Cytotoxic and Thrombolytic Activity of Leaves Extract of *Parthenium hysterophorus* (Fam: *Asteraceae*)

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Abstract

Phytochemical analysis of the extract of *Parthenium hysterophorus*, mostly active molecules considered from recent literature review are bisbenzylisoquinoline, protoberberine and indole alakaloids sesquiterpenes, quassinoids and limonoids. This review indicates that there are many antiprotozoal natural products already known that are require further scientific investigation. Pharmacological interest of these compounds promoted us to check for possible cytotoxic and thrombolytic activity. The crude extract of *Parthenium hysterophorus* showed cytotoxic activity against brine shrimp nauplii and LC_{50} value was 93.75 µg/ml. Thrombolytic effect was also screened out in our study and it was found that the average % of clot lysis were 16.52±2.91 for volunteer-1 and 19.52±4.02 for volunteer-2. The result showed significant effect with comparison to standard thrombolytic agent, streptokinase.

Keywords: *Parthenium hysterophorus* (Fam: *Asteraceae*), brine shrimp lethality bioassay, thrombolytic activity, streptokinase

Introduction

Parthenium hysterophorus, a native of tropical and subtropical America, is the most recent invader in Kathmandu valley. It has already threatened grassland ecosystems of Australia and India to a large extent (Stephen & Sowerby, 1996; Chippendale & Panetta, 1994; Goyal & Brahma, 2001). *Parthenium hysterophorus* appears to be potentially most harmful to native flora, animals and human health. Although it has already invaded grasslands of most of the urban cities and near highways in tropical to subtropical region no effort has been made to control *Parthenium hysterophorus*.

Parthenium hysterophorus L. (Family: Asteraceae; common names: Bitter weed, false ragweed, fever few, Parthenium weed, Ragweed, white top, etc; vernacular names: *Kanike ghans, Bethu ghans*, or *Padke phul*) is an annual, erect and profusely branched herb. Height varies between 50-150cm, stem highly branched; leaf simple with profusely dissected leaflets; flower heads occur on a corymb, phyllaries 10 in 2 series, ovate, dull white, 3-4mm in diameter; disc floret: numerous, dull white; stamen - 4, anther- exerted; ovary sterile; ray floret: found just opposite to inner phyllaries, only 5 ray florets per flower head, corolla obsolete, stamen-absent, stigma-parted, style short, ovary oval, dorsiventrally flattened. Fruit cypsela, each flower head bearing 5 cypsela, flat and triangular in shape with thin, white, spoon shaped appendages (Maharjan, 2006). A typical mature plant can produce from 15000 to 25000 seeds (Haseler, 1976; Joshi, 1991).

Materials and Methods

General experimental procedure

Usually the intact plant/plant part(s) is collected as a whole and sun-dried. In fresh condition, it is then oven-dried at reduced temperature (not more than 50ºC) to make suitable for grinding purpose. The coarse powder is then stored in airtight container with marking for identification and kept in cool, dark and dry place for use. Extraction can be done in two ways – cold extraction and hot extraction.

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Plant materials

Leaves of *Parthenium hysterophorus* (Fam: *Asteraceae*) was collected from Gazipur and Joydevpur area in March 2009 and were taxonomically identified by a Scientific Officer, Bangladesh National Herbarium (BNH) and one voucher specimen has been deposited there.

Extraction and isolation

Extraction of dried and powdered plant of *P. hysterophorus* was done by cold extraction process by using methanol as a solvent (Trease & Evans, 1989). The air dried and pulverized plant material (250.0gm) was cold extracted with methanol. And after that the fractions were evaporated by roto-dryer at low temperature (40 − 50°C) to dryness. Crude methanol extract was subjected to cytotoxic and thrombolytic activity study.

Antitumor Screening (brine shrimp lethality bioassay method)

Methanol extract was subjected to cytotoxic study. 1.0 mg sample was taken and a stock solution of 1000µg/ml was prepared with pure dimethyl sulfoxide (DMSO). A series of solutions of different concentrations were prepared from the stock solution by serial dilution method and the concentrations were as − 500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml, 31.25µg/ml. Then the samples were subjected to brine shrimp lethality bioassay (Goldstein & Kalkan, 1974; Meyer *et al*, 1982) for cytotoxic studies. In each test tube, containing different concentrations of test sample, 20 brine shrimp nauplii (*Artemia salina*) were added.

