

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

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### 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 (CdCl<sub>2</sub>) and resveratrol. Application of CdCl<sub>2</sub> (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 CdCl<sub>2</sub> application into isolated toad's. Pretreatment of isolated toad's hearts with RES (21μM/L) five min before CdCl<sub>2</sub> application abolished the severe bradycardia and negative inotropic effect induced by CdCl<sub>2</sub>, 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 CdCl<sub>2</sub> didn't affect these induced disorders. In conclusion, the obtained investigation indicates that RES could serve as a protective agent against some of acute ( CdCl<sub>2</sub>) toxicity on isolated toad's heart than more that its therapeutic efficacy. However, low level of resveratrol at was not effective in preventing either neither sever bradycardia nor in reducing ventricular dysfunction induced by cadmium.

**Keywords:** Resveratrol; cadmium toxicity; Cardiac muscle; Electrophysiology

**Abbreviation:** cardiovascular diseases, CVDs; cadmium chloride, CdCl<sub>2</sub>; resveratrol, RES; purkinje-ventricular junction, PVJ; C-domain of troponin C, cTnC; 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 Cd<sup>2+</sup> have been demonstrated-*in vitro*, in an experimental animal models (Sarkar *et al.*, 1995; Satarug *et al.*, 2006), and in human studies-that Cd<sup>2+</sup> causes atherosclerosis (Messner and Bernhard, 2010), edema and hypertension (Prozialeck *et al.*, 2006). *In vitro* studies, revealed that low level of Cd<sup>2+</sup> (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<sup>2+</sup> involves damaging cells, their integral proteins, and enzymatic complexes. There are proofs of the Cd<sup>2+</sup> 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<sup>2+</sup> 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<sup>2+</sup> fed groups. They also found out that rats exposed to Cd<sup>2+</sup> 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<sub>2</sub> (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  $\text{Cd}^{2+}$  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  $\text{Cd}^{2+}$  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  $\text{Cd}^{2+}$  (100-200 mM). Since  $\text{Cd}^{2+}$  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  $\text{Cd}^{2+}$ , while some PVJ sites became reversibly non-functional.

$\text{Cd}^{2+}$  was also used as a blocker of voltage-calcium channels to inhibit the stimulating effect of D-dechlorinated insecticide on rat myometrial smooth muscle cells (Juberg *et al.*, 1995). It has been reported that the toxicity of  $\text{Cd}^{2+}$  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*, 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  $\mu\text{M/L}$ ) 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  $\text{CaCl}_2$ , 0.2 gm/L  $\text{NaHCO}_3$ , 0.01 gm/L  $\text{NaPO}_4$  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  $\text{CdCl}_2$  and 21  $\mu\text{M/L}$  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-rectilinear 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  $\text{CdCl}_2$  to emphasize the cardiac disorders induced by cadmium chloride.

III- Pretreated RES and  $\text{CdCl}_2$  group:- The isolated toad's hearts pre-treated with RES (21 $\mu\text{M/L}$ ) solution for 5 min then they directly perfused with 2 mM/L  $\text{CdCl}_2$  lasted the experiment.

IV-  $\text{CdCl}_2$  pretreated and RES group:- isolated hearts were pre-treated with  $\text{CdCl}_2$  (2 mM/L) to induce cardiac abnormalities, then RES (21  $\mu\text{M/L}$ ) 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  $\pm$  standard error (SE). One-way analysis of variance (ANOVA) was performed to evaluate the eventual significant differences ( $P \leq 0.05$ ) 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  $\text{CdCl}_2$  on the cardiac muscle activity through studying the influence of RES perfusion before and after  $\text{CdCl}_2$  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  $\text{CdCl}_2$  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  $\text{CdCl}_2$  on the cardiac contractility, the amplitude of R wave decreased at 25 and 30/min after  $\text{CdCl}_2$  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  $p \leq 0.05$ .

In order to investigate the protective effect of trans-RES (21µM/L) on cadmium-induced cardio toxicity, a group of 10 isolated hearts were pretreated with RES to induce cardio protection 5 min before CdCl<sub>2</sub> application. Table (1) illustrates that pretreatment with RES rectified the decrease in HR induced by CdCl<sub>2</sub> and showed a highly significant increase when compared with the corresponding CdCl<sub>2</sub> treated group ( $p \leq 0.001$ ), 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 CdCl<sub>2</sub> application prevented prolongation of P-R interval only at 5, 20 and 25 min ( $p \leq 0.05$ ) when compared with the corresponding CdCl<sub>2</sub> 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 CdCl<sub>2</sub> and induced a strong positive inotropic effect when compared with the corresponding CdCl<sub>2</sub> treated group.

At the same time, RES pretreatment decreased the percentage cardiac disorders incidences caused by CdCl<sub>2</sub> 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 CdCl<sub>2</sub> are presented in Figures 1 and Figure 2 where RES application could abolish most ECGs abnormalities induced by CdCl<sub>2</sub>. A group of 15 hearts was pretreated with CdCl<sub>2</sub> to induce cardiac toxicity, till the appearance of cardiac abnormalities; RES (21µM/L) was added to reveal its therapeutic effect against CdCl<sub>2</sub>. 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 CdCl<sub>2</sub>.

Table 4 and Fig. 3 illustrate that application of CdCl<sub>2</sub> 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 CdCl<sub>2</sub> 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 CdCl<sub>2</sub>-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 CdCl<sub>2</sub>, 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 CdCl<sub>2</sub>, 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 CdCl<sub>2</sub> 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 CdCl<sub>2</sub> 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 (Kadabova *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 CdCl<sub>2</sub> on the heart *in vitro*, where 2 mM/L CdCl<sub>2</sub> 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 CdCl<sub>2</sub>. This indicates that toxic myogenic 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 Cd<sup>2+</sup> action on frog heart. The decrease of the heart contraction strength under the Cd<sup>2+</sup> action seems to be due to two causes: first, block of the Ca<sup>2+</sup> -channels located on the plasma membrane, for which CdCl<sub>2</sub> is inhibitor (Shen *et al.*, 2000; Wang *et al.*, 2004; Kocksk'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<sup>+</sup> 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<sub>2</sub> on Ca<sup>2+</sup> 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 21µM/L RES ameliorated the negative chronotropic and inotropic effects as well as abnormalities induced by CdCl<sub>2</sub> application, where RES perfusion before CdCl<sub>2</sub> 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<sub>2</sub>O<sub>3</sub>-induced toxicity in H9c2 cardiomyocyte cells *in vitro* and in a mouse model of As<sub>2</sub>O<sub>3</sub>-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 peroxy 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<sup>2+</sup> transients was quantitatively determined, indicating an increase in myofilament Ca<sup>2+</sup> sensitivity (Liew *et al.*, 2005).

