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RESEARCH ARTICLE

Toxicological effects of toxic metals (cadmium and mercury) on blood and the thyroid gland and pharmacological intervention by vitamin C in rabbits

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Abstract

Cadmium and mercury are non-biodegradable toxic metals that may cause many detrimental effects to the thyroid gland and blood. Vitamin C has been found to be a significant chain-breaking antioxidant and enzyme co-factor against metal toxicity and thus make them less available for animals. The current study was performed to find the effect of individual metals (cadmium and mercury), their co-administration, and the ameliorative effects of vitamin C on some of the parameters that indicate oxidative stress and thyroid dysfunction. Cadmium chloride (1.5 mg/kg), mercuric chloride (1.2 mg/kg), and vitamin C (150 mg/kg of body weight) were orally administered to eight treatment groups of the rabbits (1. control; 2. Vit C; 3. $CdCl_2$; 4. $HgCl_2$; 5. Vit C + $CdCl_2$; 6. Vit C + $HgCl_2$; 7. $CdCl_2$ + $HgCl_2$, and 8. Vit C + $CdCl_2$ + $HgCl_2$). After the biometric measurements of all experimental rabbits, biochemical parameters viz. triidothyronine (T_3), thyroxine (T_4), thyroid-stimulating hormone (TSH), and triglycerides were measured using commercially available kits. The results exhibited significant decline ($p < 0.05$) in mean hemoglobin, corpuscular hemoglobin, packed cell volume, T_3 (0.4 ± 0.0 ng/ml), and T_4 (26.3 ± 1.6 ng/ml) concentration. While, TSH (0.23 ± 0.01 nmol/l) and triglyceride (4.42 ± 0.18 nmol/l) were significantly ($p < 0.05$) increased but chemo-treatment with Vit C reduces the effects of Cd, Hg, and their co-administration but not regained the values similar to those of controls. This indicates that Vit C had a shielding effect on the possible metal toxicity. The Cd and Hg also found to accumulate in vital organs when measured by atomic absorption spectrophotometer. The metal concentration trend was observed as follows: kidney > liver > heart > lungs. It was concluded that Cd and Hg are toxic and tended to bioaccumulate in different organs and their toxic action can be subdued by vitamin C in biological systems.

Keywords Thyroid · Toxic metals · Trace elements · Bioaccumulation · Antioxidants

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Introduction

Toxic metals are non-biodegradable and their existence in environment is a major concern as they cause severe damage to the animal body even at low dosage. Various risk factors are linked with human health regarding toxic metal entry into food chain (Sarwar et al. 2017; Tay et al. 2009). Both anthropogenic and natural sources can cause toxic metal pollution. These metals seep in sloppy areas and are running water carry them downhill or runaway into sea. During extraction activities, water reservoirs are contaminated more strongly (Akinhanmi et al. 2016; Pacyna et al. 2016). The consumption of these toxic metal–contaminated plants or animals play detrimental role in humans (Sundseth et al. 2017; Truby 2003). Toxic metals can affect male and female reproductive tracts, prostate gland, breast development, cancer metabolism,

cardiovascular endocrinology, neuroendocrinology, and thyroid and also can cause obesity (Maffini et al. 2006). On the other hand, various degrees of abnormalities in male genitalia, unilateral or bilateral cryptorchidism from mild to severe hypospadias, feminization of males, and poorly developed testes are common manifestations in domestic animals like dogs, goats, and horses (Lange et al. 2002; Meyer et al. 2006). Toxic metals enter the digestive tract of terrestrial animals through food, in general, and then accumulate in various organs after transmission via blood (Javed and Usmani 2012). The blood parameters like hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC), and packed cell volume (PCV) are used to evaluate the health status of animals (Javed and Usmani 2015). However, blood parameters alone usually do not give a reliable evaluation of toxic impacts of metals' exposure (Okediran et al. 2016). Therefore, the study of other parameters like metal accumulation in different organs may need to consider.

Mercury (Hg) is a non-essential element in the nutrition of animals and plants. It is studied that mercury persists in the environmental systems and many environmental organizations have marked it as a major pollutant (Ibraheem et al. 2016). Mercury is one of the major contaminants in an aquatic environment (Pereira et al. 2009). It tends to bioaccumulate in tissues and organs of animals (Das et al. 2008).

