Electrophysiological protective and therapeutic efficacy of resveratrol against cadmium chloride: An in vitro study

Hagar E. Mohammed a, Sherifa H. Ahmed b, and Zohour I. Nabil c.

a Geological and biological Department, Faculty of Education, Suez Canal University, Al-Arish, Egypt.
b Zoology Department, Faculty of Applied Sciences, Port Said University, Port Said, Egypt.
c Zoology Department, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt.

ABSTRACT

Resveratrol (trans-3,4',5-trihydroxystilbene- RES), is a natural antioxidant found in various fruits and vegetables and is abundant in grapes. It has beneficial effects against coronary heart diseases. This study aims to explore the protective and therapeutic effects of resveratrol on cadmium chloride induced toxicity on isolated toad's hearts. Electrocardiogram (ECG) was recorded before and after direct perfusion of cadmium chloride (CdCl2) and resveratrol. Application of CdCl2 (2mM/L) into isolated toad's heart significantly decreased the heart rate (HR) accompanied by an elongation in the conduction time (P-R interval), while a non significant decrease of the ventricular contraction (R-wave amplitude) was observed within minutes (5-30 min) of CdCl2 application into isolated toad's. Pretreatment of isolated toad's hearts with RES (21µM/L) five min before CdCl2 application abolished the severe bradycardia and negative inotropic effect induced by CdCl2, this was accompanied by improvement of cardiac disorders induced by this toxic heavy metal. While, post treatment of RES after appearance of cardiac abnormalities induced by CdCl2 didn’t affect these induced disorders. In conclusion, the obtained investigation indicates that RES could serve as a protective agent against some of acute (CdCl2) toxicity on isolated toad's heart than more that its therapeutic efficacy. However, low level of resveratrol at was not effective in preventing either sever bradycardia nor in reducing ventricular dysfunction induced by cadmium.

Keywords: Resveratrol; cadmium toxicity; Cardiac muscle; Electrophysiology

Abbreviation: cardiovascular diseases, CVDs; cadmium chloride, CdCl2; resveratrol, RES; purkinje-ventricular junction, PVJ; C-domain of troponin C, cCTnC; epigallocatechin gallate, EGCg; glutathione, GSH, atrioventricular node, AVN; nitric oxide, NO.

INTRODUCTION

There has been increasing interest in the potential adverse cardiovascular effects of environmental exposures, including heavy metals (Weinhold, 2004; Bhatnagar, 2006; Houston, 2007). Cadmium is a ubiquitous environmental toxin which may plausibly contribute to cardiovascular disease (CVDs), although existing literature is limited. Today CVDs are the killer number one worldwide. In 2004, an estimated 17.1 million people died due to CVDs and this number will further increase to an estimated 23.6 million by 2030 (Messner and Bernhard, 2010).

The cardiovascular effects of Cd2+ have been demonstrated-in vitro, in an experimental animal models (Sarkar et al., 1995; Satarug et al., 2006), and in human studies-that Cd2+ causes atherosclerosis (Messner and Bernhard, 2010), edema and hypertension (Prozialeck et al., 2006). In vitro studies, revealed that low level of Cd2+ (below toxic concentrations) may contribute to the initiation of pathophysiological changes in the vessel wall (Bernhard et al., 2006). Previous studies found an association between blood cadmium and peripheral arterial disease (Navas-Acien et al., 2004) and between urinary cadmium and peripheral arterial disease and myocardial infarction (Navas-Acien et al., 2005; Everett and Frithsen, 2008). Cadmium may exert its adverse cardiovascular effects by promoting atherosclerosis and by inducing disadvantageous cardiac functional and metabolic changes (Houtman, 1993).
Pathogenesis of some CVDs induced by Cd\textsuperscript{2+} involves damaging cells, their integral proteins, and enzymatic complexes. There are proofs of the Cd\textsuperscript{2+} toxic action on channel proteins of ventricular myocytes (Wasserstrom and Vites, 1999), respiratory enzymes (Korotkov et al., 2008), and myofibrils (Berwe et al., 1987).

