

## EVALUATION OF CARDIOVASCULAR EFFECT OF *PYCNOCYCLA SPINOSA* DECNE. EXBOISS. VAR. *SPINOSA* EXTRACT IN ANAESTHETIZED RAT

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### ABSTRACT

Hydroalcoholic extract of *Pycnocycla spinosa* Decne. exBoiss. var. *spinosa* has *in vitro* spasmolytic action, and at oral dose of 250µg/kg inhibits castor oil induced diarrhoea in mice. In this investigation, effects of *P. spinosa* var. *spinosa* extract in comparison with nifedipine on blood pressure and heart rate in animal model was studied. Injection of three bolus doses of *P. spinosa* var. *spinosa* extract (100µg/kg, 500µg/kg and 1mg/kg) into the jugular vein, temporary reduced blood pressure and heart rate. However, soon after completion of extract administration blood pressure and heart rate returned to normal. Nifedipine on the other hand caused a sustained reduction in blood pressure and decreased heart rate compared with the control group. From this study it was concluded that *P. spinosa* var. *spinosa* extract at antidiarrhoeal dose has no significant effect on blood pressure and heart rate.

**Keywords:** *Pycnocycla spinosa* var. *spinosa*, Hydroalcoholic extract, Blood pressure, Heart rate, Antidiarrhoeal.

### INTRODUCTION

*Pycnocycla spinosa* Decne. exBoiss. var. *spinosa* (Fam. Umbelliferae) is an essential oil-containing wild plant growing in Iran (1, 2, 3). Hydroalcoholic extracts of *P. spinosa* var. *spinosa* is a potent relaxant of isolated ileum (4) and its antispasmodic action is very similar to that of dicyclomine (5). Yield of *P. spinosa* var. *spinosa* hydroalcoholic extract is about 14% (w/w) (4, 6), is stable and it is composed of alkaloids, flavonoids and saponins components (6). The antispasmodic action of *P. spinosa* var. *spinosa* extract is partly due to alkaloid and flavonoid fractions (6). In addition, *P. spinosa* extract is shown to have *in vivo* antidiarrhoeal action (4). Good Potency and selective pharmacological activity on ileum in comparison with bladder and uterus (7, 8) are characteristics, which make *P. spinosa* extract a suitable candidate for antidiarrhoeal activity. However, before *P. spinosa* could be recommended for clinical use it is necessary that other possible action of the extract, including cardiovascular effects to be determined. Although the extract would be given orally nevertheless, some of the extract components might be absorbed and may affect other organs. The objective of this research was to investigate the cardiovascular effects of *P. spinosa* extract.

### MATERIALS AND METHODS

#### *Plant extract*

Aerial parts of *P. spinosa* var. *spinosa* were collected in June from Isfahan University campus and identified by the botanist, Mr Mehregan, in the Biology Department at Isfahan University. A voucher specimen (A24) was authenticated and deposited in the herbarium of the Faculty of Pharmacy and Pharmaceutical Sciences (Isfahan, Iran). The aerial part of the plant was dried in shade. The total hydroalcoholic extract was obtained by percolation (9) and after evaporation of ethanol, the amount of dry crude extract (w/w) was determined.

#### *Cardiovascular study*

Male Wistar rats (220-280g) bred in Isfahan University of Medical Sciences animal house were anaesthetized by injection of ketamine (150mg/kg, i.p.). Incisions were made on the neck and jugular vein and carotid artery were identified and isolated from connective tissues. The jugular vein and the carotid artery then were cannulated using polyethylene cut down tube (4FR). The jugular cannula was then secured and connected to a syringe containing the extract, nifedipine or vehicle. The carotid cannula was filled with heparinized saline solution and connected to a pressure transducer (Harvard). The surgical

technique was based on that of Downing and Hollingsworth (10). Changes in pressure were recorded on a calibrated Harvard Universal Osillograph. To avoid blood clotting in the cannula, heparin (200iu/100g, i.v.) was injected. Initially, blood pressure was recorded for 5 minutes and then *P. spinosa* var. *spinosa* extract or nifedipine were administered via jugular cannula and recording of the blood pressure was continued. All experiments had their own control groups, which were treated with equivalent volume of the vehicle.

