

*Full Length Research Paper*

# EC50 of adrenaline-atenolol: Functional agonist assay using Langendorff isolated rabbit heart tethered to powerLab data acquisition system

Abdullah S. Shatoor<sup>1\*</sup>, Fahaid AL-Hashem<sup>2</sup>, Abbas Elkarib<sup>2</sup>, Hussein Sakr<sup>2</sup> and Mahmoud Alkhateeb<sup>2</sup>

<sup>1</sup>Department of Internal Medicine, Cardiology Section, College of Medicine, King Khalid University, Abha 61421, Saudi Arabia.

<sup>2</sup>Department of Physiology, College of Medicine, King Khalid University, Abha 61421, Saudi Arabia.

Accepted 30 March, 2012

The purpose of this study was to determine the mean EC50 of adrenaline on heart rate and force of contraction of isolated New Zealand white rabbit hearts and to determine the concentration of atenolol that completely blocks the effect of adrenaline using one of modern physiological acquisition system, PowerLab, AD instrumnets. Twelve Isolated hearts from New Zealand white rabbits were perfused through aorta in a Langendorff mode. Heart rate and contractility were determined for 5 minutes after bolus injection of 5 different concentrations of adrenaline (1.0, 2.5, 5.0, 7.5 and 10 µg/mL). The changes in heart rate and contractility after each treatment were compared with their baseline values. These data were used to calculate the mean EC50 of adrenaline on heart rate and force of contraction. This EC 50 was then used after perfusion of different concentrations of atenolol (1.0, 2.5, 5.0 and 10µg/mL). Data were collected with the help of PowerLab data acquisition and analyzed by Labchart pro7 software. Adrenaline resulted in a stimulatory effect on the heart rate and the amplitude of the heart contraction. The maximum increases in both heart rate and force of contraction were seen at adrenaline dose of 7.5 µg/mL and the plateau phase was achieved at a dose of 10 µg/mL. The average EC50 of adrenaline was 3.5 µg/mL. The positive inotropic effect of adrenaline was antagonized only at atenolol concentrations of 5.0 and 10 µg/mL and complete inhibition of adrenaline effect on heart rate was achieved at atenolol concentrations of 10 µg/mL. These data showed that atenolol must be used at a concentration no less than 7.5 µg/mL to demonstrate if β adrenergic receptors are involved in the mechanism of action of any newly tested positive inotropic or choronotroic drug.

**Key words:** Adrenaline, atenolol, isolated hearts, rabbits.

## INTRODUCTION

Isolated tissue and organ preparations have been in use for over one hundred years to evaluate pharmacological effects of drugs. These preparations provide researchers with convenient biological models that are independent of the systemic influences *in vivo* preparations (Endoh and Hori, 2006; Iglarz et al., 2008). The isolated heart model is a powerful system with many advantages. This

paradigm acts as a physiologically relevant bridge between purely *in vitro* assays and costly, resource-intensive whole animal studies. The model has been used extensively in experimental cardiovascular investigations and it is a widely accepted surrogate for the study of human cardiac function. Indeed, it has been demonstrated systematically that isolated hearts from rabbits and guinea pigs exhibit similar hemodynamic and electrophysiological responses as humans to numerous therapeutic compounds from a variety of classes (Hondeghe et al., 2001, 2003; Hondeghe and Hoffman, 2003).

\*Corresponding author. E-mail: [asshalghamdi@yahoo.com](mailto:asshalghamdi@yahoo.com). Tel: 966505268277.

In the isolated heart experiments and in order to understand the mechanism of action of any newly discovered compounds on inotropy and chronotropy of the heart, certain agonist and antagonist are usually used. These include: Adrenaline and its adrenoreceptor antagonists, such as atenolol and acetelcholine, and its muscuranic receptor antagonist, such as atropine, calcium antagonist, such as verapamil, NA/ATPase pumb blocker and many other blocking agents (Boyd, 1968; Nayler and Poole-Wilson, 1981; Dukes and Williams, 1984; Dutta, 1991).

Through our search in literature looking for the exact dose of each of these agonists and antagonist to be used in isolated rabbit's heart to study the mechanism of drugs on heart, we could not find a direct reference that mention the reference range of each of these agents to be used. Most of the studies used different and varied concentrations of these cardiac agonist and antagonist in different animal model (Armitage et al., 1957; Benforado, 1958; Brick et al., 1966; Magnussen and Kudsk, 1974; Bush et al., 1980; Kerker et al., 1985; Richardt et al., 1990; Du et al., 1993; Temsah et al., 2000; Yawar et al., 2006).

In the light of the aforementioned facts, several studies were carried out at our laboratories on New Zealand white rabbit's perfused heart to determine the  $EC_{50}$  and  $IC_{50}$  of some positive and negative inotropic and chronotropic agents (such as, adrenaline, calcium and acetylcholine) and to determine the range concentrations of some of their selected antagonist that completely block their effect at their  $EC_{50}$  or  $IC_{50}$  using PowLab data acquisition system (AD Instruments, Australia). Hoping this help student and scientists in their future cardiovascular research studies to study the mechanism of action of any drugs on the hearts isolated from this animal species. So, the present study is one of our studies and has been designed to investigate the  $EC_{50}$  of adrenaline on both force of contraction and heart rate and to determine the range concentration of atenolo, one of the most used adrenoreceptor selective blocker that completely block adrenaline effect at its  $EC_{50}$  in isolated white New Zealand white rabbit's heart.