The effect of Cd\textsuperscript{2+} on the mechanical activity of the heart was studied by several investigators. Kopp et al. (1983) reported depressed myocardial contractility in rat's heart from Cd\textsuperscript{2+} fed groups. They also found out that rats exposed to Cd\textsuperscript{2+} in drinking water developed electrocardiographic and biochemical changes in the myocardium as well as impairment of the functional status of the heart. Likewise, Nabil et al. (2002) showed that application of CdCl\textsubscript{2} (2mM) decreased the HR and power of ventricular contraction while increased the conduction time by increasing P-R interval either in vitro or in vivo studies.

In addition, Kisling et al. (1993) found that Cd\textsuperscript{2+} administration caused a reduction in myocardial contractile performance, slowing of HR and disturbances in metabolism of the heart. On the other hand, the effect of Cd\textsuperscript{2+} on the atrioventricular node (AVN) which is vital to the normal cardiac function was investigated by Hancox and Levi (1994). They reported that the action potentials recorded by current clamp from AVN were blocked by Cd\textsuperscript{2+} (100-200 mM). Since Cd\textsuperscript{2+} produces a reduction in the power of contraction, the conduction at the purkinje-ventricular junction (PVJ) sites was studied by Wiedmann et al. (1996). They proved that the conduction delay at the PVJ sites significantly increased by Cd\textsuperscript{2+}, while some PVJ sites became reversibly non-functional.

Cd\textsuperscript{2+} was also used as a blocker of voltage-calcium channels to inhibit the stimulating effect of D-dechoctrinated insecticide on rat myometrial smooth muscle cells (Juberg et al., 1995). It has been reported that the toxicity of Cd\textsuperscript{2+} is mainly due to increase of membrane lipid peroxidation and peroxidative damage (Chevion, 1991; Moustafa et al., 2000).

With the rapid advances made over the last two decades in biomedical research, there has been an unprecedented interest in unraveling the magical properties of some commonly used natural products. Consequently, a wide variety of natural products are under scrutiny for their clinical potential, both in terms of disease prevention and treatment. One remarkable compound in this list is resveratrol (RES), which is a polyphenol found in various fruits, vegetables, and is abundant in grapes. The root extracts of Polygonum cuspidatum, which is a polyphenol found in various fruits, vegetables, and is abundant in grapes. The root extracts of Polygonum cuspidatum, an important constituent of Chinese and Japanese folk medicine, is also an ample source of RES (Chen et al., 2001).

It has been speculated that RES may act as an antioxidant that modulate nitric oxide (NO) production (Hsieh et al., 1999), modulates vascular cell functions (Wallerath et al., 2002), and inhibits platelet aggregation (Olas et al., 2002). It was also reported that it reduces lipoprotein oxidation (Frankel et al., 1993), and increases high-density lipoprotein cholesterol (Bhat et al., 2001); thereby serving as a cardioprotective agent. In addition, RES exhibits anti-inflammatory, cancer chemopreventive and neuroprotective. Although it has a range of biological activities, its underlying mechanism in the protection against coronary heart disease remains unclear.

Risk reduction of cardiovascular events is one of the most well-known health promoting effects of RES. It has been shown that RES may modulate various aspects of cardiovascular diseases, including atherosclerosis, hypertension, ischemia reperfusion injury and heart failure (Huang et al., 2010 & Thandapilly et al., 2010). The present study was undertaken to elucidate the protective and therapeutic effects of trans-resveratrol on cadmium chloride-induced cardiac toxicity, for the first time, on isolated toad's hearts.

### Materials and Methods

#### i-Chemicals and solutions

RES was purchased from USA from Candlewood Stars Inc/Mega Resveratrol-Danbury, CT, 06810-6257. RES was obtained in a commercially available vegetable capsules at 500 mg polygonum cuspidatum per unit, providing 95% (500mg) RES. The RES stock and working solution (21 μM/L) were protected from light by covering the container with aluminum foil. Ringer's solution was used for the isolated heart preparations and composed of (6.5 gm/L NaCl, 0.14 gm/L KCl, 0.2 gm/L CaCl\textsubscript{2}, 0.2 gm/L NaHCO\textsubscript{3}, 0.01 gm/L NaPO\textsubscript{4} and 1gm/L glucose). Cadmium chloride was purchased from (Riedel-De Haenag- Hannover, Germany) and dissolved in Ringer's solution at a concentration of 2 mM/L (Nabil et al., 2002).

