INTRODUCTION

Cadmium (Cd) is one of the most occurring industrial and environmental pollutants. Cd is a soft, silver-white in metallic form [1]. Cd ranks seventh on Agency for Toxic Substances and Disease Registry (ATSDR)/EPA list of hazardous substances [2] and has been classified as Group I carcinogen by International Agency for Research on Cancer [3]. Moreover, the United States Environmental Protection Agency considers cadmium to be a Class B1 carcinogen [4]. The California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) has listed “cadmium and cadmium compounds” on the Proposition 65 list as carcinogens [5]. Furthermore, Cd is described as developmental toxicant and as a male reproductive toxicant [5].

Humans are exposed to Cd more by ingestion than inhalation [6]. Inhalation of Cd can occur in smokers or the people who are in occupational exposure [7]. According to the size of the particle, solubility of Cd compound inhaled and duration of exposure, absorption of Cd ranges from 10% to 50% in lungs [8]. Cigarette smoke is a big source of Cd to both smokers and non-smokers. One cigarette contains 2.0 µg of Cd, of which 2–10% is transported to cigarette smoke [9].

Cd causes tissue injury through oxidative stress. Cd stimulates the production of intracellular reactive oxygen species through mitochondrial electron transport chain retardation [10]. According to ATSDR [11], Cd led to the development of chronic obstructive pulmonary disease in smokers. Inhaling Cd for long duration may cause lung cancer [12,13]. Cd may also lead to a wide range of physiological, biochemical, and behavioral dysfunctions [14].

Curcumin (diferuloylmethane) is an active, yellow-colored component obtained from rhizomes of turmeric, Curcuma longa Linn. and a herb in ginger family (family Zingiberaceae) [15]. The scavenging and trapping potential of curcumin can be attributed to their chain breaking activity by donating hydrogen atoms probably from their phenol (OH) groups. Thus, curcumin affords protection against oxidative agents in brain, liver, lungs, kidneys, and heart [16].

Heavy metals are constant and global pollutants that generate oxidative stress and thus affecting structure and function of various organs of body. As curcumin has medicinal properties, it can be used as a protective agent against CdCl₂-induced oxidative stress in lungs of albino mice.

METHODS

Animals

Albino mice having weight of 20–22 g were obtained from Central Research Institute, Kasauli. They were acclimatized for 10–15 days and given standard pellet diet (obtained from Hindustan Liver Limited, Mumbai, India) and RO water ad libitum. The animals were handled with proper human care in accordance with the guidelines of the Institutional Animal Ethical Committee.

Chemicals

Cd chloride (CdCl₂) and curcumin were purchased from HiMedia Laboratories Pvt., Ltd., Mumbai. CdCl₂ was dissolved in distilled water and was administered to mice orally. An aqueous suspension of curcumin was made [17] and administered orally to mice.

Experimental design

Mice were divided into the following eight groups:

- Group 1 mice were kept as control and were given RO water along with pellet diet.
- Group 2 mice were received 1 mg/kg body weight of CdCl₂ orally on alternate days for 15 days. Group 3 mice were given 1 mg/kg
body weight of CdCl₂ on alternate days and 100 mg/kg body weight of curcumin daily orally for 15 days. Group 4 mice were given an oral dose of 100 mg/kg body weight of curcumin daily and were kept as positive control for 15 days.

Similar experiment was carried out for 45 days also: Groups 5, 6, 7, and 8, respectively. Autopsies were done on 15 and 45 days post-treatment. Body weights of both the controlled and treated mice were recorded before, during, and after treatment. After that animals were sacrificed, the lung tissue was removed, freed of adipose tissue, blotted dry, weighed, and was processed for biochemical analysis.

Biochemical studies
Lung homogenate was prepared with the help of tissue homogenizer in 3 ml of phosphate buffer and used for the estimation of glycogen from tissue extract with the method of Montgomery [18], cholesterol was assessed by the method of Zlatkis et al. [19], and protein by the method of Lowery et al. [20]. Cd content from the lungs tissue was estimated by the method of Ballantine and Barford [21].

Statistical analysis
The data were analyzed using the Student's t-test and Two way ANOVA.

RESULTS AND DISCUSSION
Organ weight
A significant (p<0.0001) reduction in weight of lungs was observed at 15 days and 45 days in Cd-treated Group II and Group VI in comparison to the control group of mice I and V. Cd+Curcumin treated Group III and Group VII also showed significantly decreased (p<0.001) lungs weight as compared to the control groups. Whereas, curcumin-treated Groups IV and VIII showed non-significant (p>0.05) decrease in weight of lungs at 15 and 45 days post-treatment in comparison to the control groups (Fig. 1).

Total glycogen content
In this study, glycogen content in lungs was found to be significantly decreased (p<0.0001) at both the intervals in Groups II and VI. Groups III and VII also showed significantly decreased (p<0.001) glycogen level in lungs at both intervals. Whereas, Groups IV and VIII showed non-significant (p>0.05) decrease in glycogen content in comparison to control Groups I and V (Fig. 2).

Total cholesterol
Total cholesterol content was observed to be significantly decreased (p<0.0001) in lungs in Cd-treated Groups II and VI in comparison to control Groups I and V. There was a significant decrease (p<0.001) in total cholesterol in Groups III and VII at both intervals. Group IV showed non-significant decrease (p>0.05) and Group VIII showed significant increase (p<0.01) in total cholesterol as compared to the control Groups I and V (Fig. 3).