## MATERIALS AND METHODS

### Chemical agents

Adrenaline was purchased from Sigma-Aldrich (Buchs, Switzerland). Atenolol was purchased from a local pharmacy. All drugs were used without further purification. Adrenaline and atenolol were prepared by dissolving them in the perfusion solution (Ringer-Locke) to the desired final concentrations used in this study.

### Animals

This study was performed in the Research laboratories of the Department of Physiology, Medical School, King Khalid University

between October and December, 2011. Twelve adult New Zealand white albino male rabbits weighing between 2 and 3 kg were used during the experimental procedure. The experiments were approved by Ethical Committee of the Medical School, King Khalid University, Abha, Saudi Arabia. The animals were obtained from the animal house of the College of Medicine of King Khalid University where they were fed with standard rabbit pellets and allowed free access to water. They were housed at a controlled ambient temperature of  $25 \pm 2^{\circ}\text{C}$  and  $50 \pm 10\%$  relative humidity, with 12 h light/12 h dark cycles. All studies were conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

## Experimental procedure

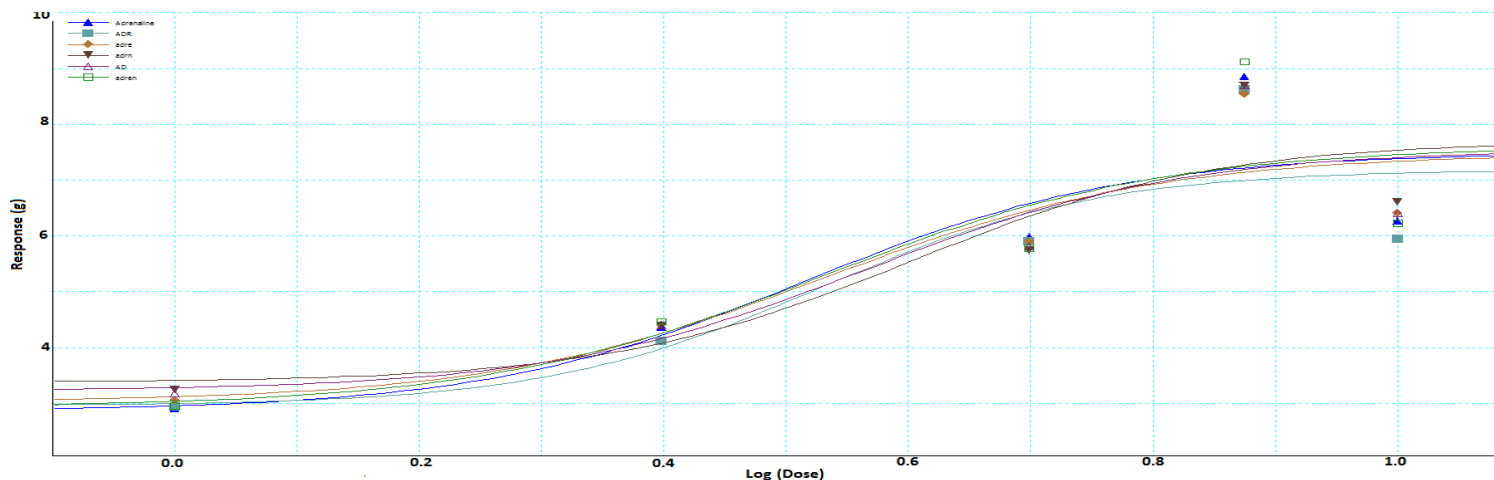
### Heart isolation and hanging

This experiment was carried out in accordance with the Langendorff (1985) procedure. Each rabbit was injected with 1000 IU of heparin intravenously through the marginal ear vein. 5 min later, a blow on the neck of the rabbit made them unconscious. Their chest was opened and the heart was dissected out with about 1 cm of aorta attached, and was quickly washed with oxygenated Ringer-Locke solution (NaCl, 45.0 g;  $\text{NaHCO}_3$ , 1.0 g; D-glucose, 5.0 g; KCl, 2.1 g;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 1.6 g; in 5 L of distilled water). The isolated heart was gently squeezed several times to remove as much residual blood as possible. The heart was then transferred to the perfusion apparatus (Radnoti isolated heart system, AD Instrument, Australia) and tied to a stainless steel canula through the aorta. The perfusion fluid was Ringer-Locke solution which was continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide, and was applied at a constant perfusion pressure of 70 mmHg. The temperature was continuously monitored by a thermo-probe inserted into the perfusion fluid tank and was maintained between 36.5 and 37.5°C.

The hearts were allowed to stabilize for 30 min before any drug interventions. The mechanical responses (force of contraction (FC) of spontaneously contracting isolated hearts were recorded by attaching one end of a thread to the apex of the heart using a Palmer clip and the other end of the thread to a force transducer (MLT 844; AD Instruments, Australia). Heart rate (HR) was calculated from the electrocardiogram (ECG) that was recorded by attaching three spring clip electrodes directly to the heart surface (MLA1210, AD Instruments, Australia). The signal from the force transducer and the ECG electrodes were filtered and amplified and sent to an analog-to-digital converter (Power Lab data acquisition and analysis system: AD Instruments, Australia) attached to a computer. The signals recorded were saved for later analysis. FC, HR and ECG were recorded and analyzed with the help of LabChartpro7 software (AD Instruments, Australia).