Two control groups were used in cytotoxicity study, to validate the test method and results obtained due to the activity of the test agent. In the study vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 µg/ml and serial dilutions were made using DMSO to get 10 µg/ml, 5 µg/ml, 2.5µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml, 0.078125 µg/ml and 0.0390 µg/ml of concentration. 30 µl of DMSO was added to each of three premarked glass vials containing 5 ml of simulated seawater and 10 shrimp nauplii to use as negative control groups. After 24 hours, the test tubes were observed and the numbers of survived nauplii in each test tube were counted and the results were noted. From this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration for each sample.

Thrombolytic activity study (Prasad *et* al, 2006)

Thrombolytic therapy reduces mortality and preserves left ventricular function in patients with myocardial infarction. Streptokinase is widely used fibrinolytic drug that was used in this study as standard. All thrombolytic agents work by activating the enzyme plasminogen that clears the cross-linked fibrin mesh. In our study 100mg of methanolic extract was used as experimental drug. 5ml of blood samples were collected from volunteer and distributed into five separate pre-weighed (W_1) microcentrifuge tubes. The blood specimen were centrifuged at 2500 rpm for five minutes and then incubated for 45 minutes at 37°C. After clotting of blood, serum was decanted and removed. Then weight of clotted blood (ΔW) was taken by subtracting the pre-weight (W_1) from the weight of clot containing tube (W_2) as - $\Delta W = W_2 - W_1$.

Then 100µl extract of *P. hysterophorus* was added to the clot containing tube. Similarly 100µl of streptokinase was added to clot of standard tube and 100µl of water was added to clot of blank tube those were used as positive and negative control respectively. Then all the tubes were incubated at 37°C for 90 minutes and weighed again for getting the weight variation among the pre weight and final weight (W_3) that was achieved for clot lyses (thrombolysis).

Result and Discussion

Antitumor Screening: In the present bioactivity study, the crude methanolic extract showed positive results indicating that the test samples are biologically active. Plotting of log of concentration (log C) versus percent mortality (% mortality) for all test samples showed an approximate linear correlation. From the graphs, the median lethal concentration (LC_{50}, the concentration at which 50% mortality of brine shrimp nauplii occurred) were determined and LC_{90} values were also determined to check the toxic level of the extract. The crude extract of *P. hysterophorus* showed significant cytotoxic activity against brine shrimp nauplii and LC_{50}

value was 93.75 µg/ml (Table 1 and Figure 1). The 90% mortality rate was also calculated to get the therapeutic index and the value was 750 µg/ml (Table 1 and Figure 1).

Table 1: Brine shrimp lethality bioassay of methanolic extract of *Parthenium hysterophorus*

Conc. (µg/ml)	Log C	% Mortality			LC_{50} (µg/ml)	LC_{90} (µg/ml)
		ME (1)	ME (2)	ME (Avg.)		
1000	3.00000	100	100	100.0	93.75	750.0
500.0	2.69897	75	80	77.5		
250.0	2.39794	60	70	65.0		
125.0	2.09691	50	50	50.0		
62.50	1.79588	45	50	47.5		
31.25	1.49485	25	25	25.0		

ME: methanolic extract

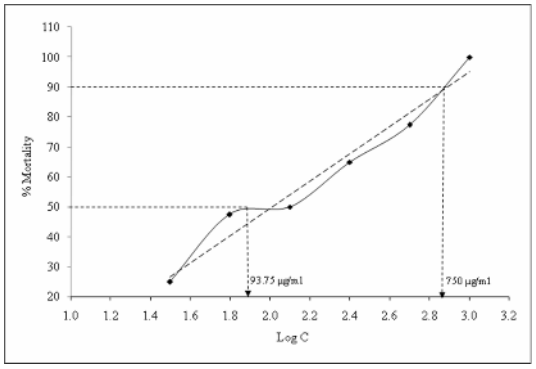

Figure 1: Determination of LC_{50} and LC_{90} of methanolic extract of *Parthenium hysterophorus*

Thrombolytic activity study: The percentage of weight loss of clot after application of extract solution was taken as the functional indication of thrombolytic activity. The study was implemented on two volunteer with five blood samples (for each) of mid-age (Table-2 and Figure 2). Average value of weight loss (in %) was calculated to examine the variation of two volunteer. Percentage of clot lysis was calculated with the following formula –

$$\% \text{ of clot lysis} = \frac{\text{weight of released clot}}{\text{clot weight}} \times 100$$

$$= \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

It was found that the average % of clot lysis were 16.52±2.91 for volunteer-1 and 19.52±4.02 for volunteer-2. There was a difference thrombolytic action between two volunteer in response to average activity due to body physiological difference (age, weight, % of clotting factor, food habit etc.).

