Tropical Plant Research
An International Journal
© Society for Tropical Plant Research

ISSN (E): 2349 – 1183
ISSN (P): 2349 – 9265
3(2): 390–395, 2016

Research article

Evaluation of *in-vitro* cytotoxic and thrombolytic activity of methanolic extract of *Xanthium indicum* L.

Muhammad Sazzad Hossain[1], Md. Hossan Sakib[1*], Mohammad Shahin Alam[1], Ahsan Ullah[2], Sadequr Rahman[3] and Limon Kanti Shill[2]

[1]Department of Pharmacy, University of Science and technology Chittagong, Bangladesh
[2]Department of Pharmacy, International Islamic University Chittagong, Bangladesh
[3]Department of Public Health, North South University, Dhaka, Bangladesh

*****Corresponding Author: sakibiiucph@gmail.com [Accepted: 16 July 2016]

Abstract: The present study was designed to investigate the cytotoxic and thrombolytic activity of methanolic extract of fruits of *Xanthium indicum*. Methanolic extract of *Xanthium indicum*, was used to evaluate its cytotoxicity in Brine shrimp lethality bioassay where vincristine sulphate was used as standard drug. Thrombolytic effect of the fraction was investigated in clot lysis experiment. In Brine shrimp lethality bioassay, LC50 value of the extract was 30.90 µg.ml^{-1} and vincristine sulphate served as the positive control showed LC50 value 10.51 µg.ml^{-1}. The extract exerted 48.47% lysis of the blood clot in thrombolytic activity test while 48.83% and 13.82% lysis were obtained for positive control (streptokinase) and negative control respectively. Compared to vincristine sulphate. It is evident that the methanolic extract of fruits of *Xanthium indicum* was cytotoxic. So, the extract possessed considerable thrombolytic activity. Which compounds is responsible for the present pharmacological actions and to know their mechanism of action, extensive pharmacological and phytochemical experiments are essential.

Keywords: *Xanthium indicum* - Cytotoxic - Thrombolytic - Vincristine - Streptokinase.

[Cite as: Hossain MS, Sakib MH, Alam MS, Ullah A, Rahman S & Shill LK (2016) Evaluation of *In-Vitro* Cytotoxic and Thrombolytic Activity of Methanolic Extract of *Xanthium indicum* L. *Tropical Plant Research* 3(2): 390–395]

INTRODUCTION

Due to the fact ancient times plants have served to be a natural source of treatments and therapies like aspirin, quinine, and coffee. Today, scientists are using these renewable resources to generate a new generation of therapeutic solutions. Plants improved by making use of biotechnology can produce the essential healthy proteins for innovative treatments for diseases like cancer, HIV, heart disease, diabetes, Alzheimer's sickness, kidney disease, Crohn's disease, cystic fibrosis, many sclerosis, spinal cord injuries, Hepatitis G, chronic obstructive pulmonary disorder (COPD), morbid obesity, arthritis and iron deficiency. The by using natural products with therapeutic properties is usually as ancient as human civilization and, for an extended time, mineral, plant and animal products were the leading sources of drugs. Vincristine in addition to vinblastine from *Catharanthus roseus*, atropine by *Atropa belladonna* and morphine and codeine by *Papaver somniferum*. It is estimated that 60% connected with anti-tumor and anti-infectious drugs already out there or under clinical trial are connected with natural origin (Rates 2001). The vast flavor these cannot yet be synthesized economically and are also still obtained from wild or developed plants. Natural compounds can be cause compounds, allowing the design and lucid planning of new drugs, biomimetic synthesis development along with the discovery of new therapeutic properties not yet assigned to known compounds (Hamburger & Hostettmann *et al*. 1991). In addition, compounds like muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicine in addition to phorbol esters, all obtained from facilities, are important tools used in medicinal, physiological and biochemical studies (Archana *et al*. 2011). Cytotoxicity is the products being toxic to cells. Cells exposed to a cytotoxic compound can respond in numerous ways. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly on account of cell lysis; they can stop rising and dividing; or they can

Received: 10 April 2016 Published online: 31 August 2016

initialize a genetic program of controlled cell phone death, termed apoptosis. Cells undergoing necrosis commonly exhibit rapid swelling, lose membrane sincerity, shut down metabolism, and release their contents into your environment upon lysis. Apoptosis is seen as a well-defined cytological and molecular event, including a big difference in the refractive index of this cell, cytoplasmic shrinkage, nuclear condensation, in addition to cleavage of DNA. Cytotoxicity assays utilized widely in drug discovery research to help predict which lead compounds might have safety concerns in humans before significant time and expense are incurred in their development. Other researchers study mechanisms of cytotoxicity as a way to gain a better understanding of the normal and abnormal biological processes that control cell growth, division, and death (Patel *et al*. 2009).

