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EVALUATION OF CYTOTOXIC AND EX-VIVO CARDIOPROTECTIVE INVESTIGATION OF ETHANOLIC EXTRACT OF *GLYCOSMIS PENTAPHYLLA* LEAVES

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ABSTRACT

The present study was designed to investigate the cytotoxic activity of methanol extract of *Glycosmis pentaphylla* on brine shrimp and thrombolytic activity of methanol extract of *Glycosmis pentaphylla* on human blood. Ethanolic extract of *G. pentaphylla* was assessed with the Brine shrimp lethality bioassay was used to evaluate cytotoxicity and ethanolic extract of *G. pentaphylla* assessed with human blood to evaluate thrombolytic effect. The extract showed remarkable cytotoxic activity, LC_{50} value of the extract was 2.8315µg/ml compared to vincristin sulphate, a reference drug. It also has evaluated as thrombolytic agent compared to streptokinase. It has significant thrombolytic effect which was about 33.144%. These findings demonstrate that the leaves extract of *G. pentaphylla* have excellent cytotoxic activity and Glycosmis pentaphylla have excellent thrombolytic effect.

KEYWORDS: *Glycosmis pentaphylla*, bioassay, cytotoxic, $LC_{50,}$ cardioprotective activity.

INTRODUCTION

From the very beginning of the civilization there is an extreme relationship between human beings and plants. In ancient period the system of treatment was not enriched like today. The ancient people used to utilize several parts of plants in different treatment purposes. Plants

are used not only as medicine but also in a number of their daily jobs (e.g. fishing, hunting etc.) eventually plants are the ultimate caretaker of environment in a sense. A single part of plant may consists of numerous medicinal values, but it has been proved that direct intake of crude plant is not good; even it may causes toxicity more cases. However plants are used as the major sources in modern and traditional system of medicine. Bangladesh has a lot of medicinal plants which has been using for a period of times locally as well as in ayurvedic & herbal medicine system; has been developing day by day. Though the plants are using unconsciously and in an improper dose, a chronic adverse effect may develop after a long run. So the proper use of plant with appropriate dose can be ensured by surveying its effect with scientific methodology. Today's medical science is employing a great concern over cancer disease, which may be defined as the abnormal cell division within different organs of the body. The major causes of cancer have been reported is either by microbial infection or by free radicals. Drugs that used in treatment of cancer are mostly cytotoxic. Plant having cytotoxic activity can be referred for further laboratory process to isolate chemicals that can be used in treating cancer.

G. pentaphylla is a species of small shrub and flowering plant in the citrus family, Rutaceae, known commonly as orange berry (English).[1] *G. pentaphylla* has a long history of usage in traditional medicine against various ailments around the world. In Ayurvedic and other traditional medicinal practices the plant has been used against diseases like bilious complaints, cough, worms, jaundice, fever, inflammation, rheumatism, anaemia and vermifuge. Phytochemicals like alkaloids, flavonoids, terpenes and sterols have been isolated.[2] The literature review says *G. pentaphylla* has hypoglycemic, anti-inflammatory,[3] anti-oxidant [4] and more recent search on this, suggest that it is more apoptotic than others.[5]

Experimentals Procedure

Plant collection: The plant was collected from Bandarban, the hilly region of Chittagong, Bangladesh in the month of October 2012. Then the plant has been identified by Dr. Shaikh Bokhtear Uddin, associate Professor, Department of Botany, University of Chittagong.

Extraction: Extraction of plant leaves was done by using organic solvent.[8] The fresh leaves of *G. pentaphylla* were cut, washed and air dried at room temperature ($24^0 \pm 2^0$ C) for about 10 days. Dried leaves were macerated into coarse powder. Dried powder (500 gm) was then extracted using ethanol. Then ethanolic extract was shaken by rotary shaking apparatus for 7

days. The extract was collected using Buckner funnel. The ethanol was evaporated at a temperature below 45^0 C and concentrated extract was weighed 29 gm, stored at 4^0C.