Cadmium is listed among the priority pollutants as well as a carcinogen of first category (IARC 2012). Cadmium causes cancerous effects on the lungs, testicles, and prostate, but there is insufficient data in the support of thyroid dysfunction due to cadmium poisoning. Cadmium seems to stimulate or activate various factors which lead to amplified cell proliferation and a decrease in normal apoptosis in thyroid cells (Buha et al. 2018). The liver, kidneys, and muscles are the most vital sites for cadmium accumulation. Biological half-life of cadmium is between 5 and 30 years because of its binding to molecules like MT and glutathione that are rich in sulfhydryl groups and thus excretes slowly. Cadmium exposure even at biologically low levels over time is related to surfeit toxic effects on the liver, kidneys, bones, testes, and the circulatory system (Matović et al. 2011; Matović et al. 2015; Mezynska and Brzóska 2018). Many researches indicate that cadmium has endocrine disrupting properties that suggest its probable role in diabetes mellitus (Edwards and Ackerman 2016) and oestrogenic activity (Pollack et al. 2011; Silva et al. 2012). Moreover, cadmium has been included in group 1 human carcinogen with adequate data for the lungs (Person et al. 2013; Hartwig 2013) and evidence for the prostate (Pal et al. 2017; Hartwig 2013), breast, and kidneys (Hartwig 2013). Some of the recent studies support the association of cadmium with other cancers like pancreatic (Buha et al. 2017), breast (Larsson et al. 2015), and urinary bladder cancer (Feki-Tounsi and Hamza-Chaffai 2014). Cadmium not only accumulates significantly in various organs but also has affinity to adversely affect the thyroid gland (Jancic and Stosic 2014). The presence of cysteine-rich proteins, metallothioneins (MT), enhances the Cd deposition in thyroid tissues as these MT bind with Cd and signify a potent intracellular cadmium detoxifier (Klaassen et al. 2009). People residing in contaminated areas have three times higher concentrations of metal(s) in their thyroid glands in comparison with those living in non-contaminated areas (Uetani et al. 2006). The thyroid hormones, including T_3, T_4, and TSH, are generally used as trustworthy gage for representation of the thyroid function in experimental animals and humans. The change in concentration of these hormones in serum indicates the disturbances in their glandular synthesis and/or secretion as well as disorders in their extra-thyroidal peripheral metabolism (Chaurasia et al. 1996; Kelly 2000). In short, the function of the thyroid gland is evaluated by measuring the levels of thyroid hormones such as T_3 and T_4 and pituitary TSH in serum. Being an imperative chain-breaking antioxidant and enzyme co-factor (Jurczuk et al. 2005; Wilson 2002), vitamin C efficiently removes hydroxyl, singlet oxygen, superoxide, hypochlorous acid, and water-soluble peroxyl radical (Smirnoff and Wheeler 2000). Vitamin C is not only a therapeutic agent against heavy metal toxicity; it can provide a remarkable solution to heavy metal toxicity and ameliorate toxicity (Bhattacharjee et al. 2003). Vitamin C binds to mercury ions (Hg^{+2}) due to its nucleophilic properties and tends to reduce mercury-induced impairment. It further potentiates its detoxification process by eradicating or diminishing free radicals formed by mercury (Herbaczynska et al. 1995). Pratima and Anand (1998) have found that the treatment with ascorbic acid restored the metal-induced reduction in hepatic 5′D-I activity and serum triiodothyronine (T_3) concentrations. The raised level of lipid peroxidation was also ameliorated by ascorbic acid. It supports that the defensive effect of ascorbic acid against cadmium-induced thyroid dysfunction is bestowed to its anti-oxidative action. Present study aims to assess the toxicological properties of cadmium chloride and mercuric chloride on biochemical/blood parameters (T_3, T_4, TSH, triglyceride, Hb, MCHC, packed cell volume) and bioaccumulation of metals in vital organs (lungs, liver, heart, and kidney). The ameliorating effects of vitamin C against metal toxicity were also evaluated using rabbits as experimental model.