Thrombolysis could be the breakdown (lysis) of blood vessels clots by pharmacological means. It is colloquially called elot busting for this reason. It functions by stimulating fibrinolysis by plasmin through infusion regarding analogs of tissue plasminogen activator (tPA), the particular protein that normally activates plasmin. Thrombolytic therapy is the usage of drugs to break up or break up blood clots, which are the main reason behind both heart attacks and stroke. Thrombolytic medications are approved for your immediate treatment of stroke and also heart attack. The most widely used drug for thrombolytic therapy is muscle plasminogen activator (tPA), but other drugs can do a similar thing (Kawsar *et al*. 2011).

Xanthium indicum L. yield Herbs perennial, monoecism or perhaps dioeciously, 25–50 cm tall. Stems climbing or erect, simple or branched, crispately pubescent. Results in alternate; nanophyllscordate or ovate, 3–10 mm; stipules lanceolate-linear, 5–7×0.5–0.8 mm, glabrous, without cystoliths; petiole 1–5 mm; Female inflorescences individual, 4–6 mm in diam.; peduncle los angeles. 1 mm; receptacle nearly orbicular, 3–5 mm inside diam.; bracts triangular; bracteoles lanceolate-linear. Men flowers 4-merous (Harnischfeger 2000).

MATERIALS & METHODS

Collection of Plant Components

The leaves of *Xanthium indicum* L. were accumulated from Chittagong local forest area; the leaves regarding *Xanthium indicum* were collected at their totally mature form. After cleaning, the results in were taken and splitting the peal, next air dried for 8 days, and kept in an oven at 45°C with 72 hours. 250 gm of dehydrated powder was cold extracted with Methanol. Dried powder soaked with methanol for 1 week. Then filtered to take the targeted extract, extract containing beaker was added to the water bath (at 40–45°C) to evaporate the solvent from your extract (Prasad 2007).

Preparation of Extraction

The extract is prepared by cold extraction process. In this process the coarse powder was submerged in ethanol (95%) since ethanol is the most common solvent for extracting most of the constituents present in herbal materials. Amber glass bottle were used for this purpose, which were kept at room temperature and allowed to stand for 7 days with occasional shaking and stirring. When the solvent became concentrated the contents were first decanted by using cotton and then filtered through Whatmann No.1 filter paper. The filtrate so obtained was then concentrated to dryness through the evaporation of solvent using rotary evaporator. Finally we got the concentrated semi-solid extract. The concentrated were then used as crude extract of respective test experiments. In our present investigation, we used methanolic extract for cytotoxic and thrombolytic activity (Mackeen *et al*. 2000)

In-vitro Cytotoxic Review

Brine shrimp lethality bioassay is widespread in the bioassay for the bioactive chemical substances. Here simple zoological organism (Artemia salina) was used to be a convenient monitor for the screening. The dried cyst on the brine shrimp were collected from an aquarium shop (Chittagong, Bangladesh) in addition to hatched in artificial seawater (3.8% NaCl alternative) with strong aeration for 24 hours day/dark cycles to mature shrimp termed nauplii. The cytotoxicity assay was conducted on brine shrimp naupli using Meyer procedure (Sarkar & Farooque 2004).

Materials

Artemia salina Leach (brine shrimp ova), Sea salt non ionized NaCl, Modest tank with perforated dividing dam to hatch this shrimp, Lamp to attract the nauplii, Pipette (1 ml in addition to 5 ml), Micropipette (1–10 minuscule liter), Glass vials (5 ml), Magnifier, Test sample for experimental plants (Sarkar & Farooque 2004).

Hatching connected with Brine Shrimp Eggs

Artemia salina Leach (brine shrimp eggs) collected on the pet shop was used as this test organism. Simulated sea water was consumed in the small tank and the shrimp ova (1.5 $g.mL^{-1}$) were included in one side of the tank and this also side was covered. The shrimps were permitted to one side of tank and that side was covered. The

shrimp were allowed for 2 days to hatch and mature seeing that nauplii (larvae). Constant oxygen supply was executed during the hatching time. The hatched shrimps were attracted to the lamp opposed to this of the divided tank through this perforated dam. These nauplii were taken due to this bioassay (Islam *et al.* 2002.)