These findings indicate a direct relation between RES and the Ca<sup>2+</sup>-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<sup>2+</sup> sensitizer through targeting C-domain of troponin C (cTnC). Recently, the polyphenol, propyl gallate, has also been identified to act as a Ca<sup>2+</sup> 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<sub>2</sub>-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

- Bernhard, D., Rossmann, A., Henderson, B., Kind, M., Seubert, A., Wick, G., 2006. Increased serum cadmium and strontium levels in young smokers: effects on arterial endothelial cell gene transcription. *Arteriosclerosis, Thrombosis and Vascular Biology* 26(4), 833–838.
- Berwe, D., Gottschalk, G., Luttgau, H.C., 1987. Effects of the calcium antagonist gallopamil (D600) upon excitation–contraction coupling in toe Muscle fibers of the frog. *J. Physiol.* 385, 693–707.
- Bhat, K.P.L., Kosmeder, J.W., Pezzuto, J.M., 2001. Biological effects of resveratrol. *Antioxid. Redox. Signal.* 3, 1041–1064.
- Bhatnagar, A., 2006. Environmental cardiology: studying mechanistic links between pollution and heart disease. *Circ. Res.* 99(7), 692–705.
- Brookes, P.S., Digerness, S.B., Parks, D.A., Darley-USmar, V., 2002. Mitochondrial function in response to cardiac ischemia-reperfusion after oral treatment with quercetin. *Free Radic. Biol. Med.* 32, 1220–1228.
- Chen, C.K., Pace-Asciak, C.R., 1996. Vasorelaxing activity of resveratrol and quercetin in isolated rat aorta. *Gen. Pharmacol.* 27, 363–366.
- Chen, F., Ding, M., Castranova, V., Shi, X.L., 2001. Carcinogenic metals and NF-kappa B activation. *Mol. Cell Biochem.* 222, 159–171.
- Chen, W.P., Su, M.J., Hung, L.M., 2007. *In vitro* electrophysiological mechanisms for antiarrhythmic efficacy

- of resveratrol, a red wine antioxidant. *Eur. J. Pharmacol.* 554, 196–204.
- Chevion, M., 1991. Protection against free radical induced and transition of metal- mediated damage; the use of "pull" and "push" mechanisms. *Free Rad. Res. Comm.* 12-13, 691-696.
- Cui, J., Cordis, G.A., Tosaki, A., Maulik, N., Das, D.K., 2002. Reduction of myocardial ischemia reperfusion injury with regular consumption of grapes. *Ann. N Y Acad. Sci.* 957, 302–307.
- Dernek, S., Ikizler, M., Erkasap, N., Ergun, B., Koken, T., Yilmaz, K., Sevin, B., Kaygisiz, Z., Kural, T., 2004 . Cardioprotection with resveratrol pretreatment—improved beneficial effects over standard treatment in rat hearts after global ischemia. *Scand. Cardiovasc. J.* 38, 245–254.
- El-Mowafy, A. M., 2002. Resveratrol activates membrane-bound guanylyl cyclase in coronary arterial smooth muscle: A novel signaling mechanism in support of coronary protection. *Biochem. Biophys. Res. Commun.* 291, 1218-1224.
- Everett, C.J., Frithsen, I.L., 2008. Association of urinary cadmium and myocardial infarction. *Environ. Res.* 106(2), 284–286.
- Frankel, E.N., Waterhouse, A.L., Kinsella, J.E., 1993. Inhibition of human LDL oxidation by resveratrol. *Lancet* 341, 1103–1104.
- Hancox, J. C., Levi, A. J., 1994. L-type calcium current in rod-shaped and spindle-shaped myocytes isolated from rabbit atrioventricular node. *Amer. J. Physiol. Heart and Circ. Physiol.* 36, 1670-1680.
- Houston, M.C., 2007. The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease, and myocardial infarction. *Altern. Ther. Health Med.* 13, S128–S133.
- Houtman, J.P., 1993. Prolonged low-level cadmium intake and atherosclerosis. *Sci. Total Environ.* 138(1–3), 31–36.
- Hsieh, T.C., Juan, G., Darzynkiewicz, Z., Wu, J.M., 1999. Resveratrol increases nitric oxide synthase, induces accumulation of p53 and p21, and suppresses cultured bovine pulmonary artery endothelial cell proliferation by perturbing progression through S to G2. *Cancer Res.* 59, 2596–2601.
- Huang, J.P., Huang, S.S., Deng, J.Y., Chang, C.C., Day, Y.J., Hung, L.M., 2010. Insulin and resveratrol act synergistically, preventing cardiac dysfunction in diabetes, but the advantage of resveratrol in diabetics with acute heart attack is antagonized by insulin. *Free Radic. Biol. Med.* 49, 1710–1721.
- Hung, L.M., Chen, J.K., Huang, S.S., Lee, R.S., Su, M.J., 2000. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. *Cardiovasc. Res.* 47, 549–555.
- Hung, L.M., Chen, J.K., Lee, R.S., Liang, H.C., Su, M.J., 2001. Beneficial effects of astringinin, a resveratrol analogue, on the ischemia and reperfusion damage in rat heart. *Free Radic. Biol. Med.* 30, 877–883.
- Hung, L.M., Su, M.J., Chu, W.K., Chiao, C.W., Chan, W.F., Chen, J.K., 2002. The protective effect of resveratrols on ischaemia-reperfusion injuries of rat hearts is correlated with antioxidant efficacy. *Br. J. Pharmacol.* 135(7), 1627–1633.
- Hung, L.M., Su, M.J., Chen, J.K., 2004. Resveratrol protects myocardial ischemia-reperfusion injury through both NO-dependent and NO-independent mechanisms. *Free Radic. Biol. Med.* 36, 774–781.
- Jang, M., Cai, L., Udeani, G.O., Slowing, K.V., Thomas, C.F., Beecher, C.W., Fong, H.H., Farnsworth, N.R., Kinghorn, A. D., Mehta, R.G., Moon, R.C., Pezzuto, J. M., 1997. Cancer chemopreventative activity of resveratrol, a natural product derived from grapes. *Science* 275, 218–220.
- Juberg, D., Stuenkel, E., Loch-Carusio, R., 1995. The chlorinated insecticide 1,1-dichloro-2,2-bis(4chlorophenyl)ethane (p,p'-DDD) increases intracellular calcium in rat myometrial smooth muscle cells. *Toxicol. Appl. Pharmacol.* 135, 147-155.
- Julian, D., Cowan, J., Mclenachan, J., 2000. Heart failure In: "Cardiology" International Edition, Harcourt Publishers Ltd. North Youkshire, China 129-153.
- Kadabova, J., Madaric, A., Ginter, E., 1992. The effect of ascorbic acid on cadmium accumulation in guinea pig tissues. *Experientia.* 48, 989–991.
- Kisling, G.M., Kopp, S.J., Paulson, D.J., Tow, J.P., 1993. Cadmium-induced attenuation of coronary blood flow in the perfused rat heart. *Toxicol. Appl. Pharmacol.* 118, 58-64.
- Kocksk'amper, J., Zima, A.V., Roderick, H.L., Pies ke, B., Blatter, L.A., Bootman, M.D., 2008. Emerging roles of inositol 1,4,5-trisphosphate signaling in cardiac myocytes. *J. Mol. Cell Cardiol.* 45, 128–147.
- Kopp, S.J., Perry, H.M., Perry, E.F., Erlanger, M., 1983. Cardiac physiologic and tissue metabolic changes following chronic low-level cadmium and cadmium plus lead ingestion in the rat. *Toxicol. Appl. Pharmacol.* 69, 149-160.
- Korotkov, S.M., Nesterov, V.P., Ryabchikov, N.N., 2008. About effect of SH-groups on the mitochondrial respiration and swelling in rat heart, *Dokl RAN* 421, 123–127.
- Liew, R., Stagg, M. A., MacLeod, K. T., Collins, P., 2005. The red wine phenol, resveratrol, exerts acute direct action on guinea-pig ventricular myocytes. *Eur. J. Pharmacol.* 519, 1-8.
- Messner, B. and Bernhard, D., (2010). Cadmium and cardiovascular diseases: cell biology, pathophysiology, and epidemiological relevance. *Bio Metals* 23 (5), 811-822.
- Moustafa, S.A., Nabil, Z.I., Ahmed, S.H., 2000. Protective effect of zinc against cadmium toxicity. *Res. Comm. Pharmacol. Toxicol.* 5(5), 3-4.
- Nabil, Z., Hussein, A., Zalut, S., Rakha, M., 1998. Mechanism of action of honey bee (*Apis mellifera* L.) venom on different

