WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 6, 433-437. Research Article ISSN 2277– 7105

POTENTIAL EVALUATION OF *EX-VIVO* CARDIO-PROTECTIVE ACTIVITY OF LEAVES OF *DESMODIUM PULCHELLEUM* (L) BENTH.

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Article Received on
02 April 2015,

Revised on 24 April 2015,
Accepted on 18 May 2015

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SUMMARY

The aim of this investigation is to find out cardioprotective drugs to treat heart block or other blood clot coagulation disorders. In the present studies, of the crude methanolic extract (86.12% ±0.1866), Ethanolic extract (43.94 %± 0.2766), Hexane soluble fraction (50.88% ± 0.3113), the Aqueous soluble fraction (46.46%± 0.1228), Distilled Water (4.84% ± 0.0845) of *D. pulchelleum* demonstrated strong cardioprotective activity, compared with Streptokinase (67.84% ± 0.0800).

KEYWORDS: *Desmodium pulchelleum*, Cardioprotective activity, Leaves, Streptokinase, Human body.

INTRODUCTION

Desmodium pulchellum (L) Benth.(Fabaceae) is known at its different local names such as Ursi (Assamese) Jatsalpan (Hindi), Jutasalpani (Bangladesh). The shrubs are 0.5 to 1.5 m tall, stem angular. Leaves are leaflets 7-10 x 4-6 cm, 3-foliolate, obovate, apex obtuse, base acute, thinly hairy above, densely below, lateral leaflets smaller than the terminal one. Racemes panicled, terminal or sub-terminal, floral leaves bifarious, to 12 mm across, orbicular, hispid, with one filiform appendage at base. Flowers bracts and bracteoles minute;

1-3 together; teeth lanceolate, calyx puberulous, shorter than the tube; standard petal 4-5 mm long, pale blue, glabrous. The pods are 7 mm long, 2-jointed, joints orbicular and pubescent.[1]

A decoction of the bark is used in diarrhoea, haemorrhage, and diseases of the eye.[2] Its roots are used for burning sensations in the abdomen, flowers are used for dental caries and stem bark is given for head-ache, biologically impact as hypotensive agent. Chinese also use *Desmodium pulchellum* to expel convulsions (in children) and rheumatic fever. They consider it good for believing it dissolves internal blood clots and builds new red cell, rheumatism and toothache. Filipinos use the leaves for pocks and ulcers. Malayans use the root decoction for puerperium.[3,4]

MATERIALS AND METHODS

Plant Material

The plant, *Desmodium Pulchellum Benth* was collected from Sylhet Division, Bangladesh during the month of September, 2012. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka and was given an accession number which was 37992. Plant leaves was collected and macerated in 2.5L methanol solvent then partitioned by the methods.[5] The leaves were pulled out and washed through water to remove all unwanted sand and plant materials, air dried under light exposure (27°C-30°C for 7 days), crushed in a mill and stored in an airtight container for further study.[5]

Preparation of Extract

The air dried and powdered fruit (500 gm) of *Desmodium Pulchellum* was grinded in 2.5 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. All the extracts were concentrated with a rotary evaporator at low temperature (40-45 ºC) with reduced pressure. The concentrated methanolic extract (ME) was fractionated by modified Kupchan partitioning method[6] and the resultant partitionates i.e., pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF), and aqueous (AQSF) soluble fractions were used for the experimental processes.

Cardio-protective

The cardio-protective activity was conducted by thrombolytic assay. The thrombolytic activity of all extracts was evaluated by the method developed by Daginawala et al.,[7]

modified by Kawsar et al.,[8] using streptokinase (SK) as the standard. Cardio-protective activity was conducted by the methods describe in Md. Reyad et al.[9]

In short, the plant extract (100 mg) suspended in 10 mL of distilled water was kept overnight. The soluble supernatant was decanted and filtered by using a 0.22-micron syringe filter. For clot lysis, venous blood (500 µl) drawn from human healthy volunteers was distributed in different sterile pre weighed microcentrifuge tube and incubated at 37°C for 45 minutes for clot formation.

After clot formation, the serum was completely removed without disturbing the clot and the clot weight was determined. 100µl aqueous solutions of different partitionates and crude extract were added separately to each microcentrifuge tube with the pre-weighed clot. Then, 100 µl (30,000 I.U) of commercial streptokinase (SK) and 100mg of distilled water were separately added to the control tube as positive and negative controls, respectively.

