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Protective Effect of Curcumin and Ascorbic acid Against Lead acetate Induced Cardiotoxicopathology in *Wistar* Rats

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ABSTRACT: Lead is a potential toxic heavy metal that exhibits deteriorating effects on various organ systems of body. Accordingly, the present study was aimed to investigate the ameliorative effect of of curcumin and ascorbic acid against lead induced cardiotoxicopathology in Wistar rats. Thirty-two Wistar rats of 6-8 weeks of age were randomly divided into four groups with eight rats each, as (Group-I) control group, (Group-II) (150 mg/kg b.wt. lead acetate), (Group-III) (150 mg/kg b.wt. lead-acetate, 400 mg/kg b.wt. curcumin and 420 mg/kg b.wt. ascorbic acid) and Group-IV (400 mg/kg b.wt. curcumin and 420 mg/kg b.wt. ascorbic acid). All the experimental groups received oral treatments for 30 consecutive days. Significant increase in blood lead level, serum AST, ALT, CKMB, cardiac troponin I (cTnI) and cardiac tissue MDA level was observed in group II rats. Significant histopathological findings (myocardial necrosis, vacuolation, fibrosis and loss of cross striations) were observed in group II rats. Administration of curcumin and ascorbic acid against lead acetate in group III rats showed significant amelioration in lead induced cardiotoxicity via decrease in cardiac troponin I and cardiac tissue MDA level along with significant improvement in histo-architecture of heart. Thus, the present study revealed that lead exposure has toxic effects on heart which disturb its functioning, while natural these antioxidants 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: Cardiotoxicity, Lead acetate, Curcumin, Ascorbic acid, ECG, Histopathology.

INTRODUCTION

Lead (Pb) is a naturally occurring systemic toxicant heavy metal, ranked high as one of the most hazardous xenobiotics in the environment as it affects several organ systems of body including hepato-renal, central nervous, hematopoietic, endocrine, cardiovascular and reproductive systems (ATSDR, 2007). Despite continuous regulations globally by various agencies, sub-toxic levels of lead exposures which may become problematic over a long period continue to occur due to sustained use of low lead levels in household products and through environmental exposure via inhalation of air or dust as well as food and water contamination (Kim *et al*., 2020). In a recent report, lead exposure accounted for 9.3% of the global burden of idiopathic intellectual disability, 4% of the global burden of ischemic heart disease and 6.6% of the global burden of stroke (Grover and Jhanda 2017). In veterinary practice, lead toxicity is a more common incidence in cattle and dogs due to their selective feeding habits (Patrick, 2006).

Cardiovascular diseases (CVDs) are the leading cause of death globally, taking an estimated 17.9 million lives each year. CVDs are a group of disorders of the heart and blood vessels and include coronary heart disease, cerebrovascular disease, rheumatic heart disease and other conditions. More than four out of five CVD

deaths are due to heart attacks and strokes and one third of these deaths occur prematurely in people under 70 years of age (Chourasia 2022). Chronic exposure of sublethal dose of lead is considered to be a potential risk for cardiovascular diseases. There is an integration between CVD, inflammation and oxidative stress (Roshan *et al*., 2011). It has been proposed that lead toxicity occurs through inflammation, generation of free radicals (reactive oxygen species) and imbalance between pro-oxidant and antioxidant mechanisms in cardiac tissue (Alghazal *et al*., 2008). Since, the pathological alterations are due to production of free radicals hence, free radical scavengers (antioxidants) like vitamin C, B_6, E, zinc etc. can be used as an important therapeutic method to prevent pathology of lead toxicity.

Conventionally, amelioration of lead toxicity is obtained with the use of chelating agents which enhance removal of accumulated lead from the tissues. Recent studies have shown that plant extract-based supplements are also useful for prevention and attenuation of lead toxicity and are very beneficial, cost effective and can be easily added to the daily diet (Zhai *et al*., 2015).