#### ii- Cardiac muscle experiments

Experiments on cardiac muscle were carried out on adult male toads of the species Bufo regularis (35-40g each) with isolated heart preparations. A dose of 2m M of CdCl\textsubscript{2} and 21 μM/L of RES was chosen and directly perfused into isolated heart preparations. ECG data were recorded directly from the surface of the heart according to Nabil et al. (1998) before and after RES and cadmium application. ECG Signals were amplified and recorded by the multi-pen-recording recorder (DBE, UK) with paper speeds of 2 and 10mm/sec. ECG was taken before any application to serve as self-control. After RES perfusion, signals were recorded each 5min for 30min.

#### iii- Experimental Design

Experiments on the cardiac muscle were carried out on the isolated toad's heart preparations. Four groups each of 10 animals except IV group (7 animals) were used.

I- Negative control group: - The isolated hearts directly perfused with Ringer's solution.

II- Cadmium chloride treated group: - The isolated hearts directly perfused with 2 mM CdCl\textsubscript{2} to emphasize the cardiac disorders induced by cadmium chloride.

III- Pretreated RES and CdCl\textsubscript{2} group: - The isolated toad's hearts pre-treated with RES (21μM/L) solution for 5 min then they directly perfused with 2 mM/L CdCl\textsubscript{2} lasted the experiment.

IV- CdCl\textsubscript{2} pretreated and RES group: - Isolated hearts were pre-treated with CdCl\textsubscript{2} (2 mM/L) to induce cardiac abnormalities, then RES (21 mM/L) was added to reveal the therapeutic effect of RES against cadmium induced-cardiotoxicity.

#### Data analysis

Responses of HR and the other electrocardiographic parameters (P-R interval and R amplitude) before and after treatment with RES were expressed as mean ± standard error (SE). One-way analysis of variance (ANOVA) was performed to evaluate the eventual significant differences (P<0.05) in the HR and different ECG parameters between control and treated groups according to Snedecor and Cochran (1980).

### RESULTS

In the present investigation, in vitro experiments were performed to investigate the protective and therapeutic effects of RES against CdCl\textsubscript{2} on the cardiac muscle activity through...
studying the influence of RES perfusion before and after CdCl₂ application on the ECG of isolated toad hearts. Normal HR and different ECG parameters were measured from the recorded ECGs of isolated hearts before any treatment to serve as self-control (0-time).

As seen in table 1, application of CdCl₂ induced severe bradycardia or −ve chronotropic effect manifested as a decrease of the HR. This was accompanied with an elongation in the P-R interval as shown in Table 2 indicating an increase in conduction time (+ve dromotropic effect). Regarding the effect of CdCl₂ on the cardiac contractility, the amplitude of R wave decreased at 25 and 30/min after CdCl₂ application reflecting a negative inotropic effect as demonstrated in Table 3. All the above mentioned changes in the HR and ECG parameters were statistically significant in comparison with the corresponding pre-treated values using Student’s paired t-test with p<0.05.

In order to investigate the protective effect of trans-RES (21µM/L) on cadmium-induced cardiac toxicity, a group of 10 isolated hearts were pretreated with RES to induce cardiac protection 5 min before CdCl₂ application. Table (1) illustrates that pretreatment with RES rectified the decrease in HR induced by CdCl₂ and showed a highly significant increase when compared with the corresponding CdCl₂ treated group (p≤ 0.001), an increase began from 10 min and lasted the end of experiments. At the same time, perfusion of isolated toad’s hearts with RES before CdCl₂ application prevented prolongation of P-R interval only at 5, 20 and 25 min (p<0.05) when compared with the corresponding CdCl₂ treated group by Student’s unpaired t-test as shown in Table 2. Likewise, the data presented in Table 3 demonstrate that pretreatment of isolated hearts with trans-RES abolished the negative inotropic effect induced by CdCl₂ and induced a strong positive inotropic effect when compared with the corresponding CdCl₂ treated group.

