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Comparative Study of Potential Anti-Cancerous and Cardio Protective Activities of Methanolic Leaf Extract of *Cyclea barbata* and *Entadapursaetha*

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ABSTRACT

The comparative study was aimed to evaluate the potential anticancerous and cardioprotective activities of methanolic leaf extract of *Cyclea barbata* and *Entadapursaetha*. The screening of anticancerous activity was performed using brine shrimp lethality bioassay while the cardioprotective activity was evaluated using the *in vitro* clot lysis model. Significant anticancerous activity was found for methanolic leaf extract of *C. barbata* whereas moderate effect was found for methanolic leaf extract of *E. pursaetha* and it was compared with the standard drug vincristine sulfate in the brine shrimp lethality bioassay. In the present study, the LC₅₀ values of methanolic crude extract of *C. barbata*, *E. pursaetha* and vincristine sulfate were 104.04, 330.31 and 12.59 µg mL⁻¹, respectively. In cardioprotective study, it was found that *C. barbata* and *E. pursaetha* showed 45.69±1.96 and 38.19±1.83% of clot lysis, respectively. Between the plants studied *C. barbata*, showed very significant (p<0.05) percentage of clot lysis than *E. pursaetha* compared to reference drug streptokinase which is 63.54±2.61%. The results of this study demonstrated that the leaf of these plants possess promising anticancerous activity on brine shrimp as well as significant cardioprotective activity *in vitro* when tested on human blood.

Key words: *Cyclea barbata*, *Entadapursaetha*, anticancerous activity, cardioprotective, brine shrimp

INTRODUCTION

Cyclea barbata (Menispermaceae) is a slender, herbaceous or woody climber, also known as *cincauhijau*, has been commonly consumed in India, Myanmar, Indo-China, Thailand, Bangladesh and Indonesia. Traditionally, it has been also used for treating gastroenteritis, typhoid, intestinal disease, high blood pressure, diarrhea, mouth sores and peptic ulcer. Scientifically, it was also proven that the leaves can be used as antihypertensive, anti-ulcer and antibacterial agents (Sulamanda, 2006; Syamsurizal, 2008). In Indonesia, the leaves of this are used to make a preparation in the form of a jelly which is consumed as a stomach medicine. A brew prepared from the dried roots is used as a prophylactic against fever (Forman, 1960). Those pharmacological effects were suspected to come from its flavonoid content. However, scientific information about phytochemical content of the leaves is still very limited (Raharjo, 2004; Arkarapanthu *et al.*, 2005).

The *E. pursaetha* (Mimosaceae), is an endemic medicinal plant, also known as Elephant creeper or immense woody climber, belongs to the family of “Fabaceae” (Priya and Srinivasa Rao, 2011). The seeds of *E. pursaetha*, have been used for many ailments according to the tribal Ayurvedic practitioners. The seed kernel is a potential source of drug for various ailments such as cancer (Liu *et al.*, 1972), dropsy, eye diseases, cuts, wounds, snake bite, respiratory problems, debility, Tuberculosis and anasarca (Vidya *et al.*, 2012). Thus, some authors have claimed that the seed kernels of the plant have medicinal importance like *in vitro* antibacterial activity, which was screened against both gram positive and gram negative bacteria. This species possess a variety of uses, including narcotic or as a tonic and also used for curing liver troubles, easing body pains, warding off colds, curing eye diseases, arthritis and paralysis (Johnson, 1999). The powdered seed kernel can be given to women for post-delivery recuperation and cures cough and stomachache. The paste obtained from the kernel is used as a contraceptive (Das *et al.*, 2003). This experiments were designed to evaluate the comparative *in vitro* anticancerous and cardioprotective activity of *C. barbata* and *E. pursaetha* methanolic leaf extracts.

MATERIALS AND METHODS

Plant collection and identification: Leaves of *C. barbata* and leaf of *E. pursaetha* were collected from different parts of Chittagong region, Bangladesh in September, 2013. These plants were identified by Dr. Shaikh Bokhtear Uddin, Taxonomist and Associate Professor, Department of Botany, University of Chittagong.

Chemicals and drugs: To the commercially available lyophilized Streptokinase vial (Durakinase, DongkookPhama. Co. Ltd, South Korea) of 15 00000 IU, 5 mL sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 μ L (30,000 IU) was used for *in vitro* thrombolysis. Absolute methanol (99.50%) and Vincristine Sulfate (VS) were purchased from Sigma-Aldrich, Munich, Germany.

Ethical consideration: The study protocol was approved by the P and D Committee (Pharmacy and drug committee institutional ethics committee), Department of Pharmacy, International Islamic University Chittagong, Bangladesh. Blood samples were collected from the students of the Department of Pharmacy, International Islamic University Chittagong. A written consent was taken from all of the volunteers.