- types of muscles. *Human and Experimental Toxicology* 17, 185-190.
- Nabil, Z.I., Moustafa, S.A., Ahmed, S.H., 2002. Effect of cadmium toxicity on cardiac and smooth muscles mechanism and protection. *J. Egypt Ger. Soc. Zool.* 38A, 1-21.
- Naderali, E. K., Doyle, P. J., Williams, G., 2000. Resveratrol induces vasorelaxation of mesenteric and uterine arteries from female guinea pigs. *Clin. Sci.* 98, 537-543.
- Nasu, T., 1983 . Spasmolytic effect of cadmium and cadmium uptake in aorta. *Br. J. Pharmacol.* 79, 751-754.
- Navas-Acien, A., Selvin, E., Sharrett, A.R., Calderon-Aranda, E., Silbergeld, E., Guallar, E., 2004. Lead, cadmium, smoking, and increased risk of peripheral arterial disease. *Circulation* 109, 3196-3201.
- Navas-Acien, A., Silbergeld, E.K., Sharrett, R., Calderon-Aranda, E., Selvin, E., Guallar, E., 2005. Metals in urine and peripheral arterial disease. *Environ Health Perspect.* 113(2), 164-169.
- Olas, B., Wachowicz, B., Saluk-Juszczak, J., Zielinski, T., 2002. Effect of resveratrol, a natural polyphenolic compound, on platelet activation induced by endotoxin or thrombin. *Thromb. Res.* 107, 141-145.
- Park, Sung-Jun; Ahmad, F., Philp, A., Baar, K., Williams, T., Luo, H., Ke, H., Rehmann, H., Taussig, R., Brown, A. L., Kim, M. K., Beaven, M. A., Burgin, A. B., Manganiello, V., Chung, J. H., 2012. Resveratrol Ameliorates Aging-Related Metabolic Phenotypes by Inhibiting cAMP Phosphodiesterases. *Cell* 148, 421-433.
- Patra, C., Rautray, A. K., Swarup, D., 2011: Oxidative Stress in Lead and Cadmium Toxicity and Its Amelioration. *Veterinary Medicine International.*
- Pervaiz S., 2003. Resveratrol: from grapevines to mammalian biology. *FASEB J.* 17, 1975-1985.
- Pineda-Sanabria, S. E., Robertson, I. M., Sykes, B. D., 2011. Structure of trans-Resveratrol in Complex with the Cardiac Regulatory Protein Troponin C. *Biochemistry* 50, 1309-1320.
- Prozialeck, W. C., Edwards, J. R., Woods, J. M., 2006. The vascular endothelium as a target of cadmium toxicity. *Life Sci*, 79, 1493-1506.
- Rakici, O., Kiziltepe, U., Coskun, B., Aslamaci, S., Akar, F., 2005. Effects of resveratrol on vascular tone and endothelial function of human saphenous vein and internal mammary artery. *In. J. Cardiol.* 105, 209-215.
- Ray, P. S., Maulik, G., Cordis, G. A., Bertelli, A. A., Bertelli, A., Das, D. K., 1999. The red wine antioxidant resveratrol protects isolated rat hearts from ischemia reperfusion injury. *Free Radic. Biol. Med.* 27, 160-169.
- Rezk, Y.A., Balulad, S.S., Keller, R.S., Bennett, J.A., 2006. Use of resveratrol to improve the effectiveness of cisplatin and doxorubicin: study in human gynecologic cancer cell lines and in rodent heart. *Am. J. Obstet. Gynecol.* 194, e23-e26.
- Sarkar, S., Yadar, P., Trivedi, R., Bansal, A.K., Bhatnagar, D., 1995. Cadmium-induced lipid peroxidation and the status of the antioxidant system in rat tissues. *J. Trace Elem. Med. Biol.* 9, 144-149.
- Satarug, S., Nishijo, M., Lasker, J.M., Edwards, R.J., Moore, M.R. 2006. Kidney dysfunction and hypertension: role for cadmium, p450 and heme oxygenases? *Tohoku J. Exp. Med.* 208,179-202.
- Sato, M., Ray, P. S., Meulki, G., Meulki, N., Engelman, R. M., Bertelli, A. A. E., Das, D. K., 2000. Myocardial protection with red wine extract. *J. Cardiovas. Pharmacol.* 35, 263-268.
- Sato, M., Cordis, G.A., Maulik, N., Das, D.K., 2000a. SAPKs regulation of ischemic preconditioning. *Am. J. Physiol.* 279, H901-H907.
- Sato, M., Maulik, G., Bagchi, D., Das, D.K., 2000b. Myocardial protection by protykin, a novel extract of trans-resveratrol and emodin. *Free Radic. Res.* 32, 135-144.
- Shemarova, I. V., Korotkov, S. M., Nesterov, V. P., 2011. Effect of oxidative processes in mitochondria on contractility of heart muscle of the frog *Rana temporaria*. *Actions of Cd<sup>2+</sup>*. *J. Evolutionary Biochem. Physiol.* 47, 360-365.
- Shen, J.B., Jiang, B., Pappano, A.J., 2000. Comparison of L-Type calcium channel blockade by nifedipine and/or cadmium in guinea pig ventricular myocytes. *J. Pharmacol. Exp. Ther.* 294, 562-570.
- Shigematsu, S., Ishida, S., Hara, M., Takahashi, N., Yoshimatsu, H., Sakata, T., Korthuis, R.J., 2003. Resveratrol, a red wine constituent polyphenol, prevents superoxide-dependent inflammatory responses induced by ischemia/reperfusion, platelet-activating factor, or oxidants. *Free Radic. Biol. Med.* 34(7):810-817.
- Snedecor, G. w., Cochran, W.G., 1980 . Statistical methods. 7<sup>th</sup> ed. Iowa State Univ. Press, U.S.A.
- Tadano, N., Morimoto, S., Takahashi-Yanaga, F., Miwa, Y., Ohtsuki, I., Sasaguri, T., 2009. Propyl Gallate, a Strong Antioxidant, Increases the Ca<sup>2+</sup> Sensitivity of Cardiac Myofilament. *J. Pharmacol. Sci.* 109,456-458.
- Tellez - Plaza, M., Navas - Acien, A., Crainiceanu, C.M., Guallar, E., 2008. Cadmium exposure and hypertension in the 1999 - 2004 National Health and Nutrition Examination Survey (NHANES). *Environ. Health Perspect.* 116, 51 - 56.
- Thandapilly, S.J., Wojciechowski, P., Behbahani, J., Louis, X.L., Yu, L., Juric, D., Kopilas, M.A., Anderson, H.D., Netticadan, T., 2010. Resveratrol prevents the development of pathological cardiac hypertrophy and contractile dysfunction in the SHR without lowering blood pressure. *Am. J. Hypertens.* 23, 192-196.
- Thirunavukkarasu, M., Penumathsa, S.V., Koneru, S., Juhasz, B., Zhan, L., Otani, H., Bagchi, D., Das, D.K., Maulik, N., 2007. Resveratrol alleviates cardiac dysfunction in streptozotocin-induced diabetes: Role of nitric oxide, thioredoxin, and heme oxygenase. *Free Radic. Biol. Med.* 43, 720-729.
- Vinson, J.A., 1998. Flavanoids in foods as *in vitro* and *in vivo* antioxidants. *Adv. Exp. Med. Biol.* 439, 151-164.