#### Drugs and solution

The following drugs were used: ketamine (Parke-Davis), Heparin (B. Braun), Nifedipine (Sigma), *Pycnocycla spinosa* extract. Nifedipine was prepared as 10mg/ml stock solution in 70% ethanol. Dried *Pycnocycla spinosa* var. *spinosa* extract was dissolved as 10mg/ml stock solution in 50% ethanol. The extract and nifedipine solutions were further diluted in saline solution for parental administration.

#### Analysis of results

Harvard Universal Osillograph was calibrated using a sphygmomanometer. Mean blood pressure were assessed as mmHg at the defined times. Heart rate was calculated at 1 min intervals as number of peaks produced by systolic blood pressure. Values are quoted as mean  $\pm$  standard error of mean (SEM). The significance of differences between means was calculated by two-tailed paired Student's t-test or by one-way analysis of variance as appropriate.

### RESULTS

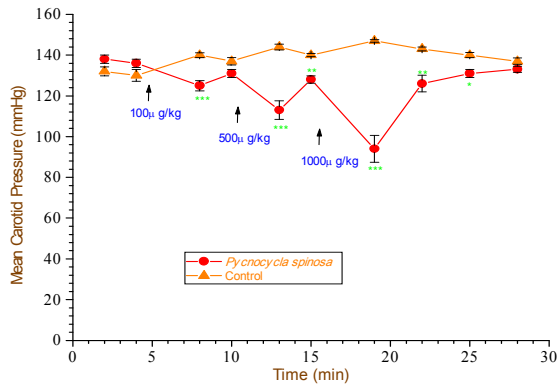
The amount of hydro-alcoholic extract yield was 14.9% on dry weight basis. Anaesthetized rats had a relatively stable blood pressure and heart rate. Bolus injection of vehicle in saline solution temporary caused a small but not significant increase in blood pressure. Slow administration of three consecutive bolus doses (100 $\mu$ g/kg, 500 $\mu$ g/kg and 1000 $\mu$ g/kg) of *P. spinosa* extract into the jugular vein, dose dependently reduced blood pressure by 9%, 17% and 31% respectively in comparison with the pre-drug control values (Figure 1). However, blood pressure returned to normal within 5 minutes. Similarly, above doses of *P. spinosa* var. *spinosa* extract reduced the heart rate by 10%, 22% and 25% respectively (Figure 2), but again soon after completion of injection, the heart rate returned to its pre-administration rate.

Intravenous injection of nifedipine into jugular vein at doses of 136mg/kg, 270mg/kg, and 400mg/kg caused a continued reduction in blood pressure by 13%, 27% and 37% respectively

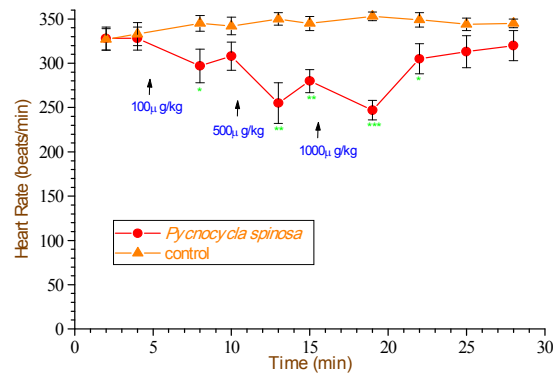
(Figure 3). At the same time heart rate was also reduced but occasionally a reflex tachycardia was occurred (Figure 4). When a dose of 540mg/kg of nifedipine was injected, there was no further reduction in blood pressure but there was an increase in heart rate (see figures 3 and 4). Higher doses of nifedipine proved to be lethal and caused sever cardiovascular depression.

