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Electrocardiographic Abnormalities in Lead Induced Cardiotoxicity and its Amelioration with Antioxidants in *Wistar* Rats

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ABSTRACT: Lead (Pb) is a well-recognized environmental pollutant and multisystemic toxin. The present work was designed to study the electrocardiographic (ECG) abnormalities in lead induced cardiotoxicity and their amelioration with antioxidants in Wistar rats. The experiment was conducted on 32 albino Wistar rats of either sex with 6-8 weeks of age having body weight of 180-200 g. These rats were maintained as per the standard CPCSEA guidelines and the study protocol was approved by IAEC, Co.V.Sc. & A.H., Jabalpur. Rats were randomly divided into four groups having 08 rats each. Group I served as control group. Group II rats received lead acetate @ 150 mg/kg b.wt. Group III rats received lead acetate @ 150 mg/kg b.wt. along with curcumin @ 400 mg/kg b.wt. and ascorbic acid @ 420 mg/kg b.wt. Group IV rats received curcumin @ 400 mg/kg b.wt. and ascorbic acid @ 420 mg/kg b.wt. All the rats were administered orally for 30 consecutive days and provided with *adlibitum* feed and water. Electrocardiography was performed in all the rats on day 30 of experimental period. Electrocardiography of rats from group II revealed significantly elevated heart rate, QRS amplitude, QT interval and significantly reduced PR interval as compared to control group. On simultaneous administration of antioxidants against lead induced cardiotoxicity, significant improvement in these ECG parameters was noticed. Several challenges have arisen during the electrocardiographic observations, including inadequate literature availability, anesthetic maintenance of rats, and ECG calibration. However, these challenges have been addressed with the assistance of veterinary cardiologists from the Department of Veterinary Medicine. Thus, the present study revealed that lead exposure has toxic effects on heart which disturb its functioning, while natural antioxidants (Curcumin and Ascorbic acid) may be preferable in reducing lead induced cardiotoxicity suggesting that chelating agents having antioxidant properties are preferred in treating cardiovascular disorders accompanying lead toxicity.

Keywords: Lead acetate, Curcumin, Ascorbic acid, Electrocardiography.

INTRODUCTION

Lead (Pb) is most hazardous among all known heavy metals. Its concentration in the environment and food chain is increasing day by day due to industrialization and excessive use of automobiles and ammunitions (Iyer *et al.,* 2015). Lead toxicity has accounted for high mortality and disabilities in human beings (ATSDR, 2007), remained a leading cause of death in bovine, and has negatively impacted growth and production worldwide (Blakley, 1984).

Cardiovascular diseases (CVDs) are the leading cause of death worldwide, leading to an estimated mortalities of 17.9 million lives per each year. More than four out of five CVD mortalities are due to cardiac attacks and strokes and one third of these mortalities occur prematurely in people under 60-70 years of age. Chronic exposure of sublethal lead dose is considered a potential risk for cardiovascular diseases (Patra *et al.,* 2006). There is an association between CVDs, inflammation and oxidative stress. It has been proposed

that lead toxicity occurs through inflammation, free radicals (reactive oxygen species) generation, and an imbalance between pro-oxidant and antioxidant mechanisms in cardiac tissue (Blakley, 1984; Patra *et al.,* 2006). Since, the pathological alterations are due to the production of free radicals, free radical scavengers (antioxidants) like vitamin C, B_6, E, zinc, etc. can be used as an important therapeutic method to prevent the pathology of lead toxicity (Chourasia, 2022).

Nearly all conventionally used chelating agents have limited use due to their adverse effects, such as nephrotoxicity, skin reactions and loss of essential metals. There are various ameliorative strategies explored by previous researchers to reduce the toxic effect of lead on animals. Amongst them, phytochemicals emerged as antioxidants that can protect cells from lead-induced damage (Dewanjee *et al.,* 2013). Curcumin, chemically known as diferuloylmethane, is an active fraction of turmeric (*Curcuma longa*) which is used as a dietary spice in foods and in traditional Indian herbal medicine since ancient times. Curcumin scavenges reactive oxygen and nitrogen species, prevents lipid peroxidation and chelates heavy metals (Patra *et al.,* 2006). Ascorbic acid, commonly known as vitamin C, is a potent antioxidant and chelating agent found naturally in the citrus family and is promisable in ameliorating the toxic effect of lead on male reproductive system (Raafat *et al.,* 2009).