Wallerath, T., Deckert, G., Ternes, T., Anderson, H., Li, H., Witte, K., Forstermann, U., 2002. Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. *Circulation* 106, 1652–1658.

Wang, X., Evans, C., Mendelowitz, D., 2004. Voltage gated P/Q and N-type calcium channels mediate the nicotinic facilitation of GABAergic and glycinergic inputs to cardiac vagal neurons. *Neuropharmacol.* 46, 372–378.

Wasserstrom, J.A., Vites, A.M., 1999. Activation of contraction in cat ventricular myocytes: Effects of Low Cd<sup>2+</sup> concentration and temperature. *Am. J. Physiol.* 277, H488–H498.

Weinhold, B., 2004. Environmental cardiology: getting to the heart of the matter. *Environ. Health Perspect.* 112(15), A880–A887.

Wiedmann, R. T., Tan, R. C., Joyner, R. W., 1996. Discontinuous conduction at Purkinje-ventricular muscle junction. *Am. J. Physiol. Heart Circ. Physiol.* 271 (4), H1507–H1516.

Wu, J.M., Wang, Z.R., Hsieh, T.C., Bruder, J. L., Zou, J.G., Huang, Y.Z., 2001. Mechanism of cardioprotection by resveratrol, a phenolic antioxidant present in red wine. *Intl. J. Mol. Med.* 8, 3-17.

Zern, T.L., Fernandez, M. L., 2005. Cardioprotective effects of dietary polyphenols. *J. Nutr.* 135, 2291-2294.

Zhao, X.Y., Li, G.Y., Liu, Y., Chai, L.M., Chen, J.X., Zhang, Y., Du, Z.M., Lu, Y.J., Yang, B.F., 2008. Resveratrol protects against arsenic trioxide-induced cardiotoxicity *in vitro* and *in vivo*. *Br. J. Pharmacol.* 154, 105–113.