Preparation of the Simulated Beach Water

38 grams sea salt seemed to be weighted accurately, dissolved in 1 liter of sterilized distilled water then filtered to get clear solution. The ph on the sea water was maintained between 8.0–8.5 applying 1n na0h solution (mosaddik *et al.* 2003).

Preparation connected with Sample Solution

At first take 19 ml distilled mineral water in beaker add 1 ml DMSO (dimethyl sulfoxide) so prepares stock solution. Clean test pipes were taken. These test tubes were for different concentration (one test tube for every single concentration) of test samples. 5 mg methanolic extracts of *Xanthium indicum* were being accurately weighed and dissolved in 4 ml stock options solution. Thus a concentration of 1000 $\mu g.ml^{-1}$ was obtained which used for extract solution. Then taking 1ml get solution from beaker & add 9 ml stock options solution In vials thus prepared remaining extract solution. From this extract alternative 12.5 $\mu g.ml^{-1}$, 25 $\mu g.ml^{-1}$, 50 $\mu g.ml^{-1}$, 100 $\mu g.ml^{-1}$, 200 $\mu g.ml^{-1}$ and 400 $\mu g.ml^{-1}$ were consumed in ten test tubes respectively and tweaked volume 5 ml sea water. Eventually 20 nauplii are then applied with each test tube (Mosaddik *et al*. 2003)

Getting ready of Control group

Control groups are used in cytotoxicity review to validate the test method and be sure that the results obtained are only a result of the activity of the test agent along with the effects of the other possible variables are nullified. Usually two types connected with control groups are used-i) Constructive control lii) Negative control (Robin *et al.* 1989)

Getting ready of Positive Control group

Positive control in cytotoxicity study is usually a widely accepted cytotoxic agent and a result of the test agent is compared while using the result obtained for the positive management. In the present study, vincristine sulphate was used for the reason that positive control. 3 mg of vincristine sulphate seemed to be dissolved in 1. 8 ml of distilled water to have a concentration of 5 $mg.ml^{-1}$. This seemed to be used as stock solution of vincristine sulphate. Through a micropipette 400, 200, 100, 50, 25 and 12.5 µl on the stock solution were transferred in 6 unique vials. NaCl solution (brine water) was included in each vial making the volume nearly 5 ml. The final concentration of vincristine sulphate from the vials became 400 $\mu g.ml^{-1}$, 200 $\mu g.ml^{-1}$, 100 $\mu g.ml^{-1}$, 50 µg.ml, 25 $\mu g.ml^{-1}$ in addition to 12.5 $\mu g.ml^{-1}$ respectively. The experiment was repeated triple (Robin *et al.* 1989)

Preparation of negative control

100 µl connected with distilled water, DMSO and ethanol was added to all of the three remarked glass vials containing 5 ml connected with simulated sea water and 20 shrimp nauplii make use of as control groups. If the brine shrimp nauplii in these vials show an immediate mortality rate, then the test is regarded as in valid as the nauplii died caused by some reason other than the cytotoxicity on the samples (Robin *et al.* 1989)

Application of Brine shrimp Naupli

Through the Pasteur pipette 20 living nauplii were added to all of the vials containing 5 ml of simulated beach water. A magnifying glass was for convenient count of nauplii. If the counting on the 20 nauplii was not be doable accurately (Robin *et al*. 1989).

Counting of your Naupli

After 24 hours, the vials are observed using a magnifying glass and the sheer numbers of survival nauplii in each vial ended up being counted and recorded. From this details, the percentage of mortality of nauplii was calculated each concentration of the sample. The median lethal concentration (LC50) of your test samples was obtained by a plot of percentage of your shrimps killed against the logarithm of your sample concentration (Robin *et al.* 1989).

In-Vitro Thrombolytic Analysis

Thrombolysis is the breakdown (lysis) with blood clots by pharmacological means. It is colloquially categorized as clot busting for this reason. It operates stimulating fibrinolysis by plasmin through infusion with analogs of tissue plasminogen activator (tPA), a protein that normally activates plasmin.

Groundwork of Extract Solution for Thrombolytic Examine

10 mg of the extract appeared to be suspended in 10ml distilled water and shaken vigorously for a vortex mixer. Then the suspension was kept overnight and decanted to eradicate the soluble supernatant, which was filtered through the filter paper (Whatman No. 1). The best was then ready for in vitro review of clot lysis activity (Mackeen *et al.* 2000)

Groundwork of Streptokinase (SK) Resolution

To the commercially available lyophilized SK vial (PolaminWerk GmbH, Herdecke, Germany) of just one, 500,000 I.U., 5 ml sterile and clean distilled water was added and compounded properly. This suspension was used for a stock from which 100 µl (31,000 I.U) was used in in vitro thrombolysis (Mackeen *et al*. 2000).