At the same time, RES pretreatment decreased the percentage cardiac disorders incidences caused by CdCl₂ such as bradycardia from 70% to 30%, ST segment elevation (ischemia) from 100% to 70% and R wave decline (negative inotropism) from 90% to 40%. Traces illustrating cardioprotective effect of RES against ECGs abnormalities induced by CdCl₂ are presented in Figures 1 and Figure 2 where RES application could abolish most ECGs abnormalities induced by CdCl₂. A group of 15 hearts was pretreated with CdCl₂ to induce cardiac toxicity, till the appearance of cardiac abnormalities: RES (21µM/L) was added to reveal its therapeutic effect against CdCl₂. Seven hearts from 15 one exhibited cardiac abnormalities after 10 min, but the others showed cardiac disorders at different times, were chosen to evaluate the therapeutic effect of RES against CdCl₂.

Table 4 and Fig. 3 illustrate that application of CdCl₂ on the isolated toad’s hearts induced a negative chronotropic effect beginning from 15min until the end of experiment accompanied by prolongation of P-R interval and reduction in the R-wave amplitude.

Cardiac arrhythmias induced by CdCl₂ as well as bradycardia, A-V heart block and negative inotropic effect and ST segment elevation. Perfusion with RES (21µM) after the incidence of the above abnormalities induced by cadmium had no therapeutic effect neither on CdCl₂–induced bradycardia nor P-R interval elongation or negative inotropic effect (noticed at 20 and 25min). The amazing thing is the ability of RES to enhance the positive dromotropic effect of CdCl₂, which was noticed at 5, 10 and 15min after RES perfusion (Table 4).

Figure 4. demonstrates four cases (I, II, III and IV) of therapeutic effect of resveratrol (21µM/L) after 2mM/L cadmium-induced cardiotoxicity, case I shows sinus arrhythmia and AV heart block. The direct perfusion of RES (21µ M) on the isolated toad’s hearts pretreated with CdCl₂, abolished this arrhythmia. Case II shows ventricular extrasystole, reflecting a ventricular focus with enhanced automaticity of the ventricle, as well as inverted P-wave, reflecting ectopic beats. Also, perfusion of RES counteracted this arrhythmia and p-wave abnormality. The surprising thing is the ability of RES to enhance the R-wave amplitude after CdCl₂ application, which was obviously noticed in both cases (I and II). Also, case III case IV show that RES had no effect on the two cases either bradycardia or R-wave decline.

Table 5 represent the percentage of incidence of ECGs abnormalities recorded from CdCl₂ pre-treated hearts before perfusion of RES. ECGs abnormalities included bradyarrhythmias as well as ST segment and AV block abnormalities (n=7/group).

Discussion

Cadmium is an ubiquitous environmental toxin which may plausibly contribute to cardiovascular diseases (Navas-Acien et al., 2004). The cardiovascular tissues, heart and blood vessels, are significant targets of cadmium toxicity (Kradarova et al., 1992). These vascular effects contribute to a variety of cardiovascular pathologic conditions including edema, hypertension (Prozialeck et al., 2006) and atherosclerosis (Messner and Bernhard, 2010).

Several studies in experimental animals have proved that oxidative stress is implicated in the toxicity of cadmium (Sarkar et al., 1995; Moustafa et al., 2000; Patra et al., 2011). Cadmium has been associated with multiple mechanisms that tend to promote vascular injury and atherosclerosis. These include the formation of reactive oxygen species, promotion of lipid peroxidation, depletion of glutathione (GSH), disruption of sulfhydryl homeostasis and down-regulation of NO (Moustafa et al., 2000; Navas-Acien et al., 2004; Tellez-Plaza et al., 2008).

The obtained results in this study revealed the effect of CdCl₂ on the heart in vitro, where 2mM/L CdCl₂ decreased the heart rate by 70%, the power of ventricular contraction by 90%, and on the contrary, increased the conduction time through increasing P-R interval by 100%. These observed effects are in agreement with the reports of the other investigators (Kopp et al., 1983; Nabil et al., 2002; Shemarova et al., 2011). It is worth noting that These effects could be related to cadmium induced alterations of calcium mediated or calcium activated physiological and biochemical pathways, or both, probably through a competition of the metal with calcium for membrane and intracellular sites linked with the contractile systems (Nasu, 1983).

It was evidenced that sinus abnormalities were the most frequent followed by different degrees of AV blocks were reported after direct administration of isolated toad’s heart with 2m M/L CdCl₂. This indicates that toxic myocardic effects on the myocardium are mostly directed to both sinoatrial (SA) and atrioventricular (AV) nodes. These pathologic cases included S-T segment depression, sinus arrhythmia, severe bradycardia, heart block and ectopic beats, which are often a feature of myocardial infarction (Julian et al., 2000).