### DISCUSSION

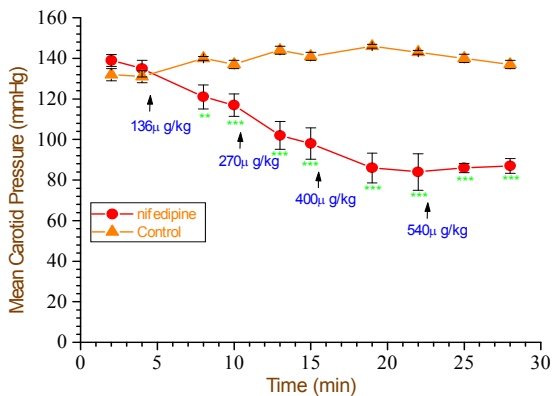
Hydroalcoholic extract of *P. spinosa* var. *spinosa* at oral dose of 250 $\mu$ g/kg has antidiarrhoeal action (4). At 1mg/kg oral dose it almost abolishes castor oil induced diarrhoea in mice (4). Loperamide and diphenoxylate are reported to inhibit castor oil induced diarrhoea in mice at dose of 3mg/kg and 2.5mg/kg respectively (11, 12). *P. spinosa* extract is mixtures of active and inactive substances and in comparative doses, it has an antidiarrhoeal action as good as that of loperamide or diphenoxylate. Nevertheless, such compounds may have other pharmacological or adverse effect, which should be investigated. If *P. spinosa* var. *spinosa* extract has no serious adverse effect, then it would be a good alternative herbal medicine for treatment of diarrhoea and abdominal spasm. In this research, the effect of *P. spinosa* extract on rat blood pressure and heart rate was studied and compared with nifedipine. Small increase in blood pressure after administration of vehicle and saline solution is most likely due to an increase in blood volume. Although the anaesthetic agent may have some effects on cardiovascular function but as all measurements were performed after anaesthetising the rats, the effect of anaesthetic drug ought to be uniform throughout the study as there were no significant changes in blood pressure or heart rate during the course of study in the vehicle treated time matched control groups. Nifedipine (an anti-hypertensive drug) caused a sustained hypotension and a decrease in heart rate. Although slight decrease in heart rate is not consistent with reported oral dose of nifedipine when it is used clinically, in this study nifedipine was administered into jugular vein and therefore, the heart initially was exposed to high concentration of nifedipine. This decrease in heart rate subsides following full drug disposition (Figure 4). In another study where cardiovascular effect of nifedipine *in vivo* and *in vitro* were compared a suppression of cardiac activity was also reported (13). The suppression of heart rate is most likely due to calcium channel blocking action of nifedipine on myocardium (14). Unlike nifedipine, *P. spinosa* extract caused a transient decrease in blood pressure and heart rate. Temporary reduction in blood pressure and heart rate during administration of *P. spinosa* extract could be due



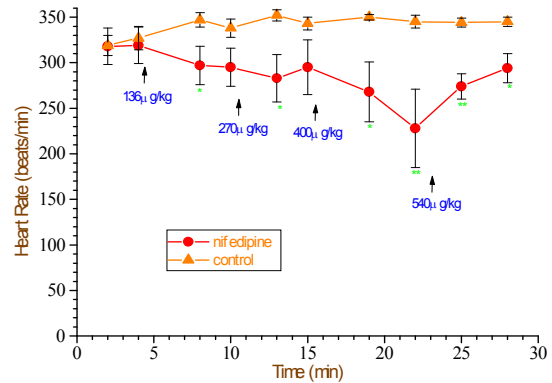
**Figure 1.** Intravenous administration of three doses of *P. spinosa* extract on mean blood pressure in anaesthetized rat. The points are mean and the vertical line shows the SEM (n=6). \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 in compare with the control group (Student's t-test).



**Figure 2.** Intravenous administration of three doses of *P. spinosa* extract on heart rate in anaesthetized rat. The points are mean and the vertical line shows the SEM (n=6). \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 in compare with the control group (Student's t-test).



**Figure 3.** Intravenous administration of four consecutive doses of nifedipine on mean blood pressure in anaesthetized rat. The points are mean and the vertical line shows the S.E.M (n=6). \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 compared with the control group (Student's t-test).