Specimen with Thrombolytic Test

3ml blood was drawn from healthy human volunteers without using history of oral contraceptive or anticoagulant therapy (with a protocol approved by the Institutional Strength Committee of Central India Institute with Medical Sciences, Nagpur). 500 µl of blood was transferred to every single ten previously weighed alpine tubes to create clots (Mackeen *et al.* 2000).

Test Procedure for Thrombolytic examines

Experiments for clot lyses were toted as reported earlier (Mackeen *et al.* 2000). Venous blood drawn from healthy volunteers was transferred within pre-weighed sterile Epen drop tube (500µl/tube) plus incubated at 37°C for 45 a matter of minutes. After clot formation, serum was wholly removed (aspirated out without troublesome the clot formed). Each tube having clot was again weighed to look for the clot weight (Clot weight = excess fat of clot containing tube - excess fat of tube alone). Each Epen drop tube containing clog was properly labeled and 100 µl of plant extract was added onto the tubes. All the tubes ended up being then incubated at 37°C for 95 minutes and observed for clot lysis. Just after incubation, fluid obtained was removed and tubes were again weighed to see the difference in weight after clog disruption. Difference obtained in weight utilized before and after clot lysis appeared to be expressed as percentage. Thrombolytic Activity with methanol extract of *Xanthium indicum* clot lysis. Streptokinase and water were used for a positive and negative (non-thrombolytic) regulate respectively. The experiment was repeated several times a day with the blood samples of several volunteers.

RESULTS

Brine Shrimp Lethality Bioassay

Brine shrimp lethality results of the fraction of *Xanthium indicum* L. leaves is shown in figure 1 and LC_{50} calculated value is recorded in table 1. The fraction showed potential cytotoxic activity with LC_{50} value of 13.56 µg.ml^{-1}. Vincristin sulphate served as the positive control for this brine shrimp lethality assay and its LC_{50} value was 10.51 µg.ml^{-1}.

Table 1. Cytotoxic activity of *Xanthium indicum.*

Con[c]	Nauplii	No.of naupli death	Log C	% of Mortality	LC_{50} µg/ml
12.5	20	9	1.096	35	
25	20	13	1.39794	45	
50	20	14	1.69897	80	
100	20	15	2	85	**13.561**
200	20	18	2.3010	90	
400	20	20	2.60205	100	

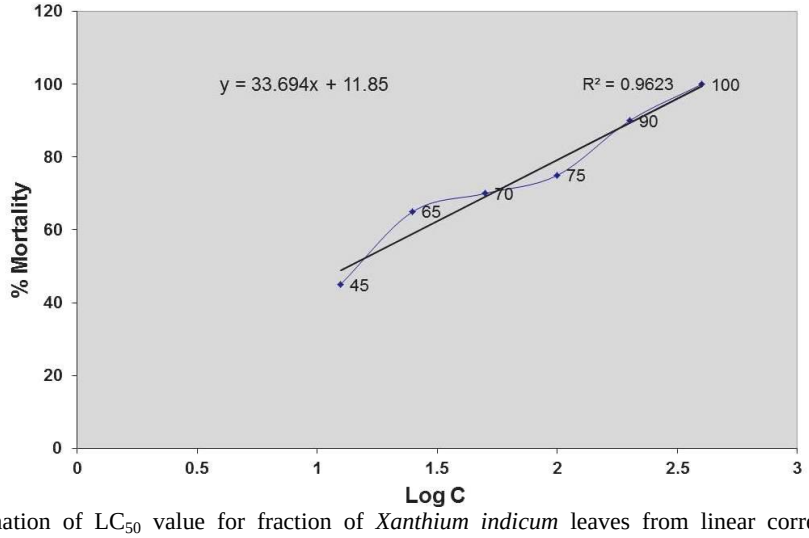

Figure 1. Determination of LC_{50} value for fraction of *Xanthium indicum* leaves from linear correlation between log concentrations versus percentage of mortality.

Thrombolytic Activity

The methanolic extract of *Xanthium indicum* leaves is exerted 27.81% lysis of the blood clot in thrombolytic activity test while 48.83% were obtained for positive control (streptokinase) and 13.82% were obtained for negative control respectively which showed in table 2. So, the extract possessed considerable thrombolytic activity (Fig. 2).