In addition, Shemarova et al. (2011) indicated a negative inotropic Cd²⁺action on frog heart. The decrease of the heart contraction strength under the Cd²⁺ action seems to be due to
two causes: first, block of the Ca^{2+}-channels located on the plasma membrane, for which CdCl\(_2\) is inhibitor (Shen et al., 2000; Wang et al., 2004; Kocks'Amper et al., 2008). The second cause is mediated by toxic effect on rat heart mitochondria, which was manifested as an increase in ion permeability of the inner mitochondrial membrane, acceleration of the energy-dependent K\(^+\) transport into the matrix of mitochondria, and inhibition of their respiratory chain (Shemarova et al., 2011).

These observations confirm the direct mechanism of action of CdCl\(_2\) on Ca\(^{2+}\) channels. Therefore, antioxidants might be beneficial in the treatment of these abnormalities. RES is a naturally occurring phenolic compound found in grape skins (Jang et al., 1997; Vinson, 1998), where it is widely consumed as a nutritional supplement (Park et al., 2012). It has been suggested that trans-RES is one of the components responsible for the potential benefits of moderate red wine consumption in reducing cardiovascular disease risk (Wu et al., 2001; Zern and Fernandez, 2005; Pineda-Sanabria et al., 2011).

The current study indicates a cardioprotective effect of RES, since pretreatment with 21\(\mu\)M/L RES ameliorated the negative chronotropic and inotropic effects as well as abnormalities induced by CdCl\(_2\) application, where RES perfusion before CdCl\(_2\) decreased the percentage of cardiac abnormalities incidence. These results coincide with Rezk et al. (2006) and Zhao et al. (2008) who proved that RES possessed protective effects on As\(_2\)O\(_3\)-induced toxicity in H\(9\)c2 cardiomyocyte cells in vitro and in a mouse model of As\(_2\)O\(_3\)-induced cardiomyopathy in vivo.

The cardioprotective effects of RES have been reported in various in vitro and in vivo studies. In vitro studies have demonstrated that RES is associated with a variety of specific benefits, including reduced platelet aggregation, protection against low-density lipoprotein oxidation and inflammation, and improved plasma lipid profiles, that could collectively contribute to the putative cardioprotective action of this compound (Wu et al., 2001; Zern and Fernandez, 2005). In addition, RES and other red wine polyphenols have vasodilatory effects when applied to isolated artery segments in vitro at pharmacologic concentrations (Chen and Pace-Asciak, 1996; Rakici et al., 2005).

Several studies have shown that vascular relaxation was achieved with RES in several vascular beds. RES-induced vessel relaxation was documented in the rat aorta, porcine coronary arteries, guinea-pig mesenteric and uterine arteries and sheep coronary arteries (Chen and Pace-Asciak, 1996; Naderali et al., 2000; El-Mowafy, 2002). These studies indicated that RES exerts both direct and indirect vasodilator effects on the blood vessel by endothelium-independent (non-NO-mediated) and endothelium-dependent (NO-mediated) mechanisms, respectively.

Furthermore, RES exerts cardioprotective effects through NO stimulation in rat hearts during ischemia/reperfusion (Hung et al., 2000, 2001). Subsequently, they also found that RES exerts anti-infarction effects through a NO-dependent mechanism; whereas the antiarrhythmic effects appear to be NO-independent (Hung et al., 2004).

Experimental studies in animal models have shown that grape juice, red wine or isolated polyphenols like flavon, resveratrol and quercetin reduce the contractile dysfunctions of the heart and protect against cellular lesion induced by cardiac ischemia (Sato et al., 2000; Brookes et al., 2002; Cui et al., 2002). These effects may be observed following an oral intake of these substances or after their perfusion in an isolated heart before the induction of an ischemia. Also, RES showed a direct cardioprotective effect in vivo on diabetic myocardium in rats with streptozotocin-induced diabetes by improving left ventricular function, reducing the size of myocardial infarcts, and increasing levels of superoxide dismutase, a powerful antioxidant (Thirunavukkarasu et al., 2007). Another studies showed that RES effectively suppresses ischemia/reperfusion-induced arrhythmia (Hung et al., 2000, 2001; Dernek et al., 2004; Chen et al., 2007) by decreasing the oxidative stress generated in ischemic-reperfused myocardium (Ray et al., 1999; Hung et al., 2002).