### Determination of $EC_{50}$ of adrenaline

In the first part of the experimental procedure, using 6 isolated rabbit's hearts, 1 ml of Ringer-Locke solution containing one concentrations of Adrenaline (1.0, 2.5, 5.0, 7.5 and 10  $\mu\text{g/ml}$ ) was injected by same person by bolus over 10 s with the aid of 1 ml syringe through the perfusion line above the aortic line, and the changes in the cardiac parameters were recorded for 5 min consecutively. After each treatment, the hearts were washed by the perfusion fluid until the baseline recording is achieved and then the second dose was then given and the effect was recorded. The recording before the direct perfusion of the drug was considered as baseline reading for each dose.  $EC_{50}$  for the effect of adrenaline on FC (g) and HR (BPM) was plotted by plotting the log of each dose



**Figure 1.** Dose response curve for the effect of adrenaline (1, 2.5, 5, 7.5 and 10  $\mu\text{g/ml}$ ) on the force of contraction of isolated rabbit's heart.  $\text{EC}_{50}$  was given as mean for group of 6 rabbit's hearts each.  $\text{EC}_{50} = 3.23 \pm 0.09 \mu\text{g/ml}$ . Dose response (g) for each dose was calculated as the average increase in force of contraction during the first 5 min after adrenaline treatment, pre-dose baseline correction was applied during the calculation. Data were collected and analyzed by Labchart pro7 software (Dose response module, AD Instruments, Australia).

against its resulted mean increase or decrease in the FC or HR (mean difference in reading after treatment and its baseline).  $\text{EC}_{50}$  which resulted in half maximum effect was calculated using dose response curve module installed in LabChartpro7 software (AD Instruments, Australia)

#### Determination of the atenolol concentrations

In the second part of the experiment, using the mean  $\text{EC}_{50}$  of adrenaline, other six isolated rabbit's hearts were used to record the heart rate and contractility. Atenolol was prepared in the perfusion fluid to a final volume of 50 ml at the following concentrations: 1.0, 2.5, 5.0 and 10  $\mu\text{g/ml}$ . In this part of the experiment, one concentration of atenolol was perfused first for 3 min and then followed by the addition of adrenaline (average  $\text{EC}_{50}$ ) by bolus and the effect on heart rate and force of contraction was recorded. Preparing of the hearts, recording and analysis of both heart rate and force of contraction were done using the same settings, procedures and condition for 5 consecutive min as described in the previously.

#### Calculations

Percent of change in FC or HR for each dose from its baseline reading at different time intervals (5 min intervals) was calculated as follows:

$$\text{Change (\%)} = \frac{\text{Mean FC (g) or HR (b/min)} - \text{Mean baseline FC or HR}}{\text{Mean baseline FC or HR}}$$

where “-Ve” sign means decrease in the value and “+Ve” sign means increase in the value.

#### Statistical analysis

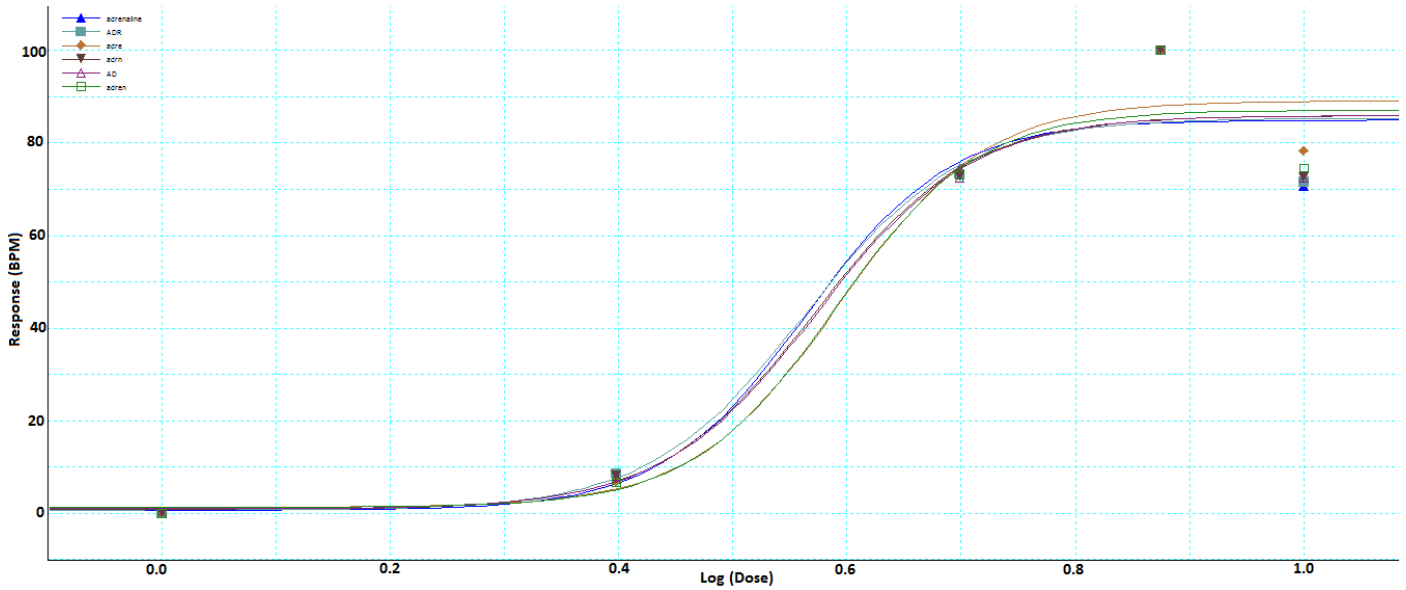
Results were expressed as the mean value  $\pm$  SD. Statistical differences between groups were assessed using the SPSS software, version 16 by One-way ANOVA test. Values of  $p < 0.05$

