. The obtained filtrate evaporates in a water bath at 60^{o} C. The selected concentration of the crude extract was suspended into 0.1 M Phosphate buffer; pH 7.4 and collected separately in sample bottles and used for further study

Blood Collection

Volunteer of either sex was used to draw the 3.0 ml blood; collected from a median cubital vein and immediately placed into the tri-sodium citrated bulb and centrifuge to obtain citrated plasma.

Preparation of plasma clot.

The method for plasma clot was adapted as mentioned by Sarkar *et al*., (2015). Freshly drawn blood was mixed immediately with 3.8% tri-sodium citrate in a ratio of 9:1 and centrifuged at 3000 rpm for 15 minutes to obtain citrated plasma. 400 μl citrated plasma was added to a pre-weighed Eppendorf tube and added to it 100 μl 0.2 M $CaCl_2$. The Eppendorf tube incubates at 37^{o} C for 30 minutes in the water bath, allowed to stand until a firm clot was obtained.

Determination of Thrombolytic Activity

Thrombolytic activity is determined as per the method mentioned in Prasad *et al*., (2006). The clot-containing Eppendorf tube was labeled as control, load with sterile distilled water, standard load with streptokinase (is one of the recognized plasminogen activators) at 1000 IU/ml concentration, and three test sample tubes i.e. Test I- 40 mg, Test II- 20 mg Test III- 10 mg of test extract, All these tubes were incubated for 90 min, at 37^{o}C in water bath. After incubation released fluid was discarded from the tube and the tube was again weighed to observe the weight of the clot released.

The given formula was used to compute the clot lysis percentage.

$$\%\ clot\ lysis = \frac{(Weight\ of\ released\ clot)}{(Weight\ of\ clot)} X100$$

Where, Weight of release clot= Weight of eppendorf tube before clot lysis-Weight of eppendorf tube after clot lysis

Weight of clot= Weight of clot containing eppendorf tube- Weight of empty eppendorf tube

Statistical Analysis

The significant % of clot lysis was compared with control. The data was expressed as mean ± standard error. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by "Bonferroni multiple comparison test" at $p< 0.05$ level of significance.

RESULTS AND DISCUSSION

Mihara *et al*., (1991), Nakajima *et al*., (2003), studied the fibrinolytic activity of lumbrokinase enzyme from *Lumbricus rubellus*, they observed that the anterior part of the alimentary canal of earthworms has good fibrinolytic activity. In the present study the in vitro thrombolytic activity of the Pre-clitellar, Clitellar, Post clitellar region, and whole body of *Eudrilus eugeniae* to confirm which region showed the highest activity. As per the results highest activity was observed in the clitellar region as compared to the pre-clitellar and Post clitellar regions of *Eudrilus eugeniae*. So, our results are corroborated with the result of Mihara *et al*., (1991) and Nakajima *et al*., (2003). On the basis of result concluded that the clitellar region extract of *Eudrilus eugeniae* has the highest activity as compared to other body region extracts of *Eudrilus eugeniae*. The present research reveals that further, study is needed to isolate the particular active principle and characterize the specific molecule, which is responsible for clot lysis from earthworm, *Eudrilus eugeniae*. This may also support pharmaceutical industries and farmers for their sustainable livelihood.

Table: 1 Clot lysis of Preclitellar, Clitellar, and Post Clitellar body region of *Eudrilus eugeniae*

Group Clot lysis	Control	Standard	Test I	Test II	Test III
Preclitellar region	1.00±0.88	14.56±2.18	3.48±2.32*	9.03±0.56**	7.7±0.82^{ns}
Clitellar region	0.025±0.2	14.56±2.18	18.92±2.23****	13.3±0.27****	26.32±0.83****
Post clitellar region	1.0±0.88	14.56±2.18	7.18±1.03****	9.70±0.50***	11.96±0.29*
Whole earthworm	1.8±0.9	14.56±2.18	19.24±0.67****	19.21±0.23****	21.08±0.31****

Note: Test I: 40 mg/ ml, Test II: 20 mg/ ml, Test III: 10 mg/ ml, Standard: Streptokinase (1000 IU/ml)

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