The current data also revealed that RES post treatment had no significant improvement of the cardiotoxic effects induced by cadmium. This agrees with the study of Hung et al. (2000) they showed that RES was not effective in preventing arrhythmia nor in reducing the mortality rate sustained coronary artery occlusion.

The cardioprotective effect of RES was also attributed to its ability to upregulate catalase activity in the myocardium. RES functions as in vivo antioxidant and can scavenge peroxyl radicals in the heart (Sato et al., 2000a; Shigematsu et al., 2003), and protects the heart from ischemia reperfusion injury (Sato et al., 2000b).

Among its cardioprotective effects, RES was shown to directly affect the contractile function of guinea pig myocytes, where it induced contraction, its relation with the Ca\(^{2+}\) transients was quantitatively determined, indicating an increase in myofilament Ca\(^{2+}\) sensitivity (Liew et al., 2005).

These findings indicate a direct relation between RES and the Ca\(^{2+}\)-regulated elements in myocytes; however, the specific mode of its action is unknown. Pineda-Sanabria et al. (2011) indicated that trans-RES act as Ca\(^{2+}\) sensitizer through targeting C-domain of troponin C (cCTnC). Recently, the polyphenol, propyl gallate, has also been identified to act as a Ca\(^{2+}\) sensitizer with strong antioxidant activity (Tadano et al., 2009), which alongside the functional and structural data for epigallocatechin gallate (EGCg) and RES, points to a common mechanism by which these natural compounds target the thin filament to protect against heart failure.

In conclusion, using an isolated toad's hearts in vitro, the current investigation confirmed the protective effects of resveratrol against CdCl\(_2\)-induced injury to cardiomyocytes. The obtained results indicate that resveratrol could serve as a protective agent against some of acute cadmium chloride toxicity on isolated toad's heart. Although, resveratrol was not effective in preventing sinus bradycardia nor in reducing ventricular dysfunction after cadmium induced cardiac disorders, it could abolish some cardiac disorders such as arrhythmia and heart block. This is might be explained by the finding of the present work.

References
pulmonary artery endothelial cell proliferation by perturbing progression from S to G2. Cancer Res. 59, 2596–2601.


Thandapilly, S.J., Wojciechowski, P., Bebbahani, J., Louis,


Table 1. Effect of direct perfusion with 21µM/L resveratrol and 2mM/L CdCl₂ on the HR of isolated toad hearts.

<table>
<thead>
<tr>
<th>TIME</th>
<th>Heart Rate (beat/min.)</th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ringer (I)</td>
<td>CdCl₂ (II)</td>
<td>RES+CdCl₂ (III)</td>
</tr>
<tr>
<td>0 min</td>
<td>52.1±1.5</td>
<td>54.13±1.4</td>
<td>57.4±2.3</td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>54.86±1.45</td>
<td>50.3±1.7</td>
<td>56.6±2.2v</td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>53.05±1.06</td>
<td>47.1±2.08</td>
<td>54.5±2.05y</td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td>53.67±2.17</td>
<td>44.3±2.4</td>
<td>51.9±1.6v</td>
<td></td>
</tr>
<tr>
<td>20 min</td>
<td>44.54±1.89</td>
<td>39.2±2.6</td>
<td>50.3±1.7av</td>
<td></td>
</tr>
<tr>
<td>25 min</td>
<td>43.49±2.34</td>
<td>31.4±2.6</td>
<td>48.2±1.5av</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>42.81±2.11</td>
<td>28.05±2.9</td>
<td>44.7±2.9av</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Effect of direct perfusion with 21µM/L resveratrol and 2mM/L CdCl₂ on the P-R interval of isolated toad hearts.