were considered significantly different (95% Confidence interval).

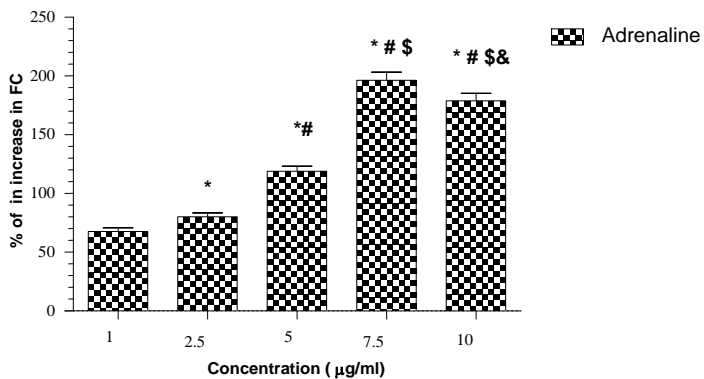
## RESULTS

All results obtained in this study are depicted in Figures 1 to 8. Cardiac parameters measured in this study were FC and HR. Figures 1 and 2 show plots of logs of different concentrations of adrenaline (1, 2.5, 5, 7.5 and 10  $\mu\text{g/ml}$ ) against the mean average resulted response in force of contraction (g) and heart rate (BPM) of 6 isolated rabbits, respectively. Adrenaline resulted in a stimulatory effect on the heart rate and the amplitude of the heart contraction. The effect was significant and dose dependent. The maximum increases in both heart rate and force of contraction were seen at adrenaline dose of 7.5  $\mu\text{g/ml}$  and the plateau phase was achieved at a dose of 10  $\mu\text{g/ml}$ . From these figures, the calculated  $\text{EC}_{50}$  of adrenaline on force of contraction was  $3.23 \pm 0.09$ , and was  $3.8 \pm 0.08 \text{ mg/ml}$  on heart rate. So, the average  $\text{EC}_{50}$  used of adrenaline in this study was 3.5  $\mu\text{g/ml}$ .

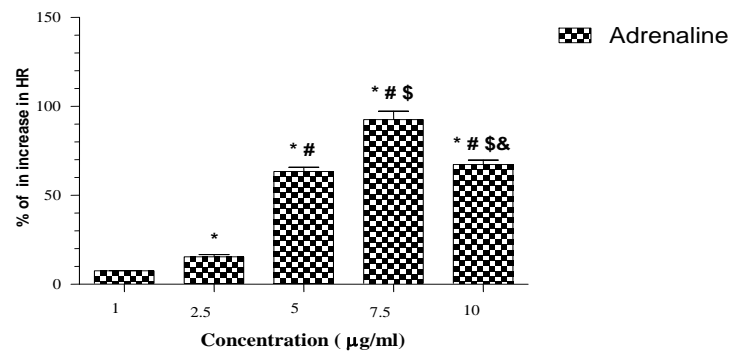
Figures 3 and 4 show the mean percents of increases in force of contraction and heart rate after adrenaline treatment over 5 min from every dose specific baseline readings, respectively. The data showed significant dose dependent mean increase in the percents of both heart rate and force of contraction with the doses of 1, 2.4, 5.0 and 7.5  $\mu\text{g/ml}$ , but at dose of 10  $\mu\text{g/ml}$ , the mean increases of heart rate and force of contraction started significantly to decline. The maximum percents of increases in force of contraction and heart rate were achieved at adrenaline dose of 7.5  $\mu\text{g/ml}$  ( $202 \pm 6.74$  and  $92.60 \pm 4.74\%$ , respectively). After atenolol (selective  $\beta$ -blocker) treatment, the positive inotropic effect of adrenaline was antagonized only at



**Figure 2.** Dose response curve for the effect of adrenaline (1, 2.5, 5, 7.5 and 10 µg/ml) on heart rate of isolated rabbit’s heart. EC<sub>50</sub> was given as mean for group of 6 rabbit’s hearts each. EC<sub>50</sub>= 3.8± 0.08 µg/ml. Dose response (g) for each dose was calculated as the average increase in heart rate during the first 5 min after adrenaline treatment, pre-dose baseline correction was applied during the calculation. Data were collected and analyzed by Labchart pro7 software (Dose response module, AD Instruments, Australia).