Table 2. Thrombolytic Activity of *Xanthium indicum.*

Exract	Positive control	Negetive control
27.806%	48.83%	13.82%

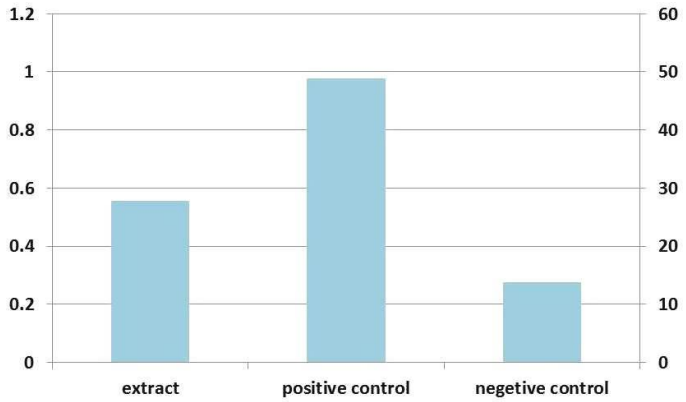

Figure 2. Methanolic extract of *Xanthium indicum* thrombolytic effect compared with standard (Streptokinase) and negative control.

DISCUSSION

Plant-derived medicines contain a long history of usage for the prevention together with treatment involving human diseases. Today, many pharmaceuticals currently approved when using the Food and Drug Administration (FDA) get hold of origins to plant sources. A number of plants source especially several leaves and vegetables are literally studied for their supplements having anticoagulant, antiplatelet and fibrinolytic activity and the way to find evidence that consuming such food brings about deterrence of coronary events and cerebrovascular event. Some of them plant products are generally modified further with recombinant technology to make them more effective and site distinct. In some of our thrombolytic assay, the comparison of positive control using negative deal with clearly demonstrated that clot dissolution won't occur when water was added over the clot. When compared with the clog lysis percentage obtained through SK alongside water, an extremely significant thrombolytic task was detected after treating the clots using C.arborea, chloroform percentage. Cell floor bound plasminogen is easily activated to be able to plasmin, which could lead to fibrinolysis. Microbial plasminogen activator: staphylokinase, streptokinase, be cofactor molecules that help with exosite formation and increase the substrate presentation on the enzyme. Staphylokinase activates plasminogen that will melt clots, also destroys the extracellular matrix alongside fibrin fibers that hold cells jointly.

Toxicity of plant materials is a serious concern to scientists and dieticians and thus cytotoxic assay was conducted in this study to think about the toxicity profile of the plant extracts across the Brine Shrimp Lethality (LC50, all day together with) test. Lagarto demonstrated a fantastic connection (r2 = 0. 91) relating into the LC50 of the brine shrimp lethality make certainly the acute oral toxicity assay throughout mice. Influenced by that correlation, brine shrimp lethality LC50< 10 $\mu g.ml^{-1}$ (LD50 involving 100 and 1000 $mg.kg^{-1}$) is certainly as the cut off value involving cytotoxicity. Depending on measured LC50 values in the extracts no one was found severely lethal or toxic to build processed as pharmaceutical products in thrombolytic employs. Yet, the extremely significant effect of *Xanthium indicum* demonstrates it to locate the best thrombolytic component for even more processing.

CONCLUSION

The results of the present study led us to the inference that the plant extract possess modest cytotoxicity and decreased level of thrombolytic properties. In the work we tried to give our maximum effort to fulfill the task successfully and in the proper way of work. The work is done within the collaboration of all the teachers and we tried to take each data carefully.

Since the extract is reported to contain a range of compounds, it is difficult to describe these observed activities to any specific group of compounds. Hence, further studies are suggested to be undertaken to pin point

the exact compound(s) and to better understand the mechanism of such actions of *Xanthium indicum* scientifically. The median lethal concentration (LC_{50}) of *Xanthium indicum was* 13.56 $\mu g.ml^{-1}$. The thrombolytic effect of *Xanthium indicum* was 27.81% at 10 mg in 10 ml conc.

ACKNOWLEDGEMENTS

Authors are grateful to Team of Pharmacy, International Islamic University Chittagong for giving all facilities to use whole project. Authors are also fortunate to Dr. Shaikh Bokhtear Uddin, Link Professor, Department of Botany, and College or university of Chittagong for identifying plants. Authors also acknowledge the volunteer blood donors for cooperation from the project. The total research work seemed to be supported by Department of Pharmacy, Overseas Islamic University Chittagong, Bangladesh. This project was an inclusive element of academic degree of M. Pharm scholar.

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