Materials and methods

Experimental animals

During the experiment, the rabbits of age 1 month (*Oryctolagus cuniculus*) were used as model animal. The other details of experimental animals, ethical statement, and chemicals were given in Ali et al. (2019)

Administration of cadmium and mercury

After an initial 2 weeks of acclimatization, 66 rabbits of both genders were randomly divided into eight groups, i.e., one control and seven treatment groups. The scheme of the experimental design is presented in Fig. 1 and the detailed procedure used for the administration of cadmium and mercury was also explained in our previous published study (Ali et al. 2019).

Hematological/biochemical parameters

The blood (5 ml) was collected aseptically through vein puncture from lateral saphenous and/or cephalic vein of each rabbit on the 14th and 28th day of experiment. The collected blood was gently transferred to an ImuMed® vacutainer 3 ml coated with EDTA.K3 and kept at 4 °C for further analyses. Analysis of hematological parameters viz. hemoglobin (Hb), packed cell volume, and mean corpuscular hemoglobin concentration (MCHC) was done in the "Hematology Unit" using the automated method with the automatic analyzer "Hematology autoanalyzer Sysmex KX-21N." The mean MCHC, concentration of Hb in a given volume of packed red cells, was calculated by the following formula (Linne and Ringsrud 1999).

$$\mathrm{MCHC\ (g/dl)} = \mathrm{Hb\ (g/dl)} \times 100/\mathrm{PCV}$$

Blood samples were centrifuged (at 2000 rpm for 10 min for analysis of different parameters). The serum was separated, and aliquots were kept at − 20 °C until analysis (Naito 1984). Triiodothyronine (T_3), triglycerides, thyroxine (T_4), and thyroid-stimulating hormone (TSH) were analyzed using commercially available kits (Tables 1 and 2).

Estimation of metal accumulation in organs

After the completion of experiments, each sample of different treatment was euthanized by intravenous applied of sodium pentobarbital overdose and the heart was removed surgically and weighed on balance. The known weight of the heart was acid-digested and concentrations of mercury and cadmium