Curcumin, the principle colouring agent present in the rhizomes of *Curcuma longa* (also known as turmeric) has many therapeutic properties including antioxidant, anti-inflammatory and anticancer activities (Kalpana *et al*., 2007). It scavenges the reactive oxygen and nitrogen species, prevents lipid peroxidation and chelates the metals. It upregulates the biosynthesis of glutathione and involved in the regulation of several cytoprotective genes that antagonize oxidative and inflammatory damage (Albasher *et al*., 2020).

Ascorbic acid (Vitamin C) is a possible lead chelator and has a property of scavenging the free radicals. It shows beneficial antioxidant property against lead induced oxidative stress in various organs of animals (Aldahmash and El-Nager 2014).

Therefore, this study was initiated to investigate the possible cardio protective effects of curcumin and ascorbic acid on lead acetate induced cardiotoxicity.

MATERIALS 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 (Nutrivet life sciences, Pune) 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 below.

1. Group I (Control group): Rats received standard feed and water for 30 consecutive days.

2. Group II: Rats were administered with Lead acetate @ 150 mg/kg b.wt., orally through intubation canula for 30 consecutive days.

3. Group III: Rats were administered with Lead acetate @ 150 mg/kg b.wt., Curcumin @ 400 mg/kg b.wt.and Ascorbic acid @ 420 mg/kg b.wt. orally through intubation canula for 30 consecutive days.

4. Group IV: Rats were administered with Curcumin @ 400 mg/kg b.wt. and Ascorbic acid @ 420 mg/k**g** b.wt. orally through intubation canula for 30 consecutive days.

Chemicals. Chemicals such as lead acetate, curcumin, L-ascorbic acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA) and all other chemicals were obtained from Sigma Chemical Inc. (St. Louis, MO, USA).

Blood sampling. After recording of the ECG, blood samples were collected from retro-orbital sinus using capillary tubes into different vacutainers, one coated with EDTA (Ethylene-Diamine-Tetra-Acetic acid) for determination of blood picture, the second coated with heparin for determination of blood lead concentration and the third tube was clot activator vial which was centrifuged at 3000 rpm for 15 minutes to separate serum. The serum was stored at -20^{o}C for determination of biochemical parameters.

Blood lead level estimation by ICP-MS (Inductively Coupled Plasma Mass Spectroscopy). ICP-MS (Thermo scientific; iCAP 7000 series) was used for the estimation of lead in digested blood samples. Argon flame was used as a fuel. Processed samples of blood were thawed to room temperature. Calibration of instrument was achieved with 6 standards of known concentrations (5, 10, 25, 50, 75 and 100 ppb) prior to analysis of unknown sample. Sample analysis was done by making work list in attached computer. Concentration of lead in blood samples were obtained in ppb which was further converted to ppm for data presentation.

Assay of cardiac marker enzymes. Serum samples were analyzed for biochemical parameters namely Aspartate aminotransferase (AST), Alanine transaminase (ALT) and Creatinine Kinase-Myocardial Band (CK-MB) using semi-automatic biochemical analyzer (Make: Erba mannheim) by using commercially available kits (Erba- Transaia Bio-Medicals LTD).

Cardiac Troponin I (cTnI) Assay. The levels of serum Troponin I was analysed by using commercial rat Troponin I ELISA kit (Biospes).

Determination of cardiac weight. Following blood collection, rats belonging to different groups were humanely sacrificed at end of study period. All the rats were subjected to detailed post mortem examination and gross changes of heart was recorded. Heart was gently separated from thymus and the weight was recorded using electronic precision balance (Aczet, CY223C) to assess the absolute organ weight. Relative

organ weight was calculated by dividing body weight of respective rat. Some portion of ventricle was kept at -20°C for oxidative stress estimation and remaining was collected for histopathological study.

Assay of cardiac lipid peroxidation. For determination of MDA in cardiac tissue, 250 mg of tissue specimens from heart were weighed and homogenized in 1:10 dilution of PBS (phosphate buffer saline). The crude tissue homogenate was centrifuged at 10,000 rpm for 15 minutes in cold centrifuge and the resultant supernatant was used for estimation (Noeman *et al*., 2011). Malonaldehyde (MDA) level in tissue homogenate was determined by the method of with suitable modifications and expressed as n moles/g tissue (Rehman, 1984).