Total protein content
Total protein concentration was found to be significantly reduced (p<0.001) in lungs in Cd treated groups II and VI in comparison to control group I and V. Group III (p>0.05) and VII (p<0.001) also showed significantly decreased total protein level in comparison to control groups. Group IV and Group VIII showed non-significant (p>0.05) decrease in protein content as compared to Group I and V mice (Fig. 4).
Thyroid hormones might also be involved in cholesterol metabolism and its increased breakdown in hyperthyroidism can cause hypocholesterolemia [45].

Figure 4: Total protein of content of lungs in control, Cd, Cd+Cur, and Cur-treated groups of mice. Values are given as mean±SEM from five mice in each group. (*Significant, NS: Non-significant) (Curcumin=Cur)

Cd content in lungs
In the present study, no Cd was detected in group I, IV, V and VIII. However, the level of Cd was found to be significantly more in Cd treated groups II and VI as compared to control group in lungs. However, a significant (p<0.0001) decrease in concentration of Cd was seen in Group III, IV, VII and VIII as compared to Cd treated Group II and VI (Fig. 5).

Figure 5: Cd content of lungs in control, Cd, Cd+Cur, and Cur-treated groups of mice. Values are given as mean±SEM from five mice in each group. (*Significant, NS: Non-significant) (Curcumin=Cur)

DISCUSSION
The effect on body weight and organ weight is one of the main symptoms of toxicological damage [22,23]. This reduced organ weight may be linked to the damaging effects of Cd on lung tissue. In animal studies, weight of organs can be taken into notice to evaluate the metal toxicity [24]. Curcumin due to its antioxidant and dopaminergic activities balances the body weight in rats [25]. Similar preventive action of curcumin by maintaining body weight of mice and rats is also reported by many other authors [26-28]. Similarly, it also reported decrease in body weight and attributed it to reduced digestion and absorption of nutrients [29] and consumption of less food and water [30] due to loss of appetite. These can be linked to the reduced lungs weight due to Cd intoxication.

Glycogen is a vital source of energy for the general metabolism of body [31]. Decrease in glycogen content is in agreement with the studies of other authors [32-38]. It was proposed that insulin decreases the blood glucose level by enhancing the sugar membrane transport and also by increasing the conversion of glucose into glycogen and triglycerides, therefore decreases the liver glucose production [39]. It was described that enhanced catabolism of biomolecules or less synthesis of biomolecules could be the reason of changes in the glycogen concentration [40]. Stress or less intake of food leads to higher level of stress hormones (cortisol/adrenaline) in blood which brings about depletion of glycogen and hence decline in glycogen content [41]. Curcumin enhances the glucokinase activity and glycogen content, however, it decreases the G6Pase and PEPCCK activities. It was also reported that curcumin inhibited the activities of gluconeogenic enzymes and upregulated the glycolytic enzymes, which led to reduction in glucose concentration and hence glycogen content in mice [42].

Decrease in cholesterol content in Cd-treated group is in agreement with the work of other authors [35,37,38,43]. Reduced cholesterol level may be linked to its increased consumption in corticosteroidogenesis and/or its de novo synthesis [44]. Thyroid hormones might also be involved in cholesterol metabolism and its increased breakdown in hyperthyroidism can cause hypocholesterolemia [45].

Reduced protein content was observed in Cd-treated group. Some authors also reported similar trend in their research findings [37,38,43,46-49]. It was suggested that decline in protein content may be due to reduced level of synthesis of protein or enhanced consumption of proteins [50]. Undesirable conditions such as inaccessibility of important enzymes and/or reduced protein synthesis sites lead to less protein synthesis [51]. Author also explained that the declined protein concentration could be due to disrupted protein metabolism and/or reduced protein concentration due to its antioxidant property and also counterbalances the damaging effects of Cd toxicity in mice [52]. It was suggested that reduced protein concentration could be due to catabolism of protein and/or hepatic malfunction [53].

Curcumin ameliorated the toxicity induced by Cd by increasing the level of proteins in lungs. It was reported the therapeutic efficacy of Curcumin in enhancing the protein synthesis and reviving the damaged liver tissue [54]. This study is also in agreement with the findings of another author [55]. It was also reported that Curcumin increased the protein concentration due to its antioxidant property and also counterbalances the damaging effects of Cd toxicity in mice [56].

Eybl et al. [55] suggested that curcumin administration leads to declined Cd accumulation in the tissues of mice. They also proposed that Cd load may be declined by curcumin through metal-ligand interaction between Cd and curcumin. This is in agreement with the observations of other researchers [57-59].

Curcumin in decreases the Cd accumulation in the organs certainly through its chelating activity. Curcumin might disturb the gastrointestinal absorption of Cd, therefore causing a reduced Cd concentration in the blood and tissues [60].

CONCLUSION
Based on the explained data, we conclude that Cd is a toxic chemical that produced significant toxic effects in lungs of treated mice as revealed by the severely affected parameters. Furthermore, this investigation fundamentally cleared the protective and/or ameliorative role played by curcumin as it normalizes the biochemical parameters disturbance caused by Cd.

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AUTHORS' CONTRIBUTIONS
Both the authors have contributed equally.

CONFLICTS OF INTEREST
The author declares that there are no conflicts of interest regarding the publication of this paper.

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