Table 2: Thrombolytic activity study of methanolic extract of *Parthenium hysterophorus*

No. of Sample	Volunteer-1				Volunteer-2			
	W_1	W_2	W_3	% clot lysis	W_1	W_2	W_3	% clot lysis
1	4.1492	4.317	4.2854	18.83	4.1215	4.2239	4.2082	15.33
2	4.1045	4.275	4.2524	13.26	4.0703	4.2665	4.2275	19.88
3	4.1035	4.183	4.1691	17.48	4.1393	4.2802	4.2473	23.35
Standard	4.1030	4.308	4.2075	49.02	4.1416	4.2538	4.1952	52.23
Blank	4.1495	4.317	4.3043	7.58	4.1326	4.3056	4.3026	1.73

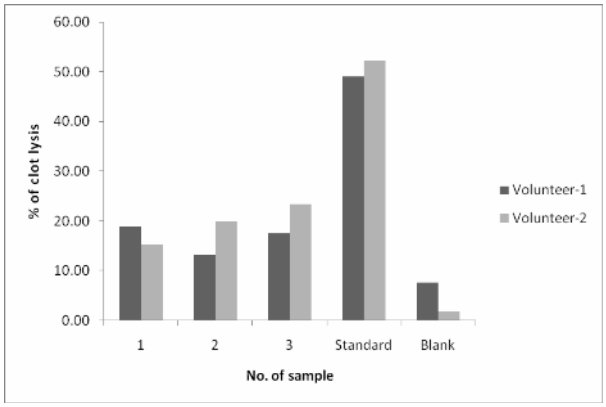

Figure 2: Comparison between the volunteer (in terms of thrombolytic effect) for methanolic extract (sample 1, 2 & 3) with standard and blank solution

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Reference

Chippendale J.F. and Panetta F.D., (1994). The cost of *Parthenium* weed to the Queensland cattle industry. *Plant Protection Quarterly*, **9**: 73-76.

Goldstein A.L. and Kalkan S.M., (1974). Principles of Drug Action, 2nd edn., Willey Biochemical Health Publications, pp. 376-381.

Goyal C.P. and Brahma B.C., (2001). A ray of hope against *Parthenium* in Rajaji National Park, *Indian Forester*, **127**(4): 409-414.

Haseler W.H., (1976). *Parthenium hysterophorus* L. in Australia, *Pest Articles & News Summaries* (PANS), **22**: 515-517.

Joshi S., (1991). Interference effects of *Cassia uniflora* Mill. On *Parthenium hysterophorus* L. *Plant and Soil*, **132**: 215-223.

Maharjan S., (2006). Phenology, leaf attributes and allelopathic potential of ***Parthenium hysterophorus L.*** a highly allergic invasive weed in Kathmandu valley, M. Sc Thesis, Central Department of Botany, Tribhuvan University.

Meyer B.B., Ferringi N.R., Futman F.J., Jacobsen L.B., Nichols D.E. and Mclaughlin J.L., (1982). Brine shrimp a convenient general bioassay for active plant constituents, *Planta Medica*, **5**: 31-34.

Prasad S., Kashyap R.S., Deopujari J.Y., Purohit H.J., Tori G.M. and Daginawala H.F., (2006). Development of an *in vitro* method to study clot lysis activity of thrombolytic drugs, *Thrombosis Journal*, **4**(2): 1-4.

Stephen W.A. and Sowerby M.S., (1996). Allelopathic potential of the weed, *Parthenium hysterophorus* L. in Australia, *Plant Protection Quarterly*, **11**: 20-23.

Trease G.E. and Evans W.C. (1989). Trease and Evans' Pharmacognosy, 13th edn.; Philadelphia; Baillire Tindall.