Histopathology. Hearts tissues from all the experimental groups were analyzed histopathologically as per the standard procedures described before(Gridley, 1960). Briefly, a portion of the ventricular tissue was fixed in 10% formalin and then paraffin-embedded heart tissues were cut into 5 µm thick sections and thereafter stained with hematoxylin and eosin stains as well as masson's trichome and mallory PTAH stains. The stained sections were viewed under a light microscope of Leica for histological changes and photomicrographs were taken.

Statistics. 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 analyse the results $p<0.05$ was considered as significant.

RESULTS

No mortality was observed in animals of any group exposed to lead acetate alone or in combination with curcumin and ascorbic acid during the treatment period of 4 weeks. Majority of rats belonging to group II showed anorexia, dullness, pale conjunctival mucous membrane, hunched back posture (Fig. 1), emaciation, ruffled feathers (Fig. 2), abdominal breathing from second week onwards till the end of the experiment. Occasional incoordination and hyperactivity were also noticed in rats of this group. During first week of administration with antioxidants, few rats of group III occasionally showed loose faeces and dullness. All the rats except of group I showed the symptoms of stress and anorexia during initial days of administration. Rats of Group I did not show any clinical signs.

Fig. 1. Hunched back posture of group II rat administered with lead acetate @ 150 mg/kg b.wt. at day 20.

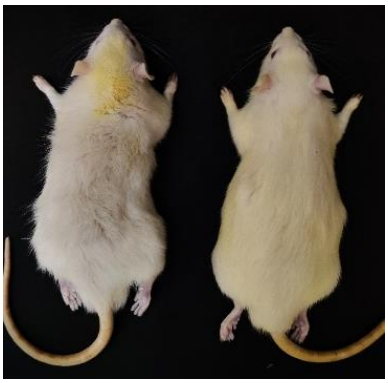

Fig. 2. Rough hair coated rat (a) of group II administered with lead acetate @ 150 mg/kg b.wt along with control rot (b) at day 30.

Weekly Body weight(g). As shown in table 01, a significant decrease in the weekly body weight was noticed in rats of lead administered group II compared to control group ($p<0.05$). However, administration of rats with curcumin and ascorbic acid against lead intoxication in group III significantly attenuated the decrease of the weekly body weight as compared to group II ($p<0.05$). There was no significant difference in body weights between group IV and control group (Group I).

Table 1: Weekly body weight (g) of rats of different groups.

Group	Weekly body weight (g) (Mean±SE)			
	I	II	III	IV
I	204.75^{a}±5.91	220.63^{a}±6.12	236.25^{a}±4.80	243.50^{a}±4.22
II	202.00^{a}±5.18	186.63^{b}±3.04	181.50^{b}±2.78	172.38^{b}±3.75
III	204.63^{a}±5.78	213.00^{a}±4.06	226.00^{a}±2.32	232.25^{a}±2.03
IV	200.88^{a}±7.17	216.88^{a}±5.68	226.75^{a}±5.40	238.38^{a}±5.46

Means with different superscripts in column differs significantly($p\leq0.05$)

Blood picture in different experimental groups. As shown in table 02, the levels of haemoglobin (Hb), total erythrocytic count (TEC), packed cell volume (PCV) and lymphocytes were significantly decreased, while total leucocyte count (TLC) along with neutrophils was increased significantly in lead exposed group II rats as compared to the control group I. Group III rats administered with antioxidants showed significant improvement in above mentioned hematological alterations as compared to the lead exposed group II. However, there was no significant difference in blood picture of group IV and control group (Group I).

Table 2: Blood picture in rats of different groups.