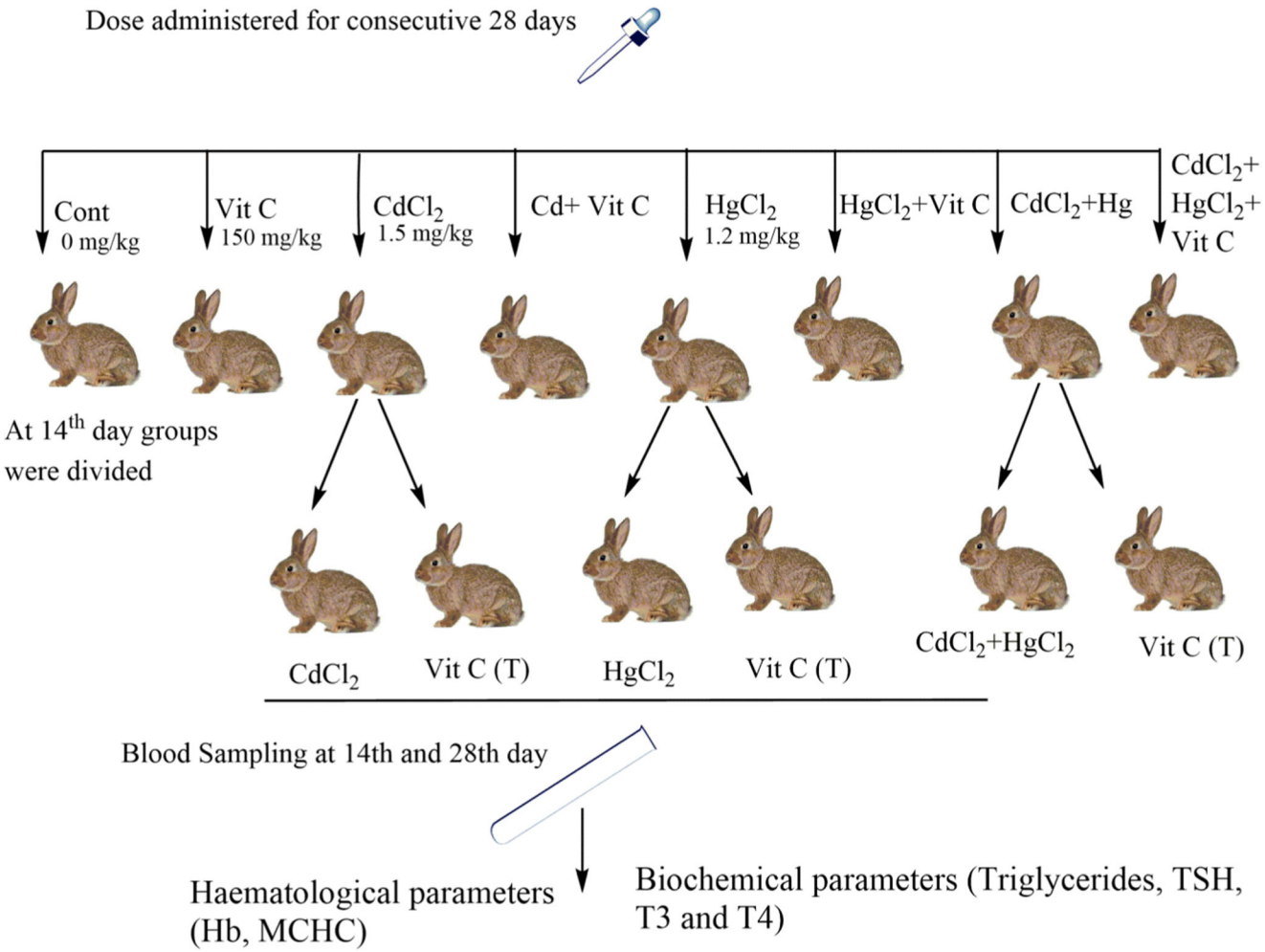

Fig. 1 A schematic presentation of animal groups, their treatment details, and experimentation

Table 1 Summary of kits used in the present study

Sr. no.	Biochemical test	Kit specifications
1	Triglycerides	Triglyceride Assay Kit-Quantification (Abcam)
2	Triiodothyronine (T_3)	The DetectX® Triiodothyronine (T_3) Enzyme Immunoassay Kit
3	Thyroxine (T_4)	Ultrasensitivity Thyroxine (U-T_4), ELISA Kit
4	Thyroid-stimulating hormone (TSH)	Thyroid-stimulating hormone ELSA, ELISA Kit

were measured through atomic absorption spectrophotometer (Du Preez and Steyn 1992). More detail is given in our recent published study (Ali et al. 2019).

Data analysis

Data were presented as mean ± SEM. Normality of the data was calculated by Kolmogorov–Smirnov test and then analyzed statistically by one-way ANOVA, with "Dunnett's Multiple Comparison Test," to identify any significant differences among the group means. GraphPad Prism version 5.0 for windows (GraphPad Software, San Diego, CA, USA) was used for analysis. Probability value ($p \leq 0.05$) was taken as significant.

Results

Effect on 3,3,5-triiodothyronine (T_3) concentration

Oral administration of cadmium chloride, mercury chloride, and their combination in rabbit for 28 alternative days showed a declined trend in the level of serum T_3. The level of T_3 was determined for various groups. Level of serum T_3 in the Cd exposed group (0.4 ± 0.0 ng/ml) showed the highest significant decrease while in the Hg exposed (0.4 ± 0.0 ng/ml) and combination of Cd + Hg group animals (0.3 ± 0.0 ng/ml), it showed a higher and the highest significant difference, respectively, as compared with that of the control group (0.7 ± 0.1 ng/ml) on the 14th day of experiment. In the prevention group (Cd + Vit C and Hg + Vit C), serum T_3 level (0.6 ± 0.0 ng/ml) showed a little higher difference as compared with the respective exposed groups. The Cd + Hg + Vit C–treated group of rabbits (0.6 ± 0.0 ng/ml) showed a higher significant difference compared with the combination of Cd + Hg (0.3 ± 0.0 ng/ml) on above the same time of sampling (Fig. 2a).