**Figure 4.** Intravenous administration of four consecutive doses of nifedipine on heart rate in anaesthetized rat. The points are mean and the vertical line shows the S.E.M (n=6). \*P<0.05, \*\*P<0.01, in compare with the control group (Student's t-test).

to direct exposure of the heart to high concentration of the extract. This small cardiac depressive effect disappears following extract disposition. Nevertheless, when the *P. spinosa* is orally administered, the extract would gradually be absorbed and the absorption may not be complete, thus, temporary reduction in blood pressure won't be expected.

From this study it may be concluded that the hydroalcoholic extract of *Pycnocycla spinosa* at anti-diarrhoea doses, has no serious effect on

blood pressure and heart rate. This study therefore confirms cardiovascular safety of *P. spinosa* hydroalcoholic extract as a useful medicinal plant for treatment of gastro-intestinal spasm and diarrhoea.

#### ACKNOWLEDGEMENT

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#### REFERENCES

1. Mozaffarian V. A dictionary of Iranian plant names. Tehran: Farhang Moaser; 1996. p. 443-444.
2. Asghari G, Houshfar G, Mahnoudi Z. Composition of the essential oil of *Pycnocycla spinosa* Decne. ex.Boiss from Isfahan. Daru 2001; 9(3&4):28-29.

3. Ahmadi L, Mirza M. Volatile constituents of the essential oil of *Pycnocycla spinosa* Decne ex. Boiss from Iran. *J Essent Oil Res* 1998; 10:197-198.
4. Sadraei H, Asghari G, Naddafi A. Relaxant effect of essential oil and hydro-alcoholic extract of *Pycnocycla spinosa* Decne. & ex Boiss on ileum contractions. *Phytother Res* 2003; 17(6):645-649.
5. Hajhashemi V, Sadraei H, Ghannadi AR, Mohseni M. Antispasmodic and antidiarrhoeal effect of *Satureja hortensis* L. essential oil. *J Ethnopharmacol* 2000; 71:187-192.
6. Sadraei H, Asghari G, Hekmatti AA. Antispasmodic effect of three fractions of hydroalcoholic extract of *Pycnocycla spinosa* var. *spinosa*. *J Ethnopharmacol* 2003; 86(2-3):187-190.
7. Sadraei H, Asghari G, Arabzadah A. Effect of hydroalcoholic extract of *Pycnocycla spinosa* on rat isolated bladder. *Iranian J Pharm Res* 2004; 4:237-241.
8. Sadraei H, Asghari G, Andishah M. *Pycnocycla spinosa* extract is a more potent relaxant of ileum than uterus smooth muscle. British Pharmacological Society Meeting, Joint Meeting with the Pharmaceutical Society of Canada Proceeding, University of Cambridge 6<sup>th</sup>–8<sup>th</sup> July 2005. p. P093.
9. Samuelsson G. *Drugs of natural origin* (4<sup>th</sup> ed.). Stockholm: Swedish Pharmaceutical Press; 1999. p. 48-49.
10. Downing SJ, Hollingsworth M. Nifedipine kinetics in the rat and relationship between its serum concentrations and uterine and cardiovascular effects. *Br J Pharmacol* 1988; 95:23-32.
11. Adzu B, Amos S, Amizan MB, Gamaniel K. Evaluation of the antidiarrhoeal effect of *Zizyphus spina-christi* stem bark in rats. *Acta Tropica* 2003; 87:245-250.
12. Sairam K, Hemalath S, Kumar A, Srinivasan T, Ganesh J, Sahnker M, Venkataraman S. Evaluation of antidiarrhoeal activity in seed extract of *Mangifera indica*. *J Ethnopharmacol* 2003; 84:11-15.
13. Nordlander M, Abrahamsson T, Akerblom B, Thalen P. Vascular versus myocardial selectivity of dihydropyridine calcium antagonist as studied *in vivo* and *in vitro*. *Pharmacol Toxicol* 1995; 76(1): 56-62.
14. Kerins DM, Robertson RM, Robertson D. Drugs used for the treatment of myocardial isohemia. In: Hardman JG, Limbird LE, Gilman AG, eds. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 10<sup>th</sup> Ed. New York: McGraw-Hill; 2001. p.843-870.