Parameter	(Mean±SE)			
	Group I	Group II	Group III	Group IV
Hb (g/dl)	14.92^{a}±0.07	10.07^{c}±0.16	14.42^{b}±0.10	14.75^{a}±0.05
TEC (x10^{6} /µl)	07.93^{a}±0.07	03.80^{c}±0.09	06.96^{b}±0.05	07.93^{a}±0.05
PCV (%)	45.02^{a}±0.61	30.10^{c}±0.47	43.10^{b}±0.21	44.16^{ab}±0.11
TLC (x10^{3} /µl)	07.82^{c}±0.14	11.25^{a}±0.13	09.28^{b}±0.17	07.64^{c}±0.14
Lymphocytes (%)	74.75^{a}±1.08	52.75^{b}±0.70	74.00^{a}±1.05	74.13^{a}±0.51
Neutrophils (%)	22.37^{b}±0.90	43.93^{a}±0.77	22.75^{b}±0.92	22.38^{b}±0.50
Monocytes (%)	01.50^{a}±0.18	01.69^{a}±0.51	01.65^{a}±0.18	01.86^{a}±0.13
Eosinophils (%)	01.37^{a}±0.26	01.87^{a}±0.22	01.62^{a}±0.18	01.63^{a}±0.26

Means with different superscripts in rows differs significantly($p<0.05$)

Blood lead level estimation. In this study, blood lead levels in the rats of lead administered group II were found to be significantly higher as compared to the control as shown in Table 3. However, when the rats administered with curcumin and ascorbic acid along with lead in group III, lead content was found to be reduced significantly as compared to group II ($p<0.05$) although still higher than control rats.

Assay of cardiac marker enzymes. The results in Table 4 showed a significant increase in values of AST, ALT and CKMB (U/L) ($p<0.05$) in group II rats administered with lead acetate alone. These elevations in values were significantly reduced in rats belonging to group III, but not equal to control rats. However, the values of group IV were in comparable to group I.

Cardiac Troponin I (cTnI) (ng/L) Assay. Rats induced with lead acetate alone (Group II) showed considerable ($p<0.05$) elevation in the levels of Cardiac Troponin I (cTnI) in serum as compared to normal control rats of group I as shown in table 05. On simultaneous administration of curcumin and ascorbic acid against lead intoxication in group III showed considerable ($p<0.05$) decrease in the levels of serum cTnI as compared with lead acetate alone induced rats. However, the values of group IV are in similar to group I.

Determination of cardiac weight. Table 6 shows the absolute heart weights of lead-administered group II rats which were significantly higher than those of control rats of group I ($p<0.05$). Administration with antioxidants for a period of 4 weeks in group III decreased significantly the weights of hearts as they did not differ from those of the control group. However, there was no significant difference in the relative organ weight of heart in all the experimental groups.

Table 3: Blood lead level (ug/dl) in rats of different groups.

Group	Blood lead level (ug/dl) (Mean±SE)
I	07.00^{c}±0.4
II	60.00^{a}±7.0
III	14.00^{b}±0.9
IV	07.00^{c}±0.5

Means with different superscripts differs significantly ($p<0.05$)

Table 4: Cardiac marker enzymes (U/L) in rats of different groups.

Parameter	Group I	Group II	Group III	Group IV
AST	74.88^{c}±0.29	115.27^{a}±1.04	83.72^{b}±0.58	75.15^{c}±0.40
ALT	26.08^{c}±0.17	69.26^{a}±1.01	33.33^{b}±0.80	28.33^{c}±1.35
CK-MB	32.04^{c}±0.49	89.05^{a}±0.84	44.28^{b}±0.52	31.73^{c}±0.57

Means with different superscripts in rows differs significantly ($p<0.05$)

Table 5: Cardiac Troponin I (ng/L) in rats of different groups.

Group	Serum Cardiac Troponin I (ng/L) (Mean±SE)
I	060.90^{b}±03.72
II	122.10^{a}±12.00
III	077.65^{b}±04.62
IV	057.52^{b}±03.90

Means with different superscripts differs significantly($p<0.05$)

Table 6: Cardiac weights (mg) in rats of different groups.