Hence, the present work was designed to explore the electrocardiographic alterations in lead induced cardiotoxicity and its amelioration with antioxidants in Wistar rats.

MATERIAL AND METHODS

Experimental animals. After the approval of experiment by the institutional animal ethics committee, Co.V.Sc. Jabalpur (02/IAEC/Vety./2022), experiment was performed on albino Wistar rats of either sex. These rats were procured from CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) certified breeder and housed in central laboratory animal house, Co.V.Sc. and A.H., Jabalpur. Rats were acclimatized for 5 days before commencement of experiment and provided with ad-libitum commercial pelleted feed and water. These rats were maintained according to the guidelines of CPCSEA. Environmental conditions like 22±3°C temperature and 12 hours light and dark cycle were provided to all the rats.

Experimental design. Study was performed in 32 albino Wistar rats weighing 180 to 200 g with age group of 6-8 weeks which were divided into following 04 groups as described in Table 1.

Table 1: Experimental design of the study.

Group	Treatment	Number of animals
I	Control	08
II	Lead acetate @ 150 mg/kg b.wt., PO for 30 days	08
III	Lead acetate @ 150 mg/kg b.wt. + Ascorbic acid @ 420 mg/kg b.wt. + Curcumin @ 400 mg/kg b.wt., PO for 30 days	08
IV	Ascorbic acid @ 420 mg/kg b.wt. + Curcumin @ 400 mg/kg b.wt., PO for 30 days	08

Chemicals. Chemicals such as lead acetate, curcumin, L-ascorbic acid were obtained from Sigma Chemical Inc. (St. Louis, MO, USA) and drugs required for anaesthesia (xylazine and ketamine) were procured from Intas pharmaceuticals pvt ltd.

Electrocardiography. Electrocardiography was performed on day 30 of experimental period of rats i.e., before their humanely sacrifice using 4-lead single channel ECG machine (BPL Technologies, 108 DIGI) as shown in figure 01. Priorly rats were stabilized in a comfortable environment for 5-10 minutes. Then rats were anasthetized with the help of Xylazine @ 10 mg/kg. b.wt., and Ketamine @ 75 mg/kg.b.wt. given intraperitoneally. The machine was calibrated at 50 mm/s paper speed and standard lead II ECG was recorded. At 50 mm/s paper speed, each big box is of 5 × 5 mm (100 milliseconds or 0.1 seconds) while the small box is of 1×1 mm (20 milliseconds or 0.002 seconds) and an amplitude of 0.1 millivolt (mV). From the ECG, parameters such as heart rate, P wave duration, PR interval, QRS amplitude and QT interval and were calculated (Fraser *et al.,* 1967).

Heart rate (bpm) was calculated with the formula: 60/RR interval (seconds).P wave duration (msec) was determined by number of small boxes covered by P wave.PR interval (msec) was the duration between starting of P wave and starting of QRS complex, and determined by number of small boxes covered within it.QRS amplitude (mV) was measured by number of small covered by the peak of QRS complex. QT interval was the duration between beginning of QRS complex and ending of T wave (Rice, 1992).

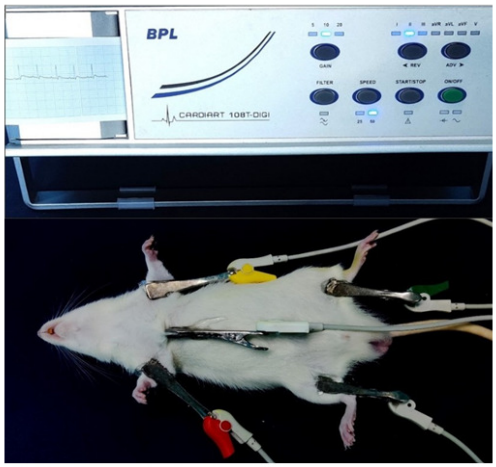

Fig. 1. Electrocardiography in rat using 4 lead single channel ECG machine.

Statistical analysis. Results were expressed as mean ± S.E. One-way analysis of variance (ANOVA) by IBM SPSS (Version 25.00) followed by Duncan's test was applied to analyze the results. P<0.05 was considered as significant.