<table>
<thead>
<tr>
<th>TIME</th>
<th>P-R (msec.)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ringer (I)</td>
<td>CdCl₂ (II)</td>
<td>RES+CdCl₂ (III)</td>
</tr>
<tr>
<td>0 min</td>
<td>280±17</td>
<td>305±8.9</td>
<td>310±24.5</td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>350±18.26</td>
<td>390±19.4</td>
<td>320±27v</td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>400±14.9</td>
<td>445±11.7</td>
<td>410±33.2</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Effect of direct perfusion with 21µM/L resveratrol and 2mM/L CdCl$_2$ on the depolarization voltage (R-wave amplitude) of isolated toad's heart.

<table>
<thead>
<tr>
<th>TIME</th>
<th>Ringer (I)</th>
<th>CdCl$_2$ (II)</th>
<th>RES+CdCl$_2$ (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0min</td>
<td>1.46±0.06</td>
<td>1.47±0.08</td>
<td>1.51±0.06</td>
</tr>
<tr>
<td>5min</td>
<td>1.62±0.09</td>
<td>1.37±0.14</td>
<td>2.22±0.17</td>
</tr>
<tr>
<td>10min</td>
<td>1.68±0.11</td>
<td>1.36±0.14</td>
<td>2.08±0.14</td>
</tr>
<tr>
<td>15min</td>
<td>1.76±0.22</td>
<td>1.28±0.12 b</td>
<td>1.85±0.17</td>
</tr>
<tr>
<td>20min</td>
<td>1.65±0.21</td>
<td>1.24±0.14</td>
<td>1.62±0.19</td>
</tr>
<tr>
<td>25min</td>
<td>1.43±0.2</td>
<td>0.98±0.13 a b</td>
<td>1.62±0.20</td>
</tr>
<tr>
<td>30min</td>
<td>1.23±0.2</td>
<td>0.71±0.1 a b</td>
<td>1.23±0.15</td>
</tr>
</tbody>
</table>

Values expressed as mean ±SE (n =10 /group).

*Significantly different between self control (0- time) and treated groups (5, 10, 15, 20, 25 & 30 min ) using Student's paired t-test (p ≤0.05).

¥ Significantly different from the control group (group I), Student's unpaired t-test (p≤0.05).

Table 4. Therapeutic effect of 21µM/L resveratrol after 2mM/L CdCl$_2$ application on the heart rate (HR), conduction velocity (P-R interval) and depolarization voltage (R-amplitude) of isolated toad's heart.

<table>
<thead>
<tr>
<th>Time</th>
<th>HR (beat/min)</th>
<th>P-R (msec)</th>
<th>R-amplitude (mv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0min</td>
<td>49.17±2.6</td>
<td>51.73±2.07</td>
<td>375.7±17.08</td>
</tr>
<tr>
<td>5min</td>
<td>48.03±1.5</td>
<td>39.78±3.4</td>
<td>425±11.2 a</td>
</tr>
<tr>
<td>10min</td>
<td>43.8±1.4</td>
<td>37.08±4.14</td>
<td>483.3±10.5 a</td>
</tr>
</tbody>
</table>

0 min, before any treatment; 5, 10, 15, 20, 25 and 30 min (I and II groups), after Ringer and CdCl$_2$ perfusion into isolated toad's heart; 5, 10, 15, 20, 25 and 30 min (III group), after application of CdCl$_2$ of RES pre-treated hearts.
Values expressed as mean ±SE (n =7/group).

* Significantly different between self control (0- time) and treated groups (5, 10, 15, 20 & 25 min ) using Student's paired t-test (p ≤0.05).

¥ Significantly different by student's unpaired t-test, CdCl2 versus CdCl2+ Res ( p≤0.02).

0 min, before any treatment; 5, 10, 15, 20 & 25 min (I group), after CdCl2 perfusion into isolated toad's heart; 5, 10, 15, 20 & 25 min (II group), after RES perfusion after 10 min of abnormalities appearance induced by CdCl2.

Table 5. Percentage of ECGs abnormalities Incidence recorded from 2mM/L CdCl2 and 21 µ M/L of resveratrol after CdCl2 on isolated toad's heart (n=7).