Group	Absolute organ weight (mg)	Relative organ weight (%)
I	678.75^{a}±6.66	0.279^{ab}±0.004
II	504.25^{c}±3.23	0.294^{a}±0.006
III	629.23^{b}±4.77	0.278^{ab}±0.003
IV	680.25^{a}±6.17	0.283^{b}±0.005

Means with different superscripts in column differs significantly ($p<0.05$)

Assay of cardiac lipid peroxidation. As shown in Table 7, lipid peroxidation, determined by MDA formation, was increased significantly($p<0.05$) in heart of the group II rats exposed to lead acetate alone while antioxidant (curcumin and ascorbic acid) administration significantly decreased the level of lipid peroxidation in heart of group III rats as compared to group II, but not in similar to control rats. However, there was no significant difference in MDA values of group IV and control group (I).

Gross pathology of heart. On gross observation, occasionally heart of group II rats showed decreased size with rounding (Fig. 3) and reduction in left ventricular cavity.

Table 7: Cardiac tissue MDA level (nmol/g tissue) in rats of different groups.

Group	MDA level (nmol/g tissue) (Mean±SE)
I	01.73^{c}±0.21
II	24.29^{a}±3.08
III	05.35^{b}±0.28
IV	01.66^{c}±0.13

Means with different superscripts differs significantly($p<0.05$)

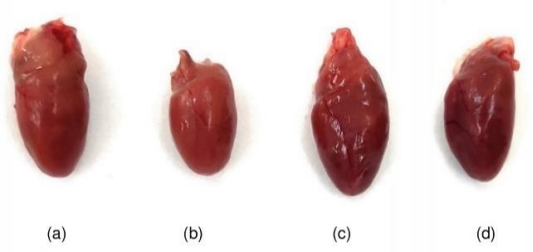

Fig. 3. Gross morphology of heart from different groups showing normal appearance in rats of group I (a), group III (c) and group IV (d) while rounding of heart in group II rat (b) exposed to lead acetate.

Histopathology. The histopathological examination of HE-stained sections of heart revealed normal histoarchitecture of heart in rats of group I (Fig. 4) and IV (Fig. 5), while significant pathological lesions were observed inlead administered rats of group II. On the other hand, co-administration of curcumin and ascorbic acid with lead in rats of group III markedly improved the severity of pathological lesions.

Heart lesions as a result of lead administration were in the form of focal epicardial thickening (Fig. 6), multifocal myocardial necrosis characterized by coagulative necrosis and vacuolation in cardiomyocytes along with the proliferation of fibrocytes and mononuclear cell infiltration (Fig. 7). Interstitial hemorrhage and congestion (Fig. 8) were observed in most of lead administered rats of group II. Fragmental degeneration of cardiomyocytes along with hyaline degeneration in form of hypereosinophilic cardiomyocytes (Fig. 9) and mild fibrosis in area of myocardial necrosis (Fig. 10) were also evidenced. The changes were more prominent towards the subendocardial region of heart. Myocardial fibres revealed moderate loss of cross striations and also a distortion in the pattern of cross striation in muscle fibres when stained with Mallory PTAH stain (Fig. 11). The heart sections of Wistar rats of group III revealed apparently normal cardiac cytoarchitecture with mild degenerative changes (Fig. 12) along with minimal vacuolation and a comparatively normal heart parenchyma. Myocardium displayed minimal and focal loss of cross striations (Fig. 13). Majority of myocardium was composed of normal cardiomyocytes with centrally located nuclei and intact arrangement of muscle fibres. These changes showed improvement of the myocardial musculature which resembles the normal myocardial muscles in most cases.

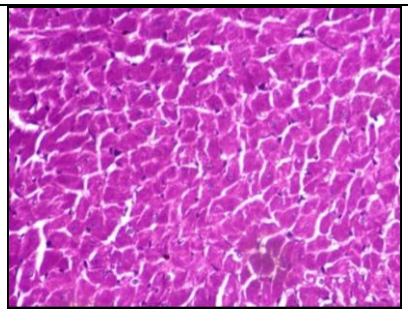

Fig. 4. Microscopic transverse section of heart of group I rat showing normal arrangement of cardiomyocytes. H&E X200.