**Table 1. Effect of direct perfusion with 21µM/L resveratrol and 2mM/L CdCl<sub>2</sub> on the HR of isolated toad hearts.**

TIME	Heart Rate (beat/min.)		
	Ringer (I)	CdCl <sub>2</sub> (II)	RES+CdCl <sub>2</sub> (III)
0 min	52.1±1.5	54.13±1.4	57.4±2.3
5 min	54.86±1.45	50.3±1.7 <sup>a b</sup>	56.6±2.2 <sup>¥</sup>
10min	53.05±1.06	47.1±2.08 <sup>a b</sup>	54.5±2.05 <sup>¥</sup>
15min	53.67±2.17	44.3±2.4 <sup>a b</sup>	51.9±1.6 <sup>¥</sup>
20min	44.54±1.89 <sup>a</sup>	39.2±2.6 <sup>a</sup>	50.3±1.7 <sup>a¥</sup>
25min	43.49±2.34 <sup>a</sup>	31.4±2.6 <sup>a b</sup>	48.2±1.5 <sup>a¥</sup>
30min	42.81±2.11 <sup>a</sup>	28.05±2.9 <sup>a b</sup>	44.7±2.9 <sup>a¥</sup>

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

TIME	P-R (msec.)		
	Ringer (I)	CdCl <sub>2</sub> (II)	RES+CdCl <sub>2</sub> (III)
0min	280±17	305±8.9	310±24.5
5min	350±18.26 <sup>a</sup>	390 ±19.4 <sup>a</sup>	320±27 <sup>¥</sup>
10min	400±14.9 <sup>a</sup>	445±11.7 <sup>a b</sup>	410±33.2 <sup>a</sup>
15min	425±26.08 <sup>a</sup>	515±22.4 <sup>a b</sup>	445±43.1 <sup>a</sup>
20min	540±28.7 <sup>a</sup>	635±36.6 <sup>a b</sup>	505±37.6 <sup>a ¥</sup>
25min	555±31.1 <sup>a</sup>	690±36.4 <sup>a b</sup>	530±42.3 <sup>a ¥</sup>
30min	580±35.9 <sup>a</sup>	720±39.6 <sup>a b</sup>	605±62.1 <sup>a</sup>

**Table 3. Effect of direct perfusion with 21μM/L resveratrol and 2mM/L CdCl<sub>2</sub> on the depolarization voltage (R-wave amplitude) of isolated toad's heart.**

TIME	R-amplitude (mv)		
	Ringer (I)	CdCl <sub>2</sub> (II)	RES+CdCl <sub>2</sub> (III)
0min	1.46±0.06	1.47±0.08	1.51±0.06
5min	1.62±0.09	1.37±0.14	2.22±0.17 <sup>a</sup> ¥
10min	1.68±0.11	1.36±0.14 <sup>b</sup>	2.08±0.14 <sup>a</sup> ¥
15min	1.76±0.22	1.28±0.12 <sup>b</sup>	1.85±0.17 <sup>a</sup> ¥
20min	1.65±0.21	1.24±0.14	1.62±0.19 <sup>a</sup>
25min	1.43±0.2	0.98±0.13 <sup>a</sup> <sup>b</sup>	1.62±0.20 <sup>¥</sup>
30min	1.23±0.2	0.71±0.1 <sup>a</sup> <sup>b</sup>	1.23±0.15 <sup>¥</sup>

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

<sup>a</sup> 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).

<sup>b</sup> Significantly different from the control group (group I), Student's unpaired *t*-test (p≤0.05).

¥ Significantly different by Student's unpaired *t*-test, CdCl<sub>2</sub> versus Res+CdCl<sub>2</sub>( p≤0.05).

0 min, before any treatment; 5, 10, 15, 20, 25 and 30 min (I and II groups), after Ringer and CdCl<sub>2</sub> perfusion into isolated toad's heart; 5, 10, 15, 20, 25 and 30 min (III group), after application of CdCl<sub>2</sub> of RES pre-treated hearts.

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

Time	HR(beat/min)		P-R (msec)		R-amplitude (mv)	
	CdCl <sub>2</sub> (I)	CdCl <sub>2</sub> +RES(II)	CdCl <sub>2</sub> (I)	CdCl <sub>2</sub> +RES(II)	CdCl <sub>2</sub> (I)	CdCl <sub>2</sub> +RES(II)
0min	49.17±2.6	51.73±2.07	375.7±17.08	407±25.42	1.4±0.07	1.73±0.15
5min	48.03±1.5	39.78±3.4	425±11.2 <sup>a</sup>	600±52.33 <sup>¥</sup>	1.33±0.18	1.54±0.13
10min	43.8±1.4	37.08±4.14	483.3±10.5 <sup>a</sup>	671±68.01 <sup>¥</sup>	1.3±0.12	1.33±0.15
15min	40.91±2.3 <sup>a</sup>	30.54±4.65	550±18.3 <sup>a</sup>	728±73.08 <sup>¥</sup>	1.2±0.13	1.13±0.15
20min	35.5±2.4 <sup>a</sup>	27.68±4.8	666.7±57.25	800±70.71	1.04±0.12 <sup>a</sup>	0.9±0.13
25min	27.44±2.2 <sup>a</sup>	26.56±4.7	741.75±43.6	829±76.26	0.8±0.1 <sup>a</sup>	0.91±0.15

Values expressed as mean ±SE (n =7 /group).