Cytotoxic activity: Cytotoxic activity was conducted by used Hatching of Brine Shrimp. Cytotoxic activity of plant extract was determined by Brine Shrimp lethality bioassay as described by Meyer et al.[6] Shrimp eggs were added to the simulated "sea water" (38 g sea salt pure NaCl was weighed, dissolved in 1 litre of distilled water adjusted to pH 8.5 using 1N NaOH and was filtered off to get clear solution) in the larger compartment of an unequally dividend tank. The chamber was kept under illumination using a table lamp for 48 h for the eggs to hatch into shrimp larvae. The illuminated compartment attracts shrimp larvae (nauplii) through perforations in the dam. Mother solution was prepared using DMSO (dimethyl sulfoxide) and sea water as solvent (20% DMSO with rest portion water to make 1:1 solution). From the stock solution 8 other concentration was prepared as, 10, 50, 80, 100, 200, 300, 400 and 500µg/ml. 8 test tube was taken with 10 brine shrimp in each of them, filled with different conc. and sea water to make total volume 5ml. A test tube of DMSO was made as control. After 24 hour each test tubes were checked. From the % of morbidity of brine shrimp LC_{50} (lethal concentration) was calculated by plotting against logarithm of concentration. Computer software "BioStat-2009" was used to calculate.[8]

Cardioprotective activity: Cardioprotective activity was conducted by thrombolytic activity assay. Experiments for clot lysis were carried as reported earlier.[7,8] Venous blood drawn from healthy volunteers was transferred in different pre-weighed sterile alpine tube (500µl/tube) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed). Each tube weighed to determine the clot weight (Clot weight = weight of clot containing tube – weight of tube alone). And again were incubated at 37°C for 90 minutes and observed for clot lysis, after addition of 100µl plant extract. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Weight variation was calculated by the following equation,

$$\%\ clot\ lysis = \left(\frac{\text{Weight of the lysis clot}}{\text{Weight of the before lysis clot}}\right) \times 100$$

Statistical analysis: The % of lethality probability unit for each concentration was calculated using "SPSS-17".[9]

RESULTS AND DISCUSSION

Percentages of lethality on brine shrimp at nine different concentrations (2.5 to 160µg/ml) of ethanolic extract of *G. pentaphylla* presented in figure-1. Plant extract shows lethality as their concentration got increased. More specifically, from lower limit to 100% death, it's a clear dose dependency manner. The % of lethality probability unit for each concentration was plotting % response or % lethality against log concentration of corresponding LC_{50} was obtained 2.8315µg/ml (figure-1).

Addition of 100µl Streptokinase, as positive control (30,000 I.U.), to the clots along with 90 minutes of incubation at 37°C, showed 75.58% clot lysis. Clots when treated with 100µl sterile distilled water (negative control) showed 4.31% of clot lysis, which is negligible. The *in vitro* thrombolytic activity study revealed that *G. pentaphylla* exhibit prominent effectiveness to lyses coagulation of blood. So we can take this herb in our important consideration when we think about new anti-coagulant molecule development for its promising result, 64.48% of clot lysis (figure-2).