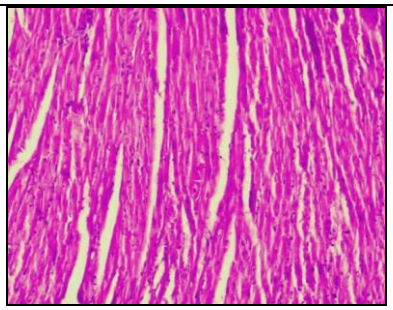

Fig. 5. Microscopic section of heart of group IV rat showing longitudinally arranged muscle fibres. H&E X100.

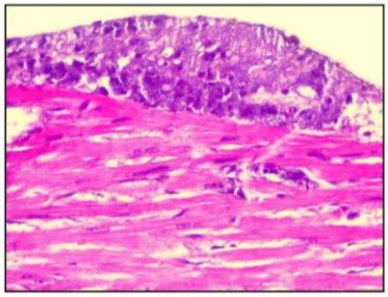

Fig. 6. Microscopic section of heart of group II rat showing thickening of epicardium H&E X400.

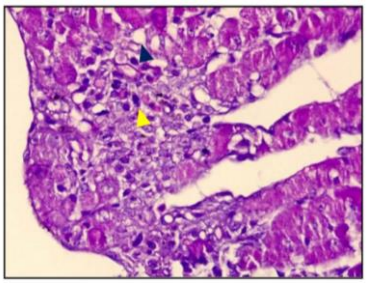

Fig. 7. Microscopic section of heart of group II rat showing necrosis of cardiomyocytes, vacuolation (black) and cellular infiltration (yellow). H&E X200.

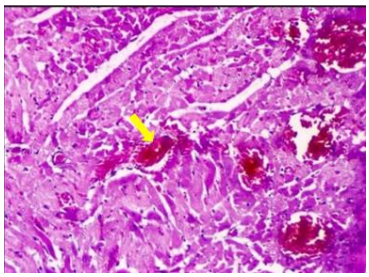

Fig. 8. Microscopic section of heart of group II rat showing myocardial congestion and haemorrhage. H&E X200.

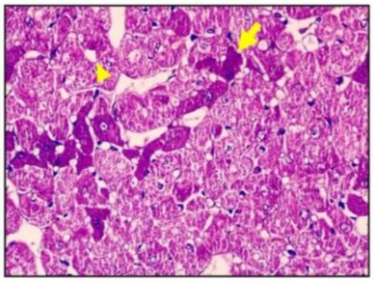

Fig. 9. Microscopic section of heart of group II rat showing vacuolation (arrow) and hyaline degeneration (arrowhead). H&E X400.

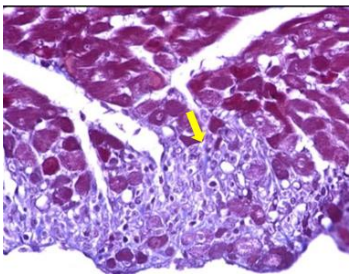

Fig. 10. Microscopic section of heart of group II rat showing myocardial necrosis and mild fibrosis (arrow) with collagen fibres stained blue. Masson's Trichome X400.

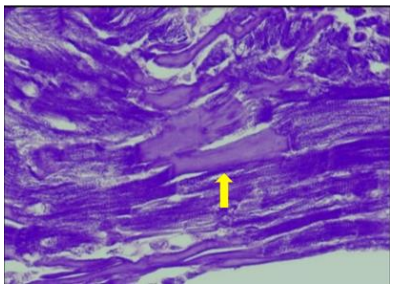

Fig. 11. Microscopic section of heart of group II rat showing muscle fibres with loss of cross striations (arrow). Mallory PTAH X400.

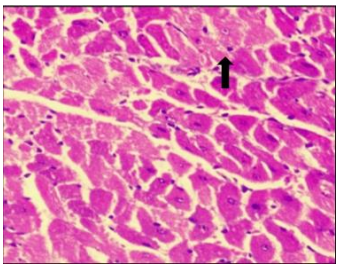

Fig. 12. Microscopic section of heart of group III rat showing minimal degeneration of cardiomyocytes (arrow). H&E X400.