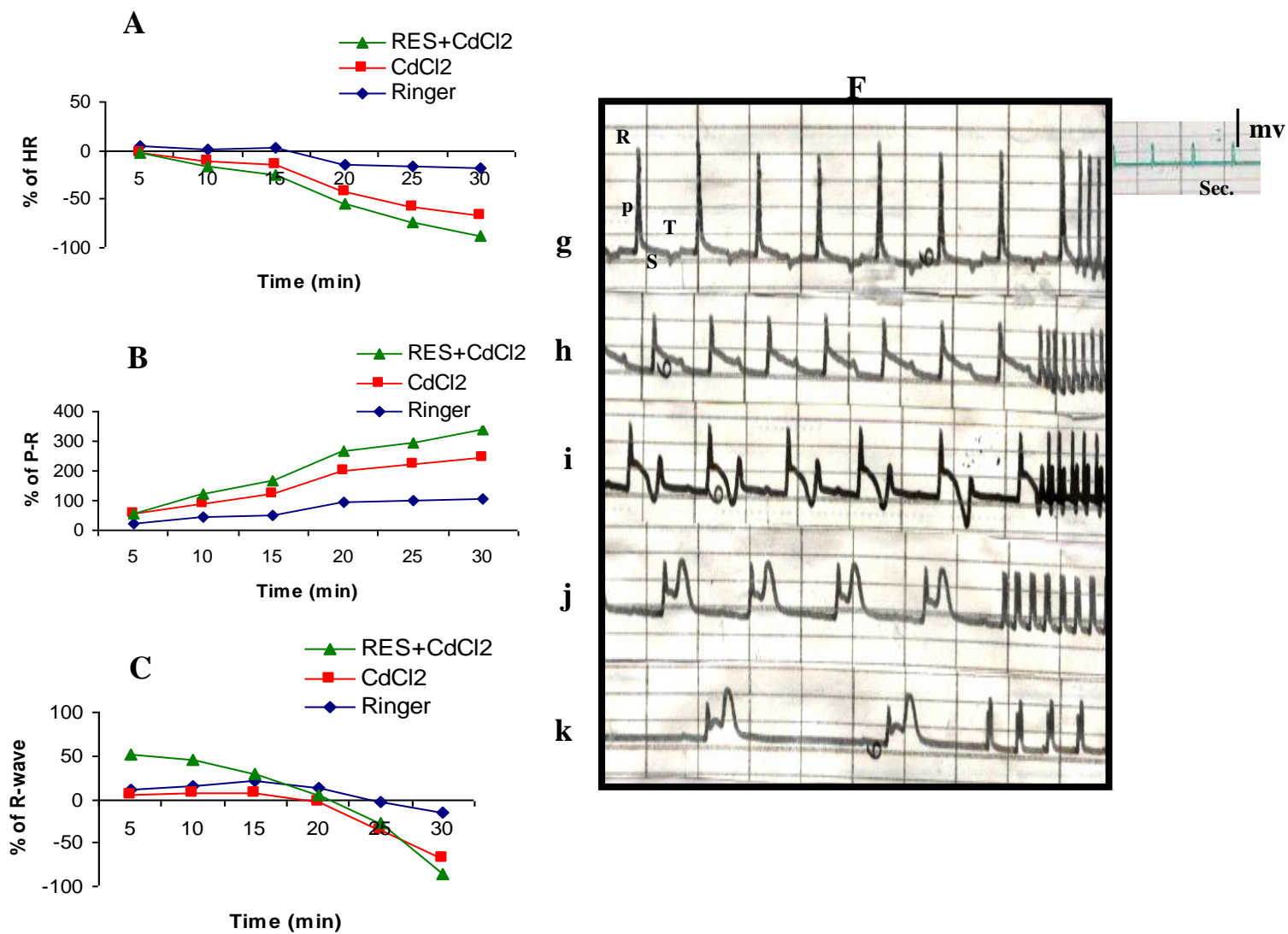
a Significantly different between self control (0- time) and treated groups (5, 10, 15, 20 & 25 min ) using Student's paired t-test ( $p \leq 0.05$ ).

¥ Significantly different by student's unpaired t-test, CdCl<sub>2</sub> versus CdCl<sub>2</sub>+ Res (  $p \leq 0.02$ ).

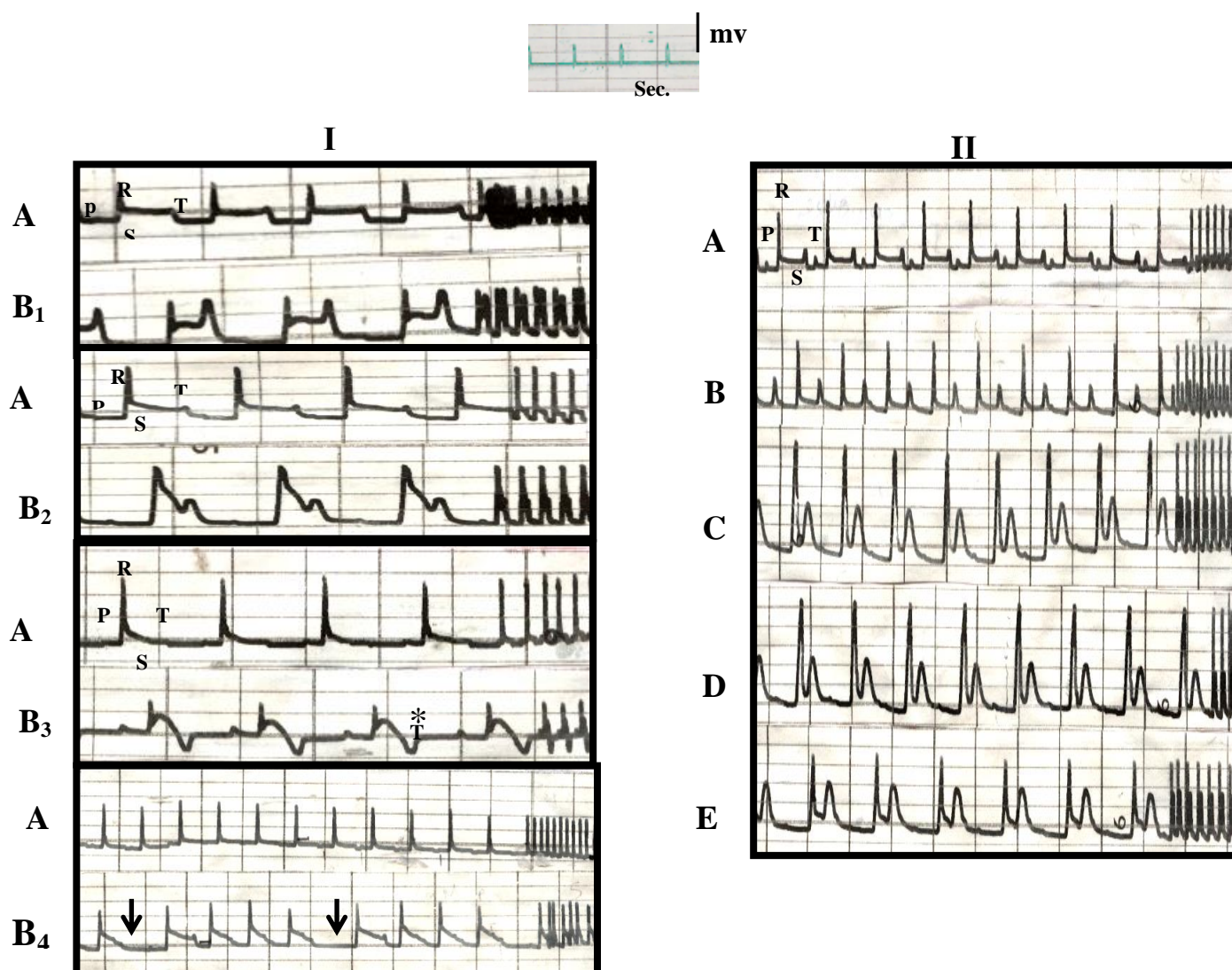
0 min, before any treatment; 5, 10, 15, 20 & 25 min (I group), after CdCl<sub>2</sub> perfusion into isolated toad's heart; 5, 10, 15, 20 & 25 min (II group), after RES perfusion after 10 min of abnormalities appearance induced by CdCl<sub>2</sub>.

