

Phytochemical Analysis and Cardiac Depressant Activity of Aqueous Methanolic Extract of *Morus nigra* L. Fruit.

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ABSTRACT

The present study was conducted to investigate the effects of aqueous methanolic extract of *Morus nigra* L. fruit on frog's heart. Force of contraction and heart rate were measured by force displacement transducer attached to Power Lab data acquisition system. Lab chart Pro 5 software was used for the acquisition and analysis of data. The extract was also screened for various phytochemical constituents using standard methods. The results indicated that the extract produced a significant dose dependent decrease in heart rate without affecting the contractility of the heart. Phytochemical analysis also revealed that the extract contained flavonoids, cardiac glycosides, alkaloids, saponins and phenolic compounds. It is conceivable therefore; that the negative chronotropic effects of aqueous methanolic extract of *Morus nigra* L. fruit may be attributed to the presence of certain pharmacologically active compounds.

INTRODUCTION

Cardiovascular diseases have been considered a serious health problem worldwide. The major risk factors for heart diseases include family history, sex, hypercholesterolemia, hypertension, obesity, cigarette smoking, and other lipid abnormalities. Most of these risk factors are prevalent in developing countries because of the lack of infrastructure. Therefore, these diseases have become a very common problem in the affluent population of the developing countries (Trivedi and Nehra, 2004). The mortality rate from cardiovascular diseases is also much higher in developing countries than the developed ones (Debashis *et al.*, 2004). Many synthetic drugs have been commonly used for the treatment of heart diseases but herbal medicines remain the popular choice in the third world countries. In Pakistan, the condition is quite similar and majority of population rely on traditional herbal medicines for the treatment of various ailments. It has been documented that various medicinal plants such as *Digitalis purpurea*, *Crataegus monogyna* (Hawthorn), *Allium sativum* and *Rauwolfia serpentina* have been used for the treatment of congestive heart failure, hypertension

and atherosclerosis (Jerie, 2007; Weng *et al.*, 1984; Sutter *et al.*, 2007). Though medicinal value of many plants has already been explored, still there is a need for more research in order to determine their therapeutic potentials. *Morus nigra* L. Fruit (Family: Moraceae), commonly known as Shahtoot has been widespread in China, Pakistan, Iran and Afghanistan. Traditionally, it has been found to be effective in reducing blood pressure (Sabeen and Ahmad, 2009). The aim of the present study was to assess the cardiovascular activity of aqueous methanolic extract of *Morus nigra* L. fruit in frog's heart.

MATERIAL AND METHODS

Plant Material

The fruits of *Morus nigra* L. were collected from Sargodha, Pakistan in the month of April, 2011. The plant was identified and authenticated by Prof. Malik Fateh Muhammad, Department of Botany, Govt. Post Graduate College, Jauharabad. The voucher specimen has been deposited in the herbarium of Faculty of Pharmacy, University of Sargodha.

Preparation of Extract

The fruits of *Morus nigra* L. were washed with water to remove any adhering dirt and then completely dried under shade.

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The fruits were grounded in herbal grinder into a coarse powder form. Aqueous methanolic (70:30) extract of *Morus nigra* L. fruit was prepared by cold maceration process. The extract was then dried on rotary evaporator and a solid mass was obtained with percentage yield of 17 % (Yuchi *et al.*, 2012).

Animals

Both male and female frogs (*Rana tigrina*) weighing 100-180g were used for this study. The study protocol was approved by ethical committee of Faculty of Pharmacy, University of Sargodha.

Preliminary Phytochemical test

The aqueous methanolic extract of *Morus nigra* L. fruit was analyzed for the presence of different phytochemical constituents such as flavonoids, Reducing sugars, tannins, phenolic compounds, saponins, alkaloids and cardiac glycosides by using standard methods (Khandelwal, 2006).

Frog Heart Preparation

Frog was pithed and the heart was exposed by a midline incision. The pericardium was removed carefully and the heart was perfused continuously with frog Ringer solution (The composition of Ringer solution in millimoles: NaCl-110; KCl-1.9; CaCl₂-1.1; NaHCO₃-2.4; NaH₂PO₄-0.06; Glucose-11.1). A clip was then attached to the apex of heart to measure the force of contraction (g) and heart rate (beats/min) by force-displacement transducer (ML221-0128). After stabilization, different doses of the extract (5mg/ml, 15mg/ml, 25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml, 500mg/ml, 600mg/ml, 700mg/ml, 800mg/ml, 900mg/ml and 1000mg/ml) were applied to assess various cardiac parameters with each heart serving as its own control. The frog heart was washed with Ringer solution after every dose of the extract till it was brought to the normal state. Power Lab data acquisition system and Lab Chart Pro 5 software were used for the recording and analysis of data (Burn, 1952).

Statistical analysis

The results were expressed as mean \pm S.E.M. Student's t-test was used for statistical analysis of data. Values of less than 0.001 were considered as significant.

RESULTS AND DISCUSSION

Medicinal plants have always been a major target for drug development. The medicinal values of traditional medicinal plants cannot be ignored and studies have been carried out in order to investigate their exact mechanism of actions. Cardiovascular research has always remained an important area in which compounds from plant sources have contributed successfully. In the present study, the aqueous methanolic extract of *Morus nigra* L. fruit produced a significant dose dependent decrease in heart rate of frog's heart. The maximum percentage reduction in heart rate was observed at 1000mg/ml when compared to control.