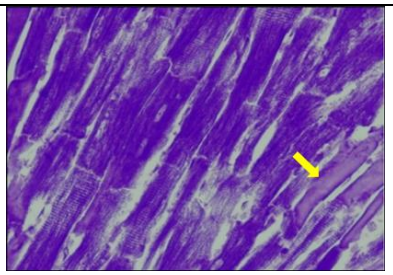

Fig. 13. Microscopic section of heart of group III rat showing minimal loss of cross striations (arrow). Mallory PTAH X400.

DISCUSSION

The present study was carried out to evaluate the protective effect of curcumin and ascorbic acid against lead acetate-induced cardiotoxicity in Wistar rats.

In present study, the continuous significant decrease in body weight of rats exposed to lead acetate is in accordance with the findings of various researchers (Ghazanfarpour *et al*., 2019). Reduction in body weight observed in the lead administered rats of group II might be due to various factors, including reduced feed and water intake along with impaired zinc-dependent enzymatic metabolic processes. It can cause partial anorexia, gastrointestinal disturbances and damage to liver and kidney eventually leading to altered metabolism (Ibrahim *et al*., 2012). Amelioration of the toxic effect of lead on body weight gain is noticed upon administration with a combination of curcumin and ascorbic acid, since these agents reduce the lead burden in gastrointestinal tract and blood by chelating lead. This also results in maintenance of the balance of

essential and trace minerals required for various enzymatic actions (Suradkar *et al*., 2010).

Alterations in blood picture as mentioned in the present study is in harmony with the findings of several researchers (Suradkar *et al*., 2010; Daku *et al*., 2019; Aladaileh *et al*. 2020). Lead inhibits several heme synthase enzymes including δ-aminoleuvulinic acid synthase, δ-ALA dehydratase and ferrochelatase that might be the reason behind reduction in erythrocytic parameters in the present study. Additionally, it binds and inhibits pyruvate kinase and pyrimidine 5 nucleotidase enzymes essential for RBC maturation resulting in shorter RBC life span (Calderon *et al*., 1993). Administration of lead causes altered inflammatory cytokines, chemokines, interleukins and other signaling molecules along with decreased immunity, which could be the reason behind leucocytosis and lymphocytopenia in lead administered rats (Alwaleedi, 2016 and Shaban *et al*., 2021). Co-administered lead acetate along with curcumin and ascorbic acid significantly improved the blood profile due to its strong antioxidant, anti-inflammatory and chelating property, thereby reducing lead-induced adverse effect on hematopoietic cells (Ibrahim *et al*., 2012).

The current data pointed out the significant elevation of blood lead concentration in rats exposed to lead acetate alone as compared to unexposed animals which is in close contrast with the studied conducted by different coworkers (Singh *et al*., 2017; Daku, 2019). Lead is a soluble non-essential heavy metal mainly transported through contaminated food, water or environment. Though GI absorption of lead is limited (1-2%) but when absorbed, a large portion of lead is bound with RBCs membrane (~99%) and remaining with serum albumin (<1% only). Systemic blood flow and soluble phosphate further distribute lead to the target organs such as brain, kidney, liver, heart, spleen, aorta and majority of skeletal system. Reduction in blood lead burden by curcumin and ascorbic acid combination has been noticed, as both the agents are potent chelators, bind with excess of lead present in gastrointestinal tract and blood further reducing the lead concentration in blood more effectively on combination (Chang, 2012 ; Abdel-Moneim *et al*., 2015).

In the current study, subacute exposure of lead acetate significantly elevated the serum levels of AST, ALT and CKMB which is in accordance with previous researchers (Ahmed and Hassanein 2013; Ansari *et al*., 2013; Baghshani and Ghahramanloo 2020). Myocardial cell damage is accountable for the release of different biomarkers such as CK-MB, AST and ALT from the cardiac myocytes into the blood. Therefore, these parameters can also act as important diagnostic tool to measure the severity of hepatic and myocardial damage (Vimal and Devaki 2004; Acikel *et al*., 2005). Simultaneous co-administration of lead acetate along with curcumin and ascorbic acid had significant improvement on altered biomarker profile, similar to previous researchers (Patra and Swarup 2004) and (Ahmed and Hassanein 2013). On account their strong antioxidant, anti-inflammatory and chelating property which thereby reduced the lead induced myocardial damage.