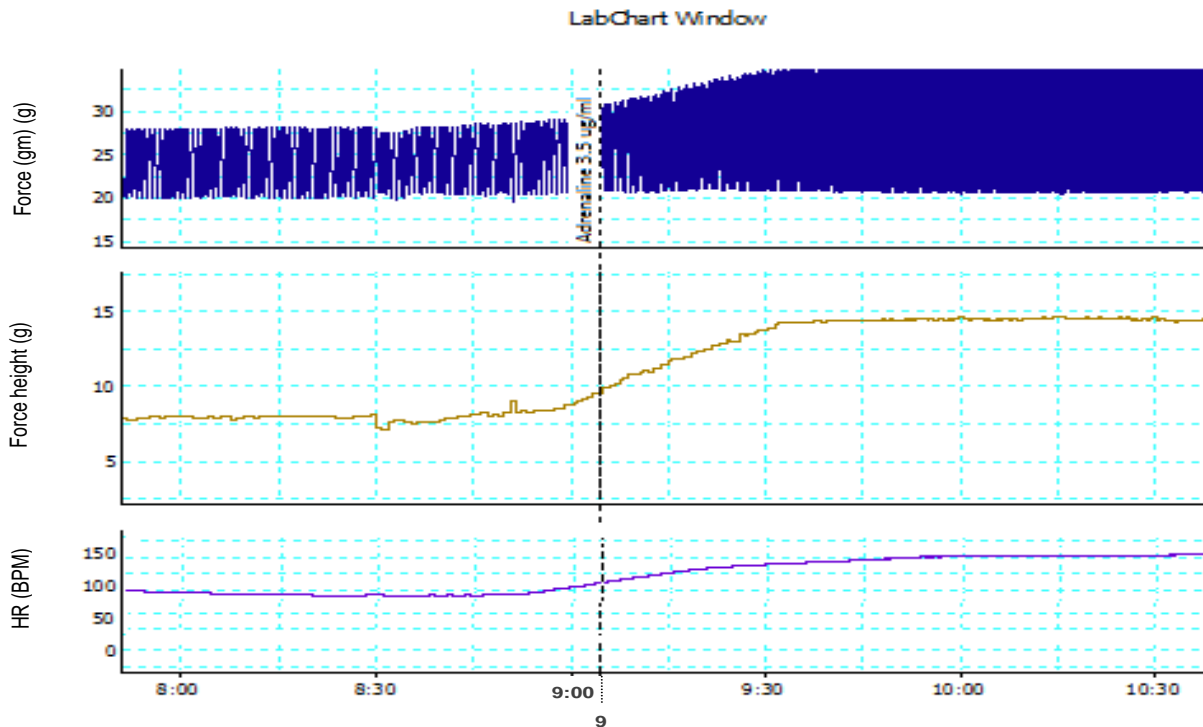
**Figure 3.** Percent of change in force of contraction of isolated hearts after adrenaline treatment (1, 2.5, 5, 7.5 and 10 µg/ml). All readings were analyzed after 5 min of recording. Values are given as Mean ± SD for groups of 6 rabbit’s hearts each. Analysis by one-way ANOVA and values were considered significantly different at P < 0.05. \*Significantly different from 1 µg/ml treated group. #, Significantly different when compared to 2.5 µg/ml treated group; \$, significantly different when compared to 5 µg/ml treated group and &, significantly different when compared to 7.5 µg/ml treated group.



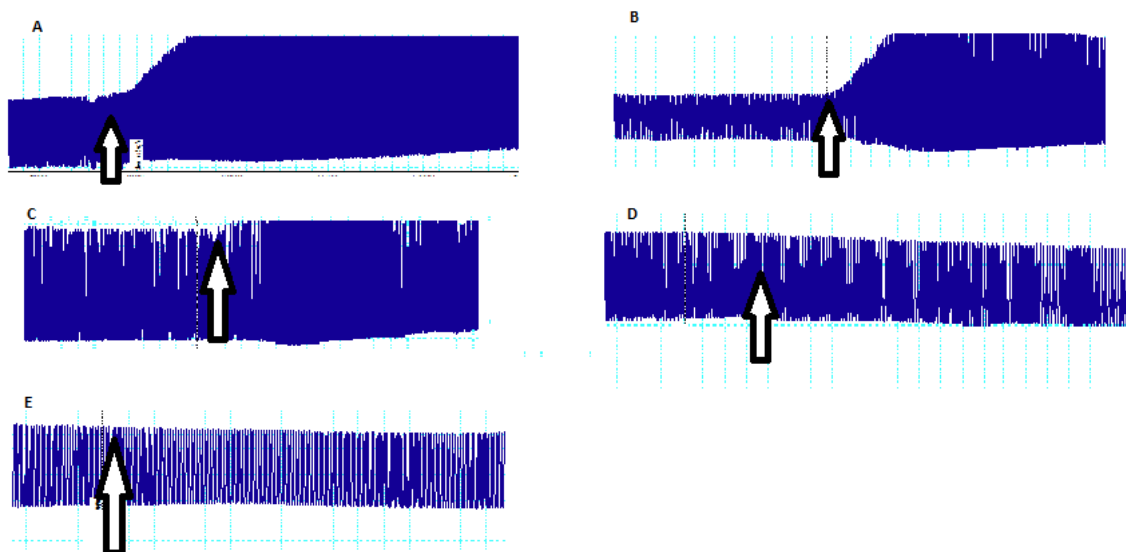
**Figure 4.** Percent of change in heart rate of isolated hearts after adrenaline treatment (1, 2.5, 5, 7.5 and 10 µg/ml). All readings were analyzed after 5 min of recording. Values are given as Mean ± SD for groups of 6 rabbit’s hearts each. Analysis by one way ANOVA and values were considered significantly different at P < 0.05; \*, Significantly different from 1 µg/ml treated group. #, Significantly different when compared to 2.5 µg/ml treated group, \$, Significantly different when compared to 5 µg/ml treated group and &, Significantly different when compared to 7.5 µg/ml treated group.