On the 28th day of exposure, the level of serum T_3 in Hg (0.46 ± 0.03 ng/ml) showed higher while the Cd (0.38 ± 0.02 ng/ml) and Cd + Hg group (0.32 ± 0.03 ng/ml) showed the highest significant decline as compared with the respective control group (0.74 ± 0.07 ng/ml). Chemo-prevention with cadmium, i.e., Cd + vit (0.68 ± 0.04 ng/ml), showed the highest significant difference while Chemo-treatment with

Table 2 Concentration of cadmium in different organs

Organs	Treatment groups						
	Cont.	Cd	Cd + Vit (P)	Cd + Vit (T)	Cd + Hg	Cd + Hg + Vit (P)	Cd + Hg + Vit (T)
Concentration of cadmium (mg/kg body weight)							
Kidney	0.0 ± 0	164.40 ± 8.25***	158.20 ± 5.95###	169.10 ± 5.92@@/$	146.07 ± 8.05***	119.17 ± 4.40&&&	131.27 ± 7.65%
Heart	0.0 ± 0	14.00 ± 1.15***	5.67 ± 0.88###	8.00 ± 1.15@@	11.67 ± 1.20***	4.330.33&&	6.670.88%
Liver	0.0 ± 0	42.33 ± 1.86***	18.00 ± 1.15###	27.00 ± 1.73@@@^{S}	30.33 ± 2.03***	11.33 ± 1.45&&&	19.33 ± 1.20%%^
Lungs	0.0 ± 0	10.00 ± 0.58***	5.00 ± 0.58###	6.67 ± 0.33@	11.33 ± 0.88***	4.33 ± 0.33&&&	6.67 ± 0.88%%
Concentration of mercury (mg/kg body weight)							
Kidney	0 ± 0	114.73 ± 7.02***	118.87 ± 6.40###	128.03 ± 7.25@/$$	94.10 ± 7.16***	69.03 ± 4.33&&	80.50 ± 8.33%
Heart	0.0 ± 0	10.33 ± 0.88***	4.00 ± 0.58###	5.67 ± 0.33@	9.67 ± 1.45***	3.67 ± 0.33&&	5.00 ± 0.88%
Liver	0.0 ± 0	27.33 ± 2.33**	14.00 ± 2.89##	17.67 ± 1.20@	24.33 ± 1.45***	8.33 ± 0.67&&&	14.33 ± 1.48%
Lungs	0.0 ± 0	8.33 ± 0.33***	4.00 ± 0.58###	5.67 ± 0.33@	8.00 ± 0.58***	3.67 ± 0.33&&&	5.67 ± 0.88

Abbreviations and keys: Cont. stands for control, Cd for cadmium, Hg for mercury, P for prevention, T for treatment. "*" indicates the significant difference between the control and cadmium, cadmium + mercury treatment groups; "#" indicates the significant difference between the cadmium and cadmium + vitamin C prevention groups; "@" indicates the significant difference between the cadmium and cadmium + vitamin C treatment groups; "$" indicates the significant difference between the cadmium + vitamin C prevention group and cadmium + vitamin C treatment group; "&" indicates the significant difference between the cadmium + mercury and cadmium + mercury + vitamin C prevention groups; "%" indicates the significant difference between the cadmium + mercury and cadmium + mercury + vitamin C treatment groups. Statistical icons: $, % = $p \leq 0.05$; @@ = $p \leq 0.01$; ***, ### = $p \leq 0.001$