Preparation of extracts: Methanolic leaf extracts of *C. barbata* and *E. pursaetha* were dried and ground (Moulinex Blender AK-241, Moulinex, France) into powder (40-80 mesh, 500 g) and soaked for 7 days with 2-3 days interval in 2.0 L of methanol at room temperature ($23\pm 0.5^\circ\text{C}$). Filtrate obtained through cheesecloth and Whatman filter paper No. 1 was concentrated under reduced pressure at the temperature below 50°C using rotary evaporator (RE 200, Sterling, UK). The extracts (yield 4.4-5.6% w/w) were all placed in glass petri dishes (90 \times 15 mm, Pyrex, Germany). A 100 mg each of the extracts was suspended in 10 mL distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22 μm syringe filter. A 100 μL of this aqueous preparation was added to the microcentrifuge tubes containing the clots to check cardioprotective activity. The same concentration (10 mg mL⁻¹) of extracts was prepared for the screening of anticancerous activity.

Anticancerous activity screening: Anticancerous activity of methanolic extracts were evaluated by the brine shrimp lethality bioassay, which is widely used for screening bioactive compounds (Meyer *et al.*, 1982; Zhao *et al.*, 1992). In this study, a simple zoological organism (*Artemia salina*) was used as a convenient monitor for the experiment. The eggs of the brine shrimp were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 h to develop into larval shrimp called nauplii. The anticancerous assay was performed on the brine shrimp nauplii using the Meyer method. The test samples (extract) were prepared by dissolving them in DMSO (not more than 50 μL in 5 mL solution) plus seawater (3.8% NaCl in water) to attain concentrations of 10, 50, 100, 200, 300 and 500 $\mu\text{g mL}^{-1}$. A vial containing 50 μL DMSO diluted to 5 mL was used as a control. Standard vincristine sulfate was used as a positive control. Mature shrimps were placed into each of the experimental vials. After 24 h, the vials were inspected using a magnifying glass and the number of surviving nauplii in each vial was counted (Alam *et al.*, 2015). From these data, the percentage of lethality of the brine shrimp nauplii was calculated for each concentration using the following equation:

$$\text{Mortality (\%)} = \frac{N_t}{N_0} \times 100$$

Where:

N_t = Number of dead nauplii after a 24 h incubation

N_0 = Number of total nauplii transferred i.e., 10

The LC_{50} (median lethal concentration) was determined from the log concentration versus percentage mortality curve.

Cardioprotective activity

Blood sample: Two milliliter of blood was drawn from five healthy human ($n = 4$). They had no history for taking any types of contraceptive or anticoagulant therapy. A 500 μL of blood was transferred to each of the three previously weighed microcentrifuge tubes to form clots.

Clot lysis: At first, three different sterile microcentrifuge tube (0.5 mL tube^{-1}) were taken and weighed. Then 2 mL venous blood was drawn from human volunteers and distributed in pre weighed sterile microcentrifuge tube. This tubes were incubated at 37°C for 45 min. In this process, serum was totally eliminated after the formation of clot without disturbing the clot. Each clot containing tube was again weighed to know the weight of clot. For the determination of clot weight, weight of tube alone were excluded from weight of clot containing tube. To each microcentrifuge tube containing pre-weighed clot, 100 μL of methanol extracts of both plants (*C. barbata* and *E. pursaetha*) were added separately. About 100 μL of streptokinase was used as a positive control and 100 μL of distilled water was used as a negative control. At last, all the tubes were incubated at 37°C for 90 min. In this way, clot lysis was observed. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference in weight before and after clot lysis was expressed as percentage of clot lysis (Prasad *et al.*, 2006). Evaluation of cardioprotective effects of both of methanolic extracts are exhibited by the equation as shown below:

$$\text{Clot lysis (\%)} = \frac{\text{Weight of released clot}}{\text{Clot weight}} \times 100$$

The experiment was repeated with the blood samples of the 4 volunteers.

Statistical analysis: The significance between percent (%) of clot lysis by streptokinase and plant extracts were tested by the paired t-test analysis using the software SPSS, version 20.0 (SPSS for Windows, Version 20.0, IBM Corporation, New York, USA). Data are expressed as Mean±Standard deviation. The mean difference between positive and negative control was considered significant at $p < 0.05$.

RESULTS

Anticancerous bioassay: Following the procedure of Meyer, the lethality of the methanol crude extract of *C. barbata* and *E. pursaetha* leaves were determined on *Artemia salina* after sample exposure for 24 h. The negative control (vehicle only) and vincristine sulfate (positive control) were also used to compare the toxic activities of the extracts. This technique was applied to determine the general toxicity of the plant extract. Percent mortality of brine shrimp at six different concentrations (10-500 $\mu\text{g mL}^{-1}$) of the extracts has been presented in Table 1. From Table 1, it is clear that the percentage of mortality is directly proportional to the extract concentrations. The LC_{50} values of the methanol extract of *C. barbata* and *E. pursaetha* obtained in the present experiment were 104.04 and 330.31 $\mu\text{g mL}^{-1}$, respectively. Therefore, the *C. barbata* demonstrated greater toxicity compared with *E. pursaetha*. The LC_{50} value for the standard drug vincristine sulfate was 12.59 $\mu\text{g mL}^{-1}$. However, no mortality was obtained for the negative control group.