atenolol concentrations of 5.0, 7.5 and 10 µg/ml (Figure 6). The percents of increase in force of contraction at its average EC<sub>50</sub> used in this study (3.5 µg/ml) was 118 ± 3.4% (Figures 5 and 6A) and the increases in this percent contraction as a result of perfusion of different atenolol doses (1.0, 2.5, 5 and 10 µg/ml) before adrenaline (3.5 µg/ml) perfusion were 124 ± 4.6, 5.0 ± 0.78, 1.8 ± 0.28 and

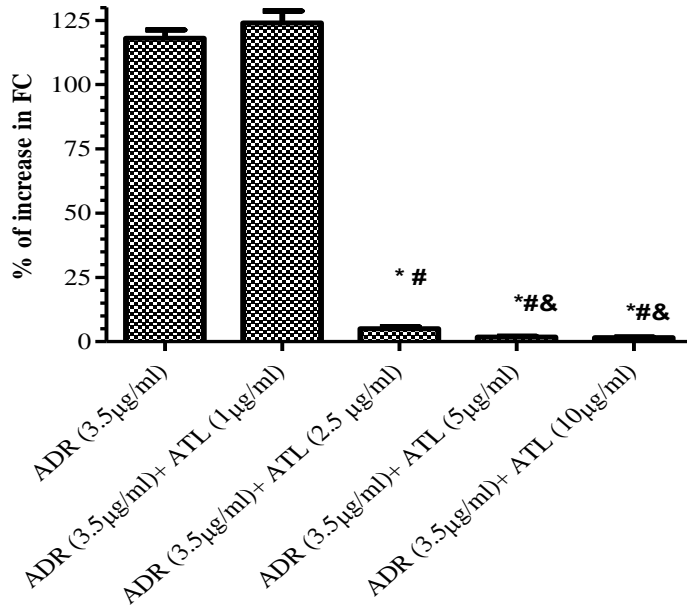
and 1.6 ± 0.16%, respectively (Figure 7). The ANOVA test revealed that there was no significant change between the percents of increase in force of contraction after adrenaline treatment with atenolol doses of 5 and 10 µg/ml. Also, as seen in Figure 8, atenolol at all dose used showed a dose response antagonistic effect against



**Figure 5.** The effect of adrenaline (at its Average  $EC_{50}$  (3.5  $\mu\text{g/ml}$ )) on heart rate and force of contraction on rabbit's isolated heart. The signal from the force transducer and the ECG electrodes were filtered and amplified and sent to an analog-to-digital converter (PowerLab data acquisition and analysis system: AD Instruments, Australia). Data were collected and analyzed by Labchartpro7 software (AD Instruments, Australia).



**Figure 6.** Recording of cardiac force of contraction and heart rate after addition of adrenaline alone (A) or adrenaline and atenolol (B-E). Atenolol of certain concentration was perfused in a final volume of 50 ml to each heart, followed by the addition of adrenaline at its average  $EC_{50}$  (3.5  $\mu\text{g/ml}$ ). A: Adrenaline 3.5  $\mu\text{g/ml}$  alone, B: Adrenaline 3.5  $\mu\text{g/ml}$  and atenolol (1  $\mu\text{g/ml}$ ), C: Adrenaline 3.5  $\mu\text{g/ml}$  and atenolol (2.5  $\mu\text{g/ml}$ ), D: Adrenaline 3.5  $\mu\text{g/ml}$  and atenolol (5  $\mu\text{g/ml}$ ) and E: Adrenaline 3.5  $\mu\text{g/ml}$  and atenolol (10  $\mu\text{g/ml}$ ). The signal from the force transducer and the ECG electrodes were filtered and amplified and sent to an analog-to-digital converter (Power Lab data acquisition and analysis system: ADInstruments, Australia). Data were collected and analyzed by Labchartpro7 software (AD Instruments, Australia).



