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# A STUDY OF CARDIOVASCULAR EFFECTS OF *AZADIRACHTA INDICA* (NEEM) ON ISOLATED PERFUSED HEART PREPARATIONS

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**Abstract :** The effects of aqueous leaf extract of *Azadirachta indica* were evaluated on isolated perfused frog and rabbit heart. Dose dependent negative inotropic and chronotropic effects were observed in both the heart preparation. An increase in coronary blood flow in isolated rabbit heart was observed. The effects were not blocked by atropine and mepyramine in both the preparations. The data suggests that *A. indica* could be of benefit in coronary artery disease and arrhythmias.

**Key words :** *azadirachta indica* inotropic chronotropic atropine mepyramine

## INTRODUCTION

*A.indica* has been reported to produce anti-inflammatory, immuno-stimulant, hypoglycaemic, antiviral and antibacterial properties (3–7). However, only few studies are available regarding the pharmacological actions of *A. indica* on cardiovascular system (8–10). The present work was undertaken to study the effects of *A. indica* leaf extract on isolated perfused heart preparations of frog and rabbit.

## METHODS

*Method of preparation of neem leaf extract (NLE):* One kg of freshly collected shade dried leaves of A. indica were ground to powder and allowed to soak overnight in 4 litres of distilled water. The suspension was centrifuged at 5200 rpm for 20 min and filtered through a Whatman No. 1 filter paper. The supernatant fluid was allowed to evaporate in glass petridishes under tubelight to provide heat and to prevent dampness so that no organism occurs. When completely dry, the powder was collected by scraping and was stored. The percentage yield was 2%. Stock solution of aqueous extract was prepared by dissolving 50 mg of the extract in 5 ml of distilled water (11). Further dilutions were also made in distilled water.

*Dose of NLE :* 1, 2, 4, & 8 mg does of aqueous extract of *A. indica* were used (dose were selected from the literature and after doing the pilot study).

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*Drugs used :* Atropine (50 μg), mepyramine (10 μg) and propranolol (5 μg) were used as muscarinic, histaminergic ($H_1$) and adrenergic receptor blockers respectively. All of these drugs were injected after diluting them in the physiological salt solutions used.

**Animal experiments**

*Isolated perfused frog heart preparation :* Common India frogs (*Rana tigrina*) of medium size (n = 7) were taken for the study. Perfusion was done by Bulbring's method as described by Burn (12). The graded dose response of NLE was taken with different doses before as well as after atropine and mepyramine. The effect of NLE was observed on heart rate, force of contraction and tone.

*Isolated perfused rabbit heart preparation :* Albino rabbits of either sex, weighing 1.5–2.5 kg (n = 7) were used. Isolated rabbit heart was perfused according to Langendorff's methods as described by Kadatz (13). The effects on heart rate, force of contraction and tone were observed with different does of NLE before as well as after atropine and mepyramine. Coronary outflow was measured with different does of NLE before as well as after pre-treatment with atropine, mepyramine and propranolol.

## RESULTS

*On isolated perfused frog heart preparation : A.indica* aqueous leaf extract (1 mg) did not produce any significant effect. However, given in the does of 2, 4 and 8 mg, it produced a significant (P<0.001) and does dependent decrease in heart rate and force of myocardial contraction. Negative inotropic and chronotropic effects persisted for 10 min and were not blocked by pre-treatment with atropine and mepyramine. Moreover, NLE failed to block the effect of adrenaline.

*On isolated perfused rabbit heart preparation : A.indica* aqueous leaf extract (2, 4, and 8 mg) produced a significant (P<0.001) and dose dependent negative inotropic and chronotropic effect. The effect persisted for a short duration (10 min). A dose dependent and significant (P<0.001) coronary vasodilator effect was also observed in the same preparation with 2, 4 and 8 mg of NLE. These effects were not blocked by pre-treatments with atropine, mepyramine and propranolol. No significant effects, however, were observed with 1 mg NLE in these isolated preparations.