Cardioprotective activity: Addition of 100 μL streptokinase (positive control) to the clots along with 90 min of incubation at 37°C, showed 63.54±2.61% clot lysis. However, distilled water (negative control) treated-clots showed only negligible clot lysis (4.21±0.73%) (Table 2). The mean difference in clot lysis percentage between positive and negative control was very significant (p -value<0.05). Treatment of clots with *C. barbata* and *E. pursaetha* extracts provided the clot lysis 45.69±1.96 and 38.19±1.83%, respectively Table 2. The mean percentage of clot lysis by *C. barbata* and *E. pursaetha* was statistically significant ($p < 0.05$). *Cyclebarbata* showed relatively higher

Table 1: Percentage of mortality of both the extract at six concentrations

Concentration ($\mu\text{g mL}^{-1}$)	LogC	Mortality (%)		
		CB	EP	Vincristine sulphate
10	1.00	10	0.00	40
50	1.699	50	10.00	80
100	2.00	60	10.00	100
200	2.301	80	30.00	100
300	2.477	80	40.00	100
500	2.699	100	80.00	100
LC_{50}		104.04	330.31	12.59

Table 2: Effect of both extracts (10 mg mL^{-1}) on *in vitro* clot lysis

Treatment	Percentage of clot lysis
Streptokinase (positive control)	63.54±2.61**
Distilled water (negative control)	4.21±0.73**
CB	45.69±1.96**
EP	38.19±1.83**

** $p < 0.05$ compared to control

percentage of clot lysis than the *E. pursaetha* although the values were significant (p -value <0.05) compared to those of both positive control (streptokinase) and negative control (water). Percent clot lysis obtained after treating the clots with both methanolic extracts and appropriate controls is shown in Table 2. Statistical representation of the effective clot lysis percentage by herbal preparations, positive cardioprotective control (Streptokinase) and negative control (sterile distilled water) done by paired t-test analysis, clot lysis % is represented as Mean \pm SD ** p <0.05 compared to control.

DISCUSSION

Toxicity profile of plant materials is mainly an important criteria to experts and medical practitioners (Singh and Singh, 2005; Fowles *et al.*, 2012; Okwuosa *et al.*, 1993) and anticancerous assay was conducted in this study to learn about both the toxicity study of plant extracts through the brine shrimp lethality (LC_{50} , 24 h) test. Logarto (Parra *et al.*, 2001) showed a great correlation ($r = 0.85$, $p < 0.05$) between the LC_{50} of brine shrimp lethality test and the severe oral toxicity assay in mice. Based on that correlation, brine shrimp lethality $LC_{50} < 10 \mu\text{g mL}^{-1}$ (LD_{50} between 100 and also 1000 mg kg^{-1}) is measured as cutoff value on anticancerousity (Parra *et al.*, 2001; Chew *et al.*, 2012). In this present experiment, significant anticancer activity was found for *C. barbata* whereas, moderate activity was found for *E. pursaetha* compared with the standard drug vincristine sulfate. In the case of cardio protective assay, *C. barbata* exhibited higher cardioprotective activity than *E. pursaetha*. These methanolic leaf extracts were compared with streptokinase as positive control and distilled water as negative control. Compared to the clot lysis percentage obtained through streptokinase and also water, a significant ($p < 0.05$) cardioprotective effect was observed after treating the clots to *C. barbata* and *E. pursaetha* leaf extract. It is established that there are some bacterial pollutants of plants that have plasminogen receptors which certain for plasminogen. Cell surface certain of plasminogen is instantly activated to plasmin that could lead to fibrinolysis (Pantzar *et al.*, 1998). Bacterial plasminogen activator such as staphylokinase, streptokinase, act as cofactor molecules that cause exosite formation and improve the substrate performance towards the enzyme. Staphylokinase activates plasminogen to be in a position to break down clots, also damages the extracellular matrix and fibrin particles that keep cell together (Parry *et al.*, 2000; Collen, 1990; Rahman *et al.*, 2013). From the above experiments, it would be alluring to investigate both the mechanism underlying clot lytic effects demonstrated by *C. barbata* and *E. pursaetha* leaf extract. However, these activities might be due to the presence of bioactive or inhibitory compounds or synergism by the existence of some compounds. Because a variety of constituents, such as saponin, polyphenols, flavonoids and alkaloids, may be present in the extracts, further extensive investigations are required to determine the specific anticancerous and cardioprotective properties present in these leaf extracts. Both of the plant extract *C. barbata* and *E. pursaetha* leaves contain promising anticancerous and cardioprotective activity *in vitro*. These plants could be incorporated as a anticancerous agent to treat anticancer as well as a cardioprotective agent for the treatment of patients suffering from cardiac diseases. Further *in vivo* investigations are needed for their active principles to elucidate.

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