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

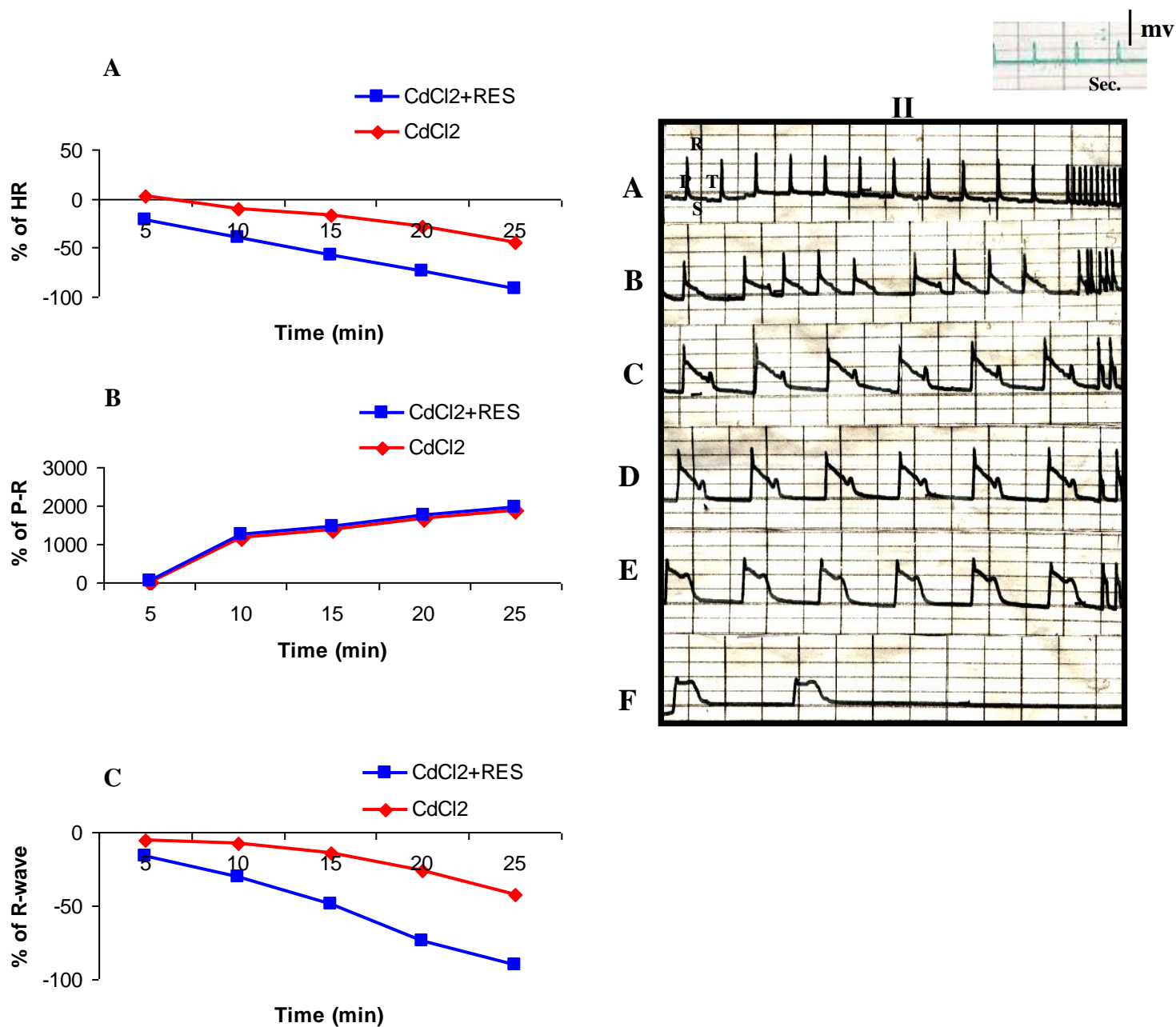
Percentage of cardiac abnormalities Incidence (%)		
ECG Abnormalities	CdCl <sub>2</sub>	CdCl <sub>2</sub> +RES
(A) Abnormal sinus rhythm:		
- Bradycardia	71 %	71 %
- Ischemia	100 %	85 %
- Sinus arrhythmia	43 %	28 %
- R wave decline	85 %	85 %
(B) Atrioventricular block:		
- First degree	100 %	85 %
- Second degree	14 %	14 %
- Complete block	-	-



**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<sub>2</sub>. (F) ECG traces showing the effect of direct application of isolated toad's heart with 2mM/L CdCl<sub>2</sub> 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<sub>2</sub> application (n=10/group).