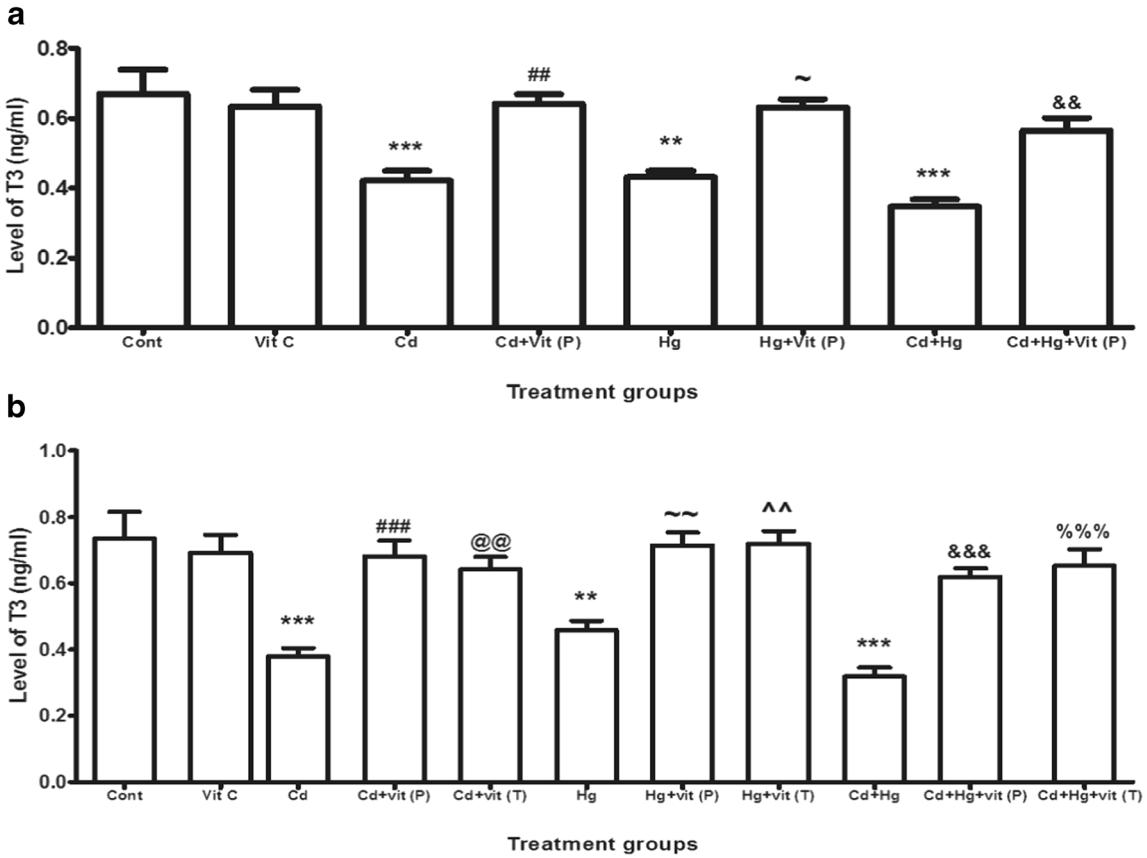

Fig. 2 Analysis of serum T_3 in the treatment groups on day 14 (**a**) and day 28 (**b**) of experiments. Abbreviation and keys: Cont. stands for control, Vit C for vitamin C, Cd for cadmium, Hg for mercury, P for prevention, T for treatment. "*" indicates the significant difference between the control and cadmium, mercury, cadmium + mercury treatment groups; "#" represents the significant difference between the cadmium and cadmium + vitamin C prevention groups; "@" indicates the significant difference between the cadmium and cadmium + vitamin C treatment groups; "~" represents the significant difference between the mercury and mercury + vitamin C prevention groups; "^" indicates the significant difference between the mercury and mercury + vitamin C treatment groups; "&" represents the significant difference between the cadmium + mercury and cadmium + mercury prevention groups, "%" indicates the significant difference between the cadmium + mercury and cadmium + mercury treatment groups. Each bar of graph represents the mean value and SEM of six replications. Statistical icons: ~ $p \leq 0.05$; **, ~~, ^^, ##, && $p \leq 0.01$; ***, &&&, %%% $p \leq 0.001$

cadmium, i.e., Cd + vit (0.64 ± 0.03 ng/ml), showed a higher significant difference as compared with the Cd exposure group (0.38 ± 0.02 ng/ml). Chemo-prevention of mercury, i.e., Hg + vit (0.72 ± 0.04 ng/ml), and chemo-treatment of mercury, i.e., Hg + vit (0.72 ± 0.03 ng/ml), both showed a higher significant difference as compared with Hg (0.46 ± 0.03 ng/ml) at the 28th day. Chemo-prevention and chemo-treatment of metal combination, i.e., Cd + Hg + vit (0.62 ± 0.02 ng/ml) and Cd + Hg + vit (0.65 ± 0.05 ng/ml), showed the highest significant difference in comparison with the combination of Cd + Hg (0.32 ± 0.03 ng/ml) on the 28th day (Fig. 2b). Serum T_3 level that was significantly decreased in all heavy metal–exposed groups became comparable with that of the blank control group in both the preventive and treatment groups.