Elevated troponin levels forecast the risk of deaths due to cardiac issues and subsequent infarction. In our study, we observed increased levels of cardiac troponin I (cTnI) in serum of lead acetate induced rats which is corroborative with several previous co-workers (Baghshani and Ghahramanloo 2020; Hegde *et al*., 2020). Cardiac troponin-I (cTnI) is a highly sensitive and specific marker of myocardial injury. Increased levels of this contractile protein is indicative of myocardial necrosis and infarction (Priscilla and Prince 2009). In the current study, significantly higher levels of serum cTnI in lead administered group II indicate leakage of this contractile protein possibly due to necrosis, change in the functional integrity and permeability of cell membrane caused by high levels of lead (Baghshani and Ghahramanloo 2020). Combination of curcumin and ascorbic acid was able to regulate the levels of cTnI in serum on lead exposure, indicating that these antioxidants may have protective potential against lead induced cardiac injury.

Further, the present study also revealed a significant decrease in absolute heart weight of lead exposed rats which is in homologous with the findings with previous researchers (Ibrahim *et al*., 2012; Ahmed and Hassanein 2013) and (Saad and Sayed 2014). Lead induced marked degenerative changes in heart, which might be the reason behind decreased absolute weight of heart. While, significant improvement in absolute heart weight on spontaneous administration of curcumin and ascorbic acid along with lead, could be attributed to their antioxidant nature. As relative weights are the proportion of absolute weight against the body weights and in present study reduction in body weight as well as heart weight might have nullified the changes in relative organ weight of heart.

In recent years, there is an increasing in interest pertaining to free radicals that have shown to modify biological molecules, which may result in various pathological conditions (Priscilla and Prince, 2009). Thus, additional natural products need to evaluate for their antioxidant potential. Increased level of oxidative stress biomarker (MDA) in lead administered rats is consistent with the hypothesis that lead toxicity appears to affect organs with low antioxidant defenses such as heart (Patrick, 2006; Gholamhosseini *et al*., 2009). Alterations in oxidative status either by overproduction of oxidant or deficit in antioxidant activity could be one of the direct consequences of lead toxicity or poisoning. MDA or thiobarbituric acid-reactive species is the end product of lipid peroxidation that plays a vital role in lipid membrane damage in cells due to increased reactive oxygen species (ROS). The second mechanism for lead induced oxidative stress is inhibition of antioxidant defence systems of cells. Curcumin and ascorbic acid, both being potent antioxidants, ceases the free radical generation, scavenge ROS and activate antioxidant defense system (Thuppil and Tannir 2013). Hence, during the present study, curcumin and ascorbic acid significantly reduced the lipid peroxidation caused by lead toxicity and sufficiently restored the MDA levels.

Lead induced histopathological alterations in heart observed in the present study are in agreement with the findings of Aksu *et al.* (2017); Feng *et al*. (2018; Hajinezhad *et al*. (2020). The histopathological lesions observed in heart might be as a result of oxidative stress, inflammation or the enhancement in programmed cell death (apoptosis) which plays an important role in development of lead-induced degenerative changes. However, administration of antioxidant combination (curcumin and ascorbic acid), reduced the cardio histopathological lesions and preserved the normal histoarchitecture of heart.

CONCLUSIONS

In conclusion, the present investigation enunciated that lead acetate induced the significant cardiotoxicity due to the excess generation of free radicals and impairment of antioxidant defences that subsequently resulted in significant alterations in several parameters pertaining to heart. Use of curcumin and ascorbic acid countered the adverse effects of lead induced cardiotoxicity to a major extent suggesting its antioxidant potential owing to depletion of tissue pool of MDA. 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 protective effect of curcumin and ascorbic acid against lead acetate induced cardiotoxicopathology in wistar Rats. Future comprehensive research may be done to determine the protective effect of these antioxidantsin lead induced ultrastructural changes on heart along with lead induced genotoxic and carcinogenic changes.

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Conflict of Interest. None.

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