However, there was no significant effect on the contractility of frog heart (Figure 1). Phytochemical analysis also revealed that the extract contained certain active principles such as saponins, alkaloids, phenolic compounds, cardiac glycosides and flavonoids (Table 1). These findings indicated that the extract could have compounds similar to Ivabradine, that cause bradycardia in frog heart. Newer agents like Ivabradine, and Zatebradine are known to inhibit sinus node pacemaker current without direct effect on contraction and are recently gaining more attention for treating patients with Ischemic heart disease (Gillam, 1965; Goethals *et al.*, 1993; Lechat, 1998; Borer *et al.*, 2003). Previously, it has been reported that saponins are associated with multiple interesting pharmacologic effects including negative chronotropic, diuretic, antibacterial and anti-inflammatory actions (Francis *et al.*, 2002; Lacaille-Dubois and Wagner, 1996). Experimental studies have also demonstrated that various plants such as *Digitalis*, *Strophanthus*, *Acokanthera*, *Urginea*, *Apocynum*, *Convallaria* contain cardiac glycosides that are endowed with negative chronotropic effects (Usmanghani, 1989). Similarly, flavonoids and phenolic compounds have also been reported to produce a decrease in heart rate and vasodilation in animal models (Zhou *et al.*, 2012; Carusio *et al.*, 2008). It is also well established that black mulberry fruit contains flavonoids such as kaempferol-3-O-rutinoside and 5-O-caffeoylquinic acid (Pawlowska *et al.*, 2008). Thus it could be inferred from the previous findings that a decrease in heart rate produced by the extract might be due to the presence of these active compounds.

Table 1: Phytochemical analysis of aqueous methanolic extract of *Morus nigra* L. fruit.

Compounds	Inferences
Alkaloids	+
Saponins	+
Flavonoids	+
Phenols	+
Cardiac glycosides	+
Tannins	-
Reducing sugars	-

KEY

+ = Present, - = Absent

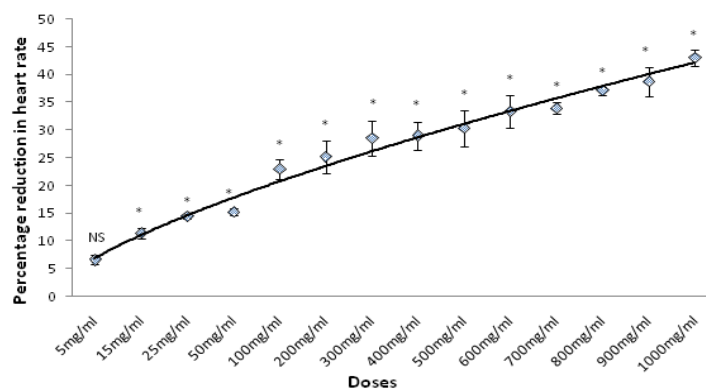


Fig. 1: Effect of aqueous methanolic extract of *Morus nigra* L. fruit on heart rate of frog heart (n = 6), where * = P < 0.001 and NS = Non-significant vs. control.

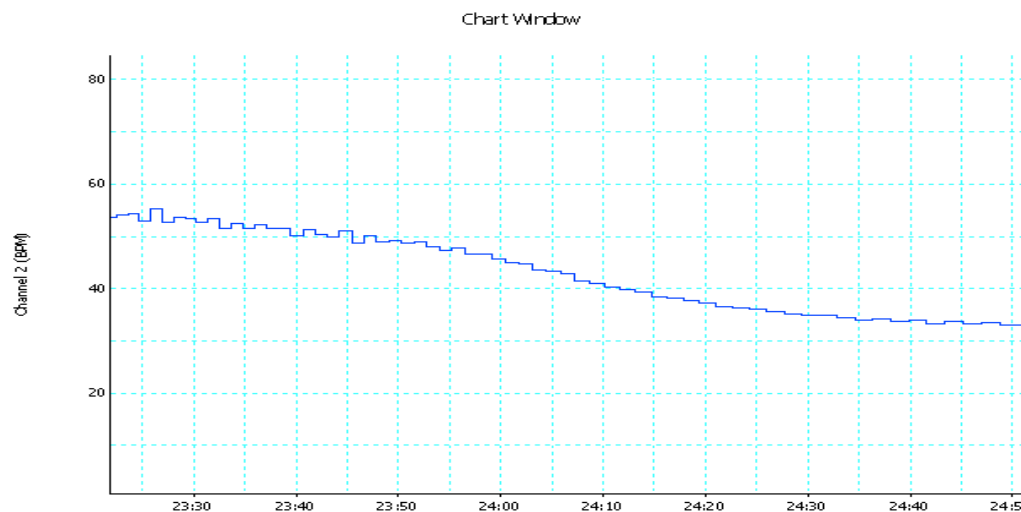


Fig. 2: Effect of aqueous methanolic extract of *Morus nigra* L. fruit on heart rate of frog heart. Data were collected and analyzed by Lab chart Pro 5 software (AD instruments, Australia).

CONCLUSION

It is concluded from this study that the aqueous methanolic extract of *Morus nigra* L. fruit contain certain active principles that may be responsible for the negative chronotropic effects in frog heart. Further studies are therefore required to isolate these compounds and determine their exact mechanism of actions.

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