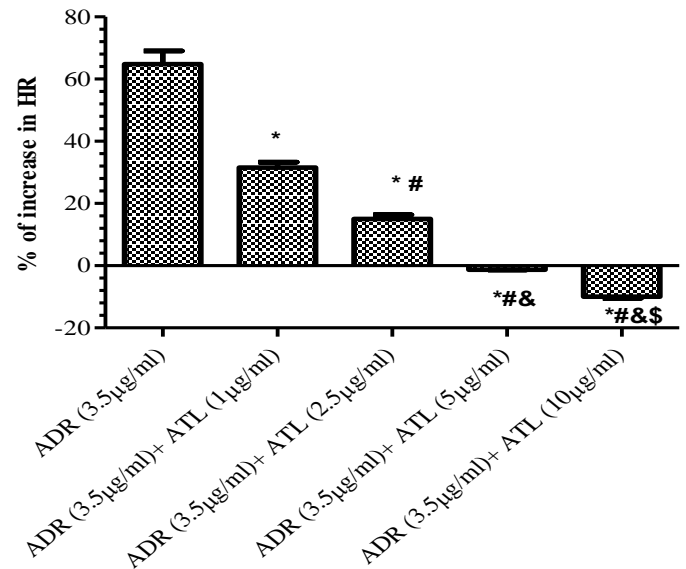
**Figure 7.** Percent of change in force of contraction of isolated hearts after adrenaline (3.5 µg/ml) with or without atenolol treatment. Atenolol (ALT) of certain concentration was perfused in a final volume of 50 ml to each heart, followed by the addition of adrenaline (ADR). All readings were analyzed after 5 min of recording. Values are given as Mean ± SD for groups of 6 rabbit's hearts each. Analysis by one-way ANOVA and values were considered significantly different at  $p < 0.05$ ; \*, Significantly different from adrenaline treated group; #, significantly different when compared to adrenaline (3.5 µg/ml) and atenolol (1 µg/ml) treated group, \$, significantly different when to adrenaline (3.5 µg/ml) and atenolol (2.5 µg/ml) treated group.

adrenaline. Adrenaline alone resulted in about  $64 \pm 2.4$  µg/ml increases in heart rate, while atenolol at doses of 1.0, 2.5, 5 and 10 µg/ml resulted in about  $31.5 \pm 1.8$ ,  $15 \pm 1.3$ ,  $-1.3 \pm 0.08$ ,  $-10 \pm 0.45\%$ , respectively, increases in heart rate. From the data, it is clear that the complete inhibition of adrenaline effect on heart rate was achieved at atenolol concentrations of 10 µg/ml.

## DISCUSSION

The isolated heart experiments possess numerous advantages as a cost-effective and informative screen for the cardiovascular effect and safety assessment of new drug candidates. Literature is almost silent regarding the exact pharmacological doses of different cardiac antagonist that are usually used in assessing the mechanism of action of any newly discovered drugs in isolated rabbit's hearts.

The present study, as single part of series studies to examine different concentrations of cardiac antagonists, has been conducted to determine the exact concentration of one of the most sympathomimetic agonists, adrenaline that completely blocks the positive inotropic and



**Figure 8.** Percent of change in heart rate of isolated hearts after adrenaline (3.5 µg/ml) with or without atenolol treatment. Atenolol (ATL) of certain concentration was perfused in a final volume of 50 ml to each heart followed by the addition of adrenaline (ADR). All readings were analyzed after 5 minutes of recording. Values are given as Mean ± SD for groups of 6 rabbit's hearts each. Analysis by one way ANOVA and values were considered significantly different at  $P < 0.05$ ; \*Significantly different from adrenaline treated group. #, Significantly different when compared to adrenaline (3.5 µg/ml) and atenolol (2.5 µg/ml) treated group; \$, significantly different when to adrenaline (3.5 µg/ml) and atenolol (10 µg/ml) treated group.

chronotropic effect of adrenaline at its  $EC_{50}$  in isolated rabbits heart. The results obtained in this study were collected and analyzed using one of the most modern and highly accurate and sensitive data acquisition system in the markets, PowerLab system and Lab chart software. Atenolol was mostly used in isolated hearts from rats at a concentration of 10 µM (2.66 µg/ml) (Richardt et al., 1990; Du et al., 1993). Through our search, little is known regarding atenolol exact concentration to be used to completely block adrenoreceptors in isolated rabbit's hearts. Hence, in this study, average  $EC_{50}$  of adrenaline on both heart rate and force of contraction was determined first on exposed heart of the white New Zealand white rabbits hearts, and the effects of adrenaline at its average  $EC_{50}$  on these cardiac parameters were recorded after perfusion of the isolated heart with different concentration of atenolol. Adrenoreceptors include alpha and beta adrenergic receptors. Beta receptors are usually sensitive to activators than alpha receptors

In the present study, as expected, adrenaline produced a stimulatory effect on both heart rate and force of contraction of isolated hearts, probably through an activation of beta, -adrenoceptors (Ariens and Simonis, 1983). The dose response of adrenaline on both force

of contraction and heart rate was almost close to each other ( $3.23 \pm 0.09$  and  $3.8 \pm 0.08$   $\mu\text{g/ml}$ , respectively), thus, the average  $\text{EC}_{50}$  was shown to be  $3.5$   $\mu\text{g/ml}$ .

On the other hand, chronic blocker of stimulatory  $\beta$ -Adrenergic receptors, that is, atenolol leads to a coordinate trans-regulation of inhibitory receptors and Gi-proteins (Boyd, 1968). It slows down the strength of heart's contractions and reduces its oxygen requirements (Stephenson, 1973). Atenolol was administered to isolated hearts by perfusion not by bolus and this was based in a previous study which reported that drugs with low lipophilicity such as atenolol are ineffective in inhibiting the adrenoceptor agonist when given by bolus, but are effective if given by infusion in isolated heart experiments (Nakasone et al., 1988). In this experiment, atenolol only at higher concentrations of  $10$   $\mu\text{g/ml}$  showed complete antagonistic effect of adrenaline at its  $\text{EC}_{50}$ .