RESULTS

Heart rate: In the present study, heart rate (bpm) in lead administered group II rats was found to be significantly (P≤0.05) increased in comparison with rats of the control group (Group I) as shown in figure 02. Whereas, amelioration of lead-intoxicated animals with curcumin and ascorbic acid (Group III) has significantly decreased the heart rate as compared to lead administered animals (Group II). No significant statistical difference was observed between heart rate of rats of group IV and group I.

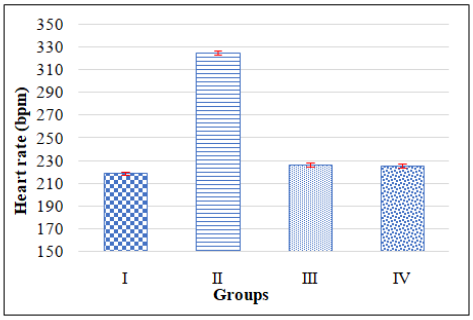

Fig. 2. Heart rate (bpm) in rats of different groups.

P wave duration: The mean±S.E. values of P wave duration (msec) of groups I, II, III and IV animals were depicted in Fig. 3. No significance statistical differences were recorded in the P wave duration of different treatment groups as compared to control group in our study.

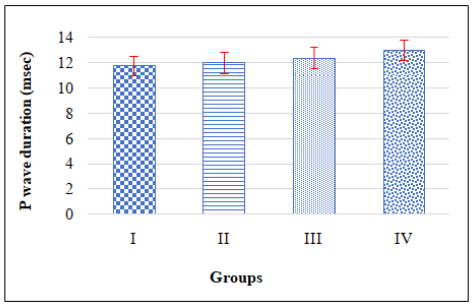

Fig. 3. P wave duration (msec) in rats of different group**.**

PR interval: Fig. 4 represents the mean±S.E. values of PR interval (msec) of groups I, II, III and IV animals. In present study, a significant decrease in the duration of PR interval (P≤0.05) has been observed in rats of group II in which lead acetate was administered as compared to control group (I).

A significant improvement in PR interval has been observed in group III when compared with group II indicating the ameliorative effect of curcumin and ascorbic acid. However, no significant difference was observed in PR interval duration of rats between group IV and group I.

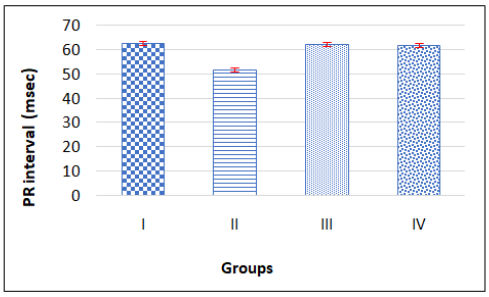

Fig. 4. PR interval (msec) in rats of different groups.

QRS Complex: In the present study, rats of group II revealed a significant increase (P≤0.05) in QRS amplitude when compared with rats of group I (control group) as depicted in Fig. 5, denoting the toxic effects of lead. However, on simultaneous administration of the antioxidants (curcumin and ascorbic acid), a significant amelioration was observed in rats of group III when compared to group II. No significant difference was observed between rats of group IV and group I in our study.

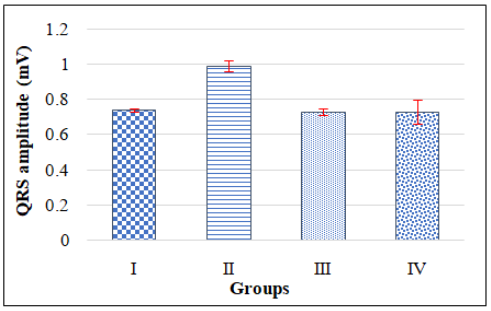

Fig. 5. QRS amplitude (mV) in rats of different groups.

QT interval: The mean±S.E. values recorded for QT interval (msec) in group IIare found to be significantly increased (P≤0.05) in rats of group II when compared with the control group (I). On the other hand, group III animals administered with curcumin and ascorbic acid along with lead showed significant improvement in QT interval when compared to lead acetate treated rats of group II.