<table>
<thead>
<tr>
<th>Percentage of cardiac abnormalities Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ECG Abnormalities</strong></td>
</tr>
<tr>
<td>(A) Abnormal sinus rhythm:</td>
</tr>
<tr>
<td>- Bradycardia</td>
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<tr>
<td>- Ischemia</td>
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<tr>
<td>- Sinus arrhythmia</td>
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<tr>
<td>- R wave decline</td>
</tr>
<tr>
<td>(B) Atrioventricular block:</td>
</tr>
<tr>
<td>- First degree</td>
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<tr>
<td>- Second degree</td>
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<td>- Complete block</td>
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Figure 1. Percent of change of (A) heart rate, (B) P-R interval and (C) R-amplitude of isolated toad's heart after perfusion of RES and CdCl$_2$. (F) ECG traces showing the effect of direct application of isolated toad's heart with 2mM/L CdCl$_2$ on the ECG isolated toad's heart at different time intervals. g- Before treatment. h, i, j and k- after 5, 10, 20 and 30 min after CdCl$_2$ application (n=10/group).
Figure 2. I- ECGs recorders showing examples of cardiac disorders induced by direct application of isolated toad's heart with 2m M/L of cadmium chloride. A-Before treatment, B-After treatment, B1- Ischemia (S-T segment elevation), B2- QRS Widen, B3- *ST segment depression, B4- Sinus arrhythmia (↓). II- ECG traces showing the protective of direct perfusion of isolated toad's heart with 21µM/L resveratrol against CdCl₂ on the ECG isolated toad's heart at different time intervals. A- Before treatment. (B, C, D and E; 5, 10, 20 and 30 min after CdCl₂ application of RES pre-treated hearts, respectively)
Figure 3. I- Percent of change of (A) heart rate, (B) P-R interval and (C) R-wave of therapeutic effect of RES against CdCl₂ pretreated toad's heart (n=7/group).
II- Therapeutic effects of direct perfusion of isolated toad's heart with 21µM/L resveratrol against CdCl₂ on the ECG isolated toad's heart at different time intervals. Trace A- Before treatment; trace B- 5 min after CdCl₂ application; traces C, D, E and F are 5, 10, 20 and 30 min from RES perfusion after appearance of abnormalities induced by CdCl₂.
Figure 4. ECG traces showing the therapeutic effect of resveratrol (21µM/L) after CdCl$_2$ (2mM/L) application of isolated toad's.

B- After CdCl$_2$ application
C- After resveratrol perfusion.
I - Sinus arrhythmia (arrow) & AV block (*)
II - Ventricular extra systole with inverted P- wave.
III - Bradycardia
IV- ST elevation and R-wave decline.
الملخص العربي:

الريسفيراترول من الفلافونيدات الموجودة بكثرة في العنب والذي يستخدم كمقاوم للشوارد الحرة ومضاد للأكسدة وله تأثير نافع على أمراض القلب التاجية. تهدف الدراسة إلى توضيح التأثير الوقائي والعلاجى للريسفيراترول على تأثير الكادميوم السام على القلب المفصول للضفدع.

تم تسجيل رسم القلب قبل وبعد إضافة كلوريد الكادميوم و الريسفيراترول. اضافة الكادميوم (2 ملي مولر / لتر) على القلب المفصول أدى إلى تناقص معنوي في معدل نبضات القلب والتي مرتبطة بزيادة في وقت التوصيلية . كما أدى إلى تناقص غير معنوي في قوة انقباض البطين (R-wave amplitude). كما ظهرت بعض التغيرات في رسم القلب بعد إضافة كلوريد الكادميوم منها عدم انتظام النبضات القلبية، والانسداد القلبى. لدراسة التأثير الوقائي للريسفيراترول تم إضافة 21 ميكرومول / لتر منه قبل إضافة كلوريد الكادميوم بخمس دقائق و الذي أزالت التأثير الضار للكلوريد الكادميوم المتمثل في ضعف القلب الانتقاضية، ونقص ضربات القلب وقلل من العلامات المرضية في رسم القلب الكهربائي بينما لم يظهر التأثير العلاجي للريسفيراترول أي تحسن في تغير بعض المعايير الإلكتروفسيولوجية مثل معدل نبضات القلب وقوة البطين الانتقاضية. و نتيجة لذلك فإن الريسفيراترول قد يكون له تأثير وقائي على السمية القلبية لكلوريد الكادميوم أكثر من التأثير العلاجي له حيث أنه لم يمنع تناقص ضربات القلب وضعف قوة البطين الانتقاضية المستحدثة باضافة الكادميوم.