In summary, perfusion of atenolol to isolated New Zealand white rabbit's heart at a concentration of  $10$   $\mu\text{g/ml}$  completely inhibit adrenaline positive inotropic and chronotropic effect at its  $\text{EC}_{50}$ . This finding may help many researchers in the field to use this drug at this concentration in their researches.

## REFERENCES

- Ariens EJ, Simonis AM (1983). Physiological and pharmacological aspects of adrenergic receptor classification, *Biochem. Pharmacol.*, 32: 1539-1545
- Armitage AK, Burn JH, Gunning AJ (1957). Ventricular Fibrillation In The Isolated Rabbit Heart. *Brit. J. Pharmacol.*, 12: 215.
- Benforado JM (1958). A Depressant Effect Of Acetylcholine On The Idio-Ventricular- Pacemaker Of The Isolated Perfused Rabbit Heart. *Brit. J. Pharmacol.*, 13: 15.
- Brick I, Hutchison KJ, Roddie IC (1966). The Effect Of Propranolol On The Response Of The Rat Isolated Heart To Noradrenaline. *Br. J. Pharma. Chemother.*, 28: 324-327.
- Boyd IA (1968). Homoeopathy through the eyes of a physiologist. *Brit. Hom. J.*, 57: 86-95.
- Bush LR, Haack DW, Schlafer M, Lucchesi BR (1980). Protective Effects of adrenergic blockade in isolated ischemic hearts. *Eur. J. Pharmacol.*, 67: 209-217.
- Du XJ, Riemersma RA, Fox KAA, Dart AM (1993). Propranolol and lidocaine inhibit neural norepinephrine release in hearts with increased extracellular potassium and ischemia. *Circulation*, 88 (1): 1885-1892.
- Dutta AC (1991). Homeopathy: A light isotopic treatment. In "Homeopathy a light isotopic treatment" Edit: Dutta AC. 1st ed Jain Publishers, New Dehli, pp. 30-40.
- Endoh M, Hori M (2006). Acute heart failure: Inotropic agents and their clinical uses. *Expert Opin. Pharmacother.*, 7 (16): 2179-2202.
- Hondeghem LM, Carlsson L, Duker G (2001). Instability and triangulation of the action potential predict serious proarrhythmia, but action potential duration prolongation is antiarrhythmic. *Circulation*, 103: 2004-2013..
- Hondeghem LM, Hoffman P (2003). Blinded test in isolated female rabbit heart reliably identifies action potential duration and prolongation and proarrhythmic drugs: importance of triangulation, reverse use dependence and instability. *J. Cardiovasc. Pharmacol.*, 41: 14-24.
- Hondeghem LM, Lu HR, van Rossem K, De Clerck F (2003). Detection of proarrhythmia in the female rabbit heart: blinded variation. *J. Cardiovasc. Electrophysiol.*, 14: 287-294.
- Iglarz M, Binkert C, Morrison K, Fischli W, Gatfield J, Treiber A, Weller T, Bolli MH, Boss C, Buchmann S (2008). Pharmacology of macitentan, an orally active tissue-targeting dual endothelin receptor antagonist. *J. Pharmacol. Exp. Ther.*, 327: 736.
- Kerker JP, Ruigrok TJC, Nieuwenhuijs JHM, Borst C, Meijler FL (1985). Post-extra systolic potentiation: Influence of calcium and verapamil in rat and rabbit hearts. *Eur. Heart J.*, 6: 532-538.
- Langendorff O (1985). Unverschungen und Uber lebenden Saugil hurherzen Pfluger. *Arch. Gis. Physiol.*, 61: 291.
- Magnussen F, Kudsk N (1974). Effects of Verapamil and Propranolol on ontractility, Frequency, Coronary Flow and Oxygen Consumption in the Isolated Rabbit Heart. *Acta Pharmacologica et Toxicologica.*, 34 (3); 141-151.
- Nakasone J, Kato T, Noguchi K, Nagam F, Sakanash IM (1988). Effects of Atenolol and Some Other Beta-Adrenoceptor Blocking Agents on Norepinephrine-Induced Responses in Isolated and Perfused Rat Hearts. *Japan. J. Pharmacol.*, 47: 387-395.
- Naylor WG, Poole-Wilson P (1981). Calcium antagonists: definition and mode of action. *Basic Res. Cardiol.*, 76: 1-15.
- Richardt G, Lumpp U, Haass M, Scho'mig A (1990). Propranolol inhibits nonexocytotic noradrenaline release in myocardial ischemia. *Naunyn-Schmiedeberg's. Arch. Pharmacol.*, 341: 50-55.
- Stephenson J (1973). Substance in dilution greater than 10-24. A review of investigation into their action. *Brit. Hom. J.*, 62: 3-8.
- Temsah RM, Dyck C, Netticadan T, Chapman D, Elimban V, Dhalla NS (2000). Effect of  $\beta$ -Adrenoceptor Blockers on Sarcoplasmic Reticular Function and Gene Expression in the Ischemic-Reperfused Heart. *The journal of pharmacology and experimental therapeutics. JPET*, 293: 15-23.
- Yawar E, Abdul Azeem M, Savanur A (2006). Comparison Of The Effect Of Sympathetic Agonist And Antagonist On Rabbits Perfused Heart: A Study On Simple And Succussed Dilutions. *Pak. J. Physiol.*, 2(1): 14-17.