All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, the released fluid was removed and tubes were reweighed to observe the difference in weight after clot disruption. Percentage of clot lysis was determined from below formula.

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

Drugs and chemicals

The drugs such as Streptokinase were collected from Opsonin Pharmaceuticals Ltd, Dhaka, Bangladesh as gift sample. Chemicals were obtained from Merck limited.

RESULTS AND DISCUSSION

As part of our cardio-protective investigation we were evaluate the leaves extract of *D. pulchelleum.* In this investigation, the results demonstrate significant cardio-protective activity (Figure-1). In the present studies, the crude methanolic extract (86.12% ±0.1866), Ethanolic extract (43.94 %± 0.2766), Hexane soluble fraction (50.88% ± 0.3113), the Aqueous soluble fraction (46.46%± 0.1228), Distilled Water (4.84% ± 0.0845) of *D. pulchelleum* demonstrated strong cardioprotective activity, compared with Streptokinase (67.84% ± 0.0800).

The thrombolytic activity is demonstrate due to the presence of plant`s diverse composition like tannins, flavonoids and terpenoids.[10] Further investigation on identification, isolation

and purification of active moieties of the plant responsible for these therapeutic properties may lead to new drug development.

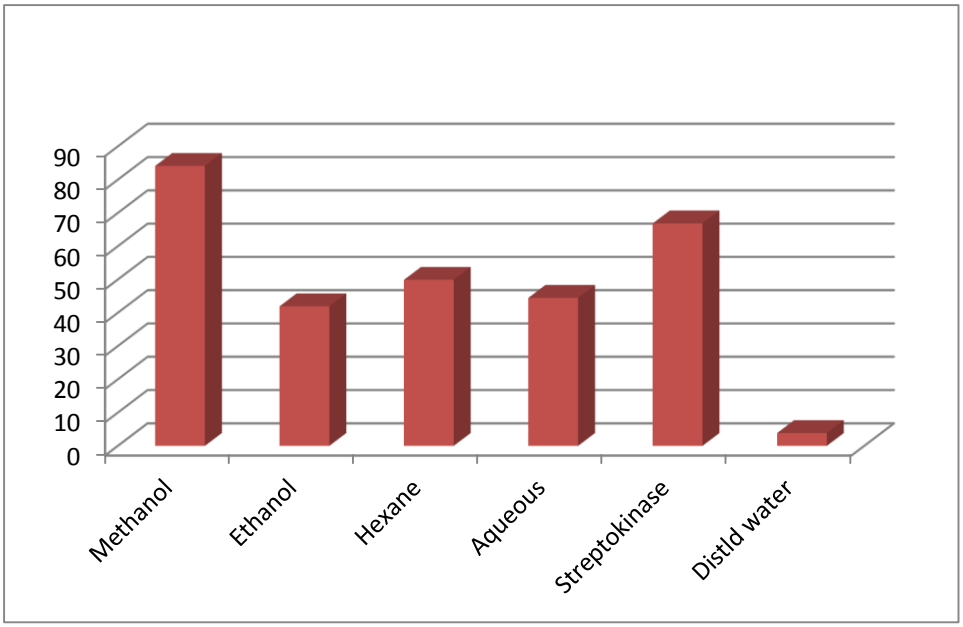

Figure-1: Cardioprotective activity of crude extract and different fractionsof *D. pulchelleum*.

(ME = Methanolic extract; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF= chloroform soluble fraction; AQSF = Aqueous soluble fraction of the methanolic extract of *D. pulchelleum*. SK =Streptokinase).

CONCLUSION

Results of the present investigation indicate that the *D. pulchelleum* leaves possess significant cardio-protective activity and suggest that the plant may be an economical, safe and easily available source of natural agents used in cardiovascular disorders involving blood clot.

This activity may occur due to the presence of active lead compounds in different fraction of this plant leaves extracts.

Further investigation on isolation, purification and identification of active moieties of the plant responsible for these therapeutic properties may lead to new drug development.

ACKNOWLEDGEMENT

We are acknowledged to the department of Pharmacy at Progati Medical Institute for their endeavor support to complete this experiment successfully.

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