Effect on thyroxine (T_4) concentration

Oral administration of cadmium chloride, mercury chloride, and their co-administration for 28 alternative days declined the level of serum T_4. Level of serum T_4 in Cd (26.3 ± 1.6 ng/ml), Hg (21.3 ± 1.1 ng/ml), and co-administration of Cd + Hg (27.3 ± 1.3 ng/ml) showed a comparable significant decrease with that of the control group (42.2 ± 1.7 ng/ml) sampled at 14th day of experiment. Prevention of Cd + vit (36.0 ± 2.1 ng/ml) and Hg + vit (31.5 ± 1.2 ng/ml) showed a higher significant increase in the serum level of T_4 as compared with their respective Cd (26.3 ± 1.6 ng/ml) and Hg (21.3 ± 1.1 ng/ml) exposed rabbits but that was less than the respective blank control. Similarly, the Cd + Hg + vit (35.8 ± 2.1 ng/ml) group showed a significant increase compared with the combination of Cd + Hg (27.3 ± 1.3 ng/ml) on day 14 (Fig. 3a).

At the 28th day, the level of serum T_4 in the Cd (25.8 ± 1.4 ng/ml), Hg (23.2 ± 1.6 ng/ml), and Cd + Hg group (27.3 ± 1.3 ng/ml) showed the highest significant decline as compared with the control group (45.5 ± 1.5 ng/ml). Chemo-prevention with cadmium, i.e., Cd + Vit C (38.2 ± 1.5 ng/ml), showed the highest significant difference while chemo-treatment with