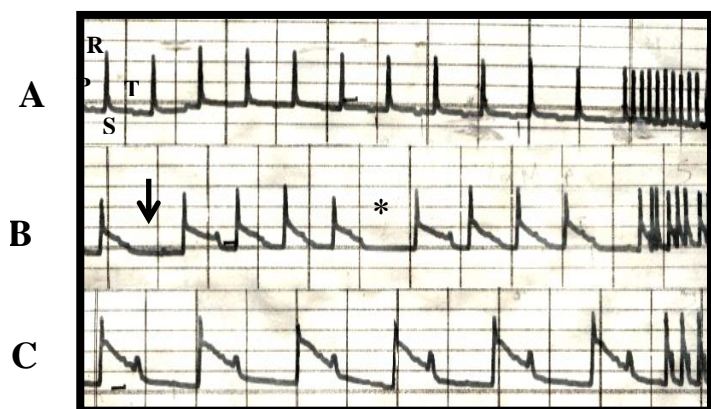


**Figure 2. I-** ECGs recorders showing examples of cardiac disorders induced by direct application of isolated toad's heart with 2m M/L of cadmium chloride. **A-**Before treatment, **B-** After treatment, B<sub>1</sub>- Ischemia (S-T segment elevation), B<sub>2</sub>- QRS Widen, B<sub>3</sub>- \*ST segment depression, B<sub>4</sub>- Sinus arrhythmia (↓),. **II-** ECG traces showing the protective of direct perfusion of isolated toad's heart with 21μM/L resveratrol against CdCl<sub>2</sub> 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 CdCl<sub>2</sub> application of RES pre-treated hearts, respectively)

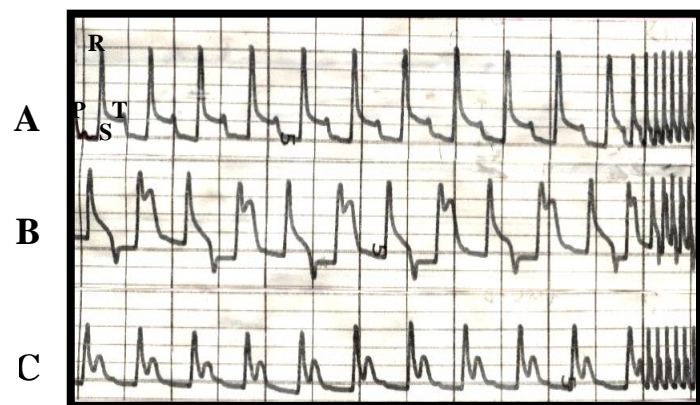




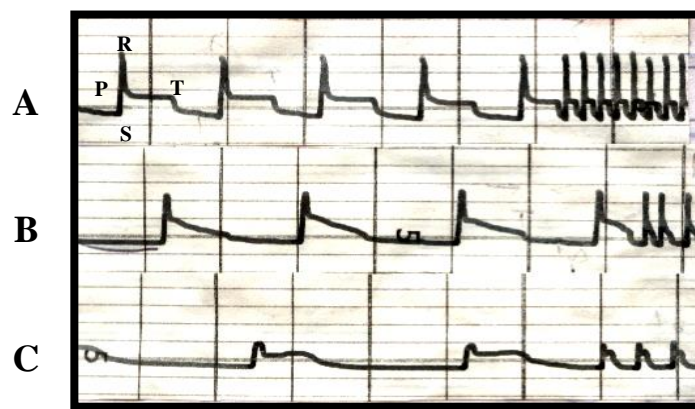
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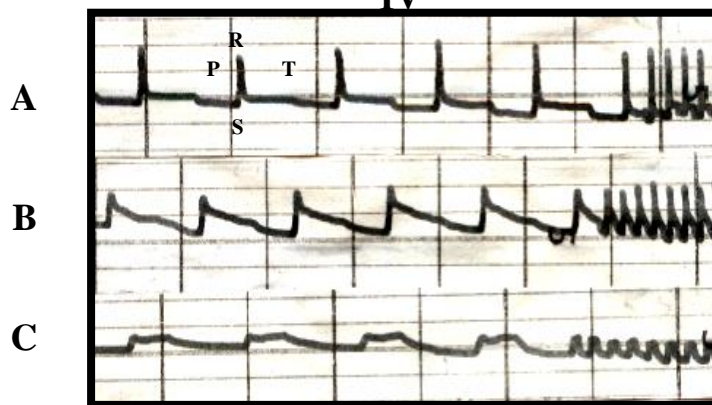
II



III



IV



**Figure 4. ECG traces showing the therapeutic effect of resveratrol (21 $\mu$ M/L) after CdCl<sub>2</sub> (2mM/L) application of isolated toad's.**

A- Before treatment.

B- After CdCl<sub>2</sub> 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 امراض القلب التاجية. تهدف الدراسة الى توضيح التأثير الوقائي والعلاجي للريسفيراترول على تأثير الكاديوم السام على القلب المفصول للضفدعة.

تم تسجيل رسم القلب قبل وبعد اضافة كلوريد الكاديوم و الريسفيراترول. اضافة الكاديوم (٢ مللى مولر / لتر) على القلب المفصول ادى الى تناقص معنوى فى معدل نبضات القلب و التى مرتبطة بزيادة فى وقت التوصيلية . كما ادى الى تناقص غير معنوى فى قوة انقباض البطين (R-wave amplitude). كما ظهرت بعض التغيرات فى رسم القلب بعد اضافة كلوريد الكاديوم منها عدم انتظام النبضات القلبية والانسداد القلبي. لدراسة التأثير الوقائي للريسفيراترول تم اضافة ٢١ ميكرومولر/ لتر منه قبل اضافة كلوريد الكاديوم بخمس دقائق و الذى ازال التأثير الضار للكاديوم المتمثل فى ضعف القوم الانقباضية ونقص ضربات القلب و قلل من العلامات المرضية فى رسم القلب الكهربى بينما لم يظهر التأثير العلاجى للريسفيراترول اى تحسن فى تغير بعض المعايير الالكتروفسيولوجية مثل معدل نبضات القلب و قوة البطين الانقباضية . و نتيجة لذلك فان الريسفيراترول قد يكون له تأثير وقائى على السمية القلبية لكلوريد الكاديوم أكثر من التأثير العلاجى له حيث انه لم يمنع تناقص ضربات القلب وضعف قوة البطين الانقباضية المستحدثة باضافة الكاديوم.