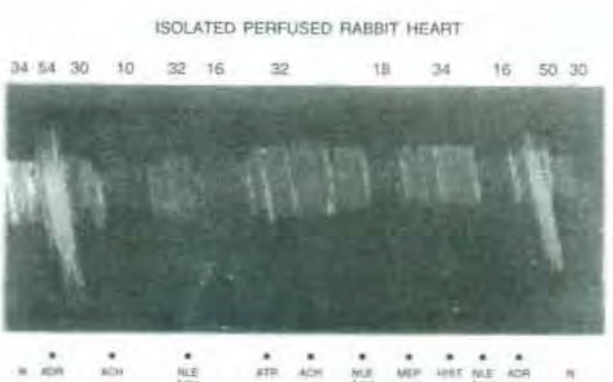

TABLE I : Effect of neem leaf extract (NLE) on isolated perfused Frog and Rabbit heart.

| | *Control* | *1 mg NLE* | *2 mg NLE* | *4 mg NLE* | *8 mg NLE* | *Atropine* | *4 mg NLE* | *Mepyramine* | *4 mg NLE* |
|---|---|---|---|---|---|---|---|---|---|
| *Frog's perfused heart* | | | | | | | | | |
| H.R. (Beats/min.) Mean ± SEM | 55.42±084 | 54.85±0.33* | 48.57±1.04* | 45.14±0.76** | 39.71±1.11** | 55.62±1.42* | 46.14±0.86** | 54.92±0.62* | 44.82±0.48** |
| *Rabbit's perfused heart* | | | | | | | | | |
| H.R. (Beats/min.) Mean ± SEM | 33.14±0.88 | 31.71±0.95* | 27.71±0.84** | 23.42±0.80** | 16.85±1.02** | 34.12±0.84* | 24.12±0.10** | 32.86±1.26* | 23.60±0.14** |
| *Vol. of perfused* | | | | | | | | | |
| Fluid (ml/min.) Mean ± SEM | 2.37±0.15 | 2.41±0.02* | 2.60±0.05** | 2.90±0.60** | 3.15±0.07** | 2.34±0.08* | 2.86±0.07** | 2.46±0.10* | 2.80±0.05** |

*P>0.05, **P<0.001 when compared with control (Student's T test)

## DISCUSSION

The present study was conducted to see the effects of *A. indica* on cardiovascular system using isolated perfused heart preparations.

The study shows a does dependent negative inotropic and chronotropic effect of *A. indica* on isolated perfused frog and rabbit heart. The effects were not blocked by pretreatment with atropine and mepyramine. These results are supported by some earlier reports which show the cardiodepressant effects of *A. indica* (8–10). Our results also indicate that muscarinic and histaminergic receptors are not involved in negative inotropic and chronotropic effects of NLE. Furthermore, NLE pre-treatment failed to reverse the positive chronotropic and inotropic effects of adrenaline, thereby showing that NLE does not possess β-adrenergic receptor antagonistic activity.

In the study, it has also been observed that NLE produces a coronary vasodilator effect which is not blocked by prior administration of atropine, mepyramine and propranolol. These observations further suggest that coronary vasodilator action of *A. indica* is independent of muscarinic, histaminergic and β-adrenergic receptors. Therefore, it might be possible that the negative inotropic, chronotropic and coronary vasodilator effects shown by *A. indica* are due to its direct actions on the heart and coronary arteries.

It may be suggested, in keeping the cardiovascular effects observed in our study, that *A. indica* may be of benefit in coronary artery disease and arrhythmic states.

The results of our study also indicate considerable scope of avoiding the cardiovascular effects of *A. indica*, when used in lower does, for its potential benefits in disease states like diabetes, hyperlipidemia, etc. (3–7). A limited scope of this study, however, needs further elaboration by elucidating the effects of the active principles involved, in future studies.

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