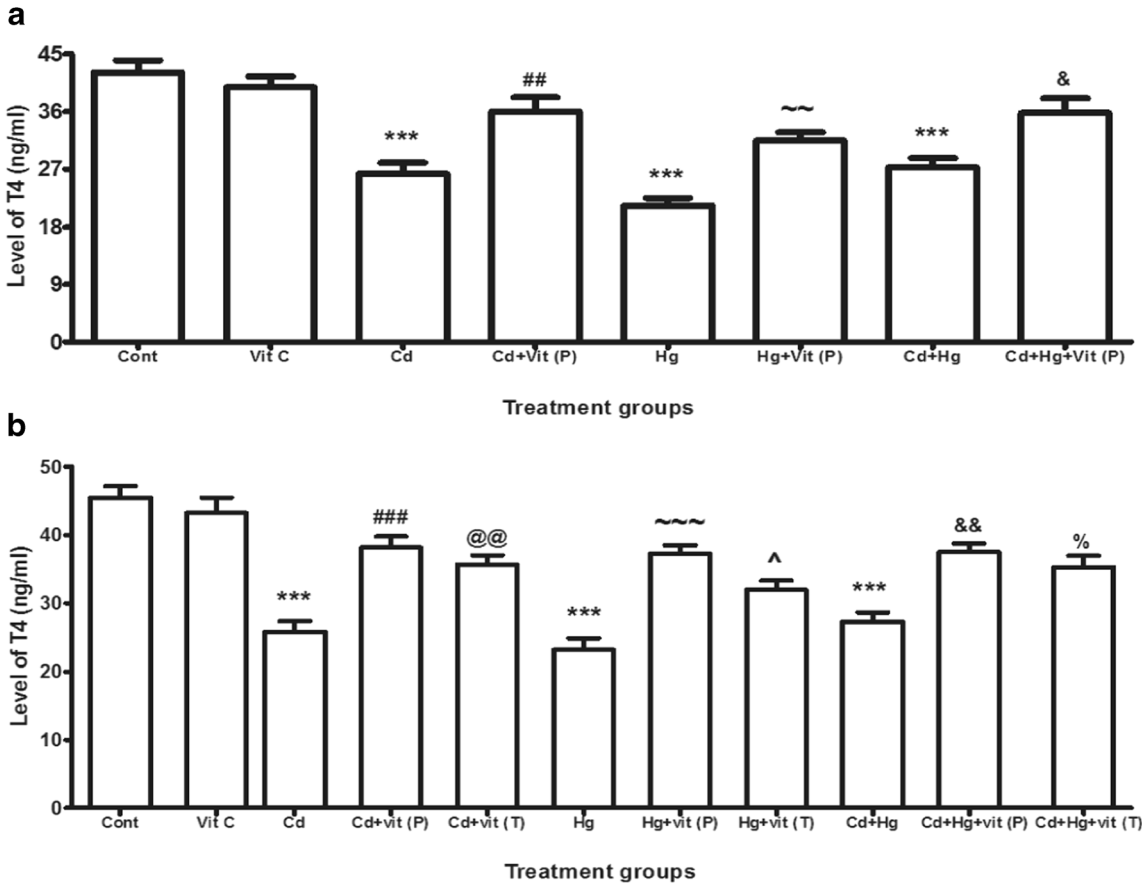

Fig. 3 Analysis of serum T_4 in the treatment groups on day 14 (**a**) and day 28 (**b**) of experiments. For abbreviation and keys, see Fig. 2

cadmium, i.e., Cd + Vit C (35.7 ± 1.2 ng/ml), showed a higher significant difference as compared with Cd (25.8 ± 1.4 ng/ml). Chemo-prevention of mercury, i.e., Hg + Vit C (37.3 ± 1.1 ng/ml), showed the highest significant difference and chemo-treatment of mercury, i.e., Hg + Vit C (32.0 ± 1.2 ng/ml), showed a significant difference as compared with Hg (23.2 ± 1.6 ng/ml) at the 28th day. Chemo-prevention and chemo-treatment of metal combination, i.e., Cd + Hg + Vit C (37.5 ± 1.2 ng/ml) and Cd + Hg + Vit C (35.3 ± 1.5 ng/ml), showed a higher significant difference and a significant difference respectively in comparison with the combination of Cd + Hg (27.3 ± 1.3 ng/ml) on the 28th day (Fig. 3b).

Effect on thyroid-stimulating hormone concentration

Oral administration of cadmium chloride, mercury chloride, and their co-administration for 28 alternative days declined the level of serum TSH. Level of serum TSH in Cd (0.17 ± 0.01 nmol/l), Hg (0.19 ± 0.01 nmol/l), and co-administration of Cd + Hg (0.17 ± 0.01 nmol/l) showed the highest significant difference as compared with the control group (0.06 ± 0.01 nmol/l) at the 14th day. Prevention of Cd + vit (0.08 ± 0.00 nmol/l) and Hg + Vit C (0.13 ± 0.01 nmol/l) showed a higher significant difference as compared with Cd (0.17 ± 0.01 nmol/l) and Hg (0.19 ± 0.01 nmol/l) respectively. Cd + Hg + Vit C (0.13 ± 0.01 nmol/l) showed higher significant differences compared with the combination of Cd + Hg (0.17 ± 0.01 nmol/l) at the 14th day (Fig. 4a).

At the 28th day, the level of serum TSH in the Cd (0.19 ± 0.01 nmol/l), Hg (0.23 ± 0.01 nmol/l), and Cd + Hg group (0.20 ± 0.01 nmol/l) showed the highest significant increase as compared with the control group (0.09 ± 0.01 nmol/l). Chemo-prevention with cadmium, i.e., Cd + Vit C (0.11 ± 0.01 nmol/l), showed the highest significant difference while chemo-treatment with cadmium, i.e., Cd + Vit C (0.14 ± 0.01 nmol/l), showed only a significant difference as compared with Cd (0.19 ± 0.01 nmol/l). Chemo-prevention of mercury, i.e., Hg + Vit C (0.13 ± 0.01 nmol/l), showed the highest significant difference and chemo-treatment of mercury, i.e., Hg + Vit C (0.17 ± 0.01 nmol/l), showed a higher significant difference as compared with Hg (0.23 ± 0.01 nmol/l) at the 28th day. Chemo-prevention and chemo-treatment of metal combination, i.e., Cd + Hg + Vit C (0.13 ± 0.01 nmol/l) and Cd + Hg + Vit C (0.15 ± 0.01 