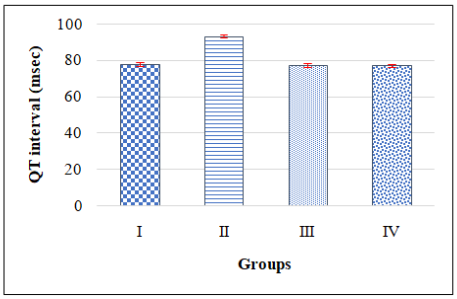

Fig. 6. QT interval (msec) in rats of different groups.

Alterations of ECG as observed in our research are correlated with the studies conducted by Lal *et al.* (1991) (Increased heart rate and QT interval), Reza *et*

al. (2008) (Increased heart rate and QRS duration), Ahmed and Hassanein (2013) (Increased heart rate), Saad and Sayed (2014) (Increased heart rate and QRS amplitude), Wildemann *et al*. (2014) (Increased heart rate and QT interval) and Protsenko *et al*. (2018) (elevated QRS amplitude). Whereas, significant increase in PR interval as observed by Lal *et al*. (1991); Wildemann *et al*. (2014), significant decrease in QT interval as reported by Saad and Sayed (2014) and significant decrease in heart rate observed by Protsenko *et al*. (2018) were in contrast with the findings of the present study.

DISCUSSION

Electrocardiogram (ECG) is one of the vital tests that precisely records the electrical activity of heart along with the cardiac rhythm. ECG is employed as a crucial diagnostic test to determine any abnormalities associated with cardiac conduction system. The electrical impulses are represented in the form of a wave as P wave and QRS complex which indicate atrial and ventricular depolarization respectively while the T wave indicates ventricular repolarization (Konopelski and Ufnal 2016).

In the current study, the observed positive chronotropy (elevated heart rate) in lead exposed rats may be due to its ability to alter cellular metabolism of calcium ions. Studies on calcium and vascular effects of lead have shown that intracellular calciumion binding sites are involved in the action of lead on vascular smooth muscles (Reza *et al.,* 2008). Lead alters the calcium transport systems including activation of protein kinase C (that activates Ca^{+2} channel opening) and inhibition of Na-K ATPases which is responsible for increase in calcium influx and elevation of intracellular levels of calcium. The increased HR and cardiac contractility following the augmented intracellular calcium concentrations cause hypertension due to increased cardiac output. However, how far this increased in calcium influx in cardiomyocytes contributes to the ECG changes in lead-exposed animals needs further investigation (Mackiewicze *et al.,* 2014).

The PR interval represents atrial depolarization and conduction through the AV node. It denotes the duration of impulse transmission from atrium to ventricle i.e., time from onset of atrial depolarization to onset of ventricular depolarization. Existence of an accessory pathway between atria and ventricles allows atrial impulse to bypass the AV node. Hence, ventricular depolarization starts earlier than expected which makes the shortened PR interval (Rice, 1992).

The QRS complex in the ECG is produced by ventricular depolarization and alteration in QRS complex represents an abnormality in heart function. The electric currents generated by ventricular myocardium are proportional to ventricular muscle mass (Farraj *et al.,* 2011). The QT interval represents the time of depolarization and repolarization of ventricular cardiomyocytes, the period of electric systole which determines the functional integrity of the myocardium (Thippeswamy *et al.,* 2009). The pathological duration of QRS and QT interval is indicative of the disturbances in electrical activity of heart as a result of intrinsic heart diseases or other pathological conditions including myocardial infarction and ischemia (Mackiewicze *et al.,* 2014).

CONCLUSIONS

In conclusion, the present investigation enunciated that lead acetate induced the significant cardiotoxicity elicited by significant alterations in electrocardiographic parameters of heart. Use of curcumin and ascorbic acid countered the adverse effects of lead induced cardiotoxicity to a major extent suggesting its antioxidant potential. Also, this study suggested that lead chelating agents having antioxidant properties are preferred in treating cardiovascular disorders accompanying lead toxicity.

FUTURE SCOPE

Thus, the current research offers pertinent data on the electrocardiographic abnormalities in lead induced cardiotoxicity and its amelioration with antioxidants in Wistar rats. Future comprehensive research may be done to determine the lead induced ultrastructural changes on heart along with echocardiographic alterations in lead induced cardiotoxicity which favours in understanding of qualitative pathology.

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Conflict of Interest. None.

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