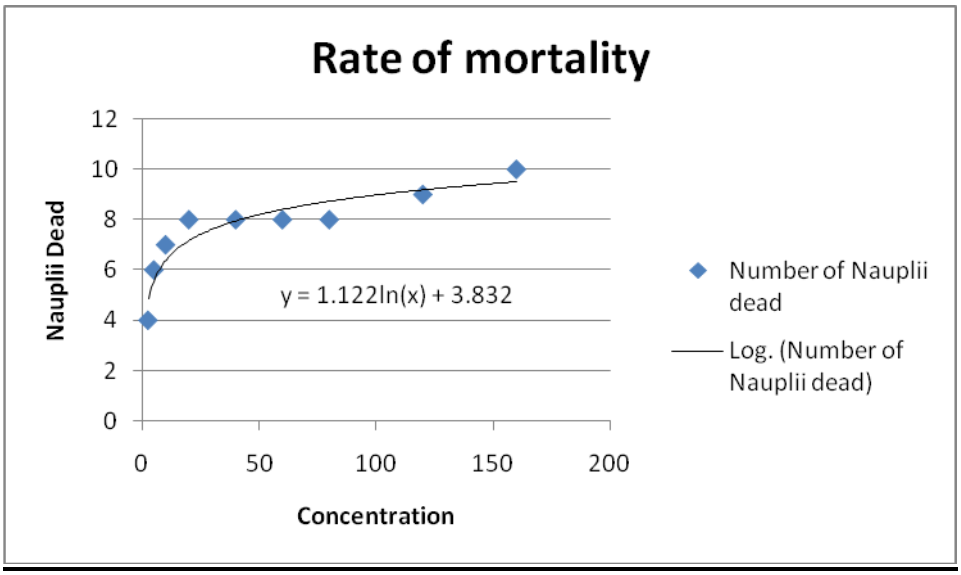

Figure-1: Graphical analysis for determination of LC_{50} of ethanolic extract of *G. pentaphylla*

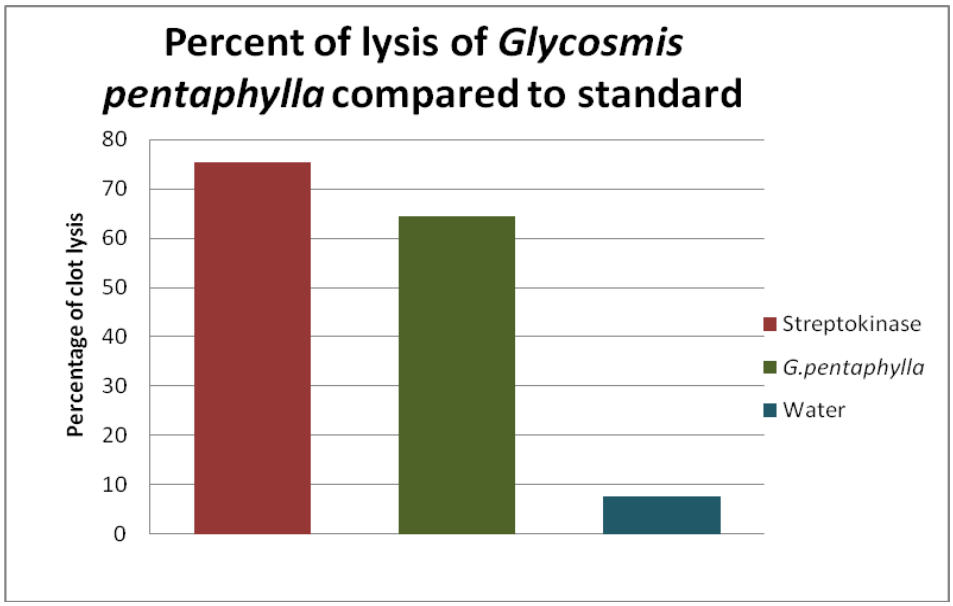

Figure-2: Percent of lysis caused by *G. pentaphylla* compared with Streptokinase (standard)

CONCLUSION

From the above study it can be concluded that ethanolic extract of *G. pentaphylla* may be a potential candidate for future thrombolytic and cytotoxic agent. It was observed that the activity increased with increasing amount of the concentration of extract. LC_{50} of ethanolic extract of *G. pentaphylla* was found 2.8135µg/ml. Further more study and isolation is needed to obtain site specific and more potent agent that causing this effect.

In spite of the beneficial effects of thrombolytic therapy are now well established [11] and the biochemical mechanisms of thrombolytic therapy have been make clear, but the search for alternative and complimentary therapy is still continuing due to some reasons including availability and diversity of natural resources, easy access and affordability. My present study showed that the leaves of *G. pentaphylla* showed significant thrombolytic activity. The percentage of weight loss due to clot lysis induced by *G. pentaphylla* was 64.48%. That's why this plant is seemed to potent sources for further investigation to find the responsible lead compounds for thrombolytic activity. I suggest, avoid *G. pentaphylla*, not to use as food supplement, since it is toxic (as found by brine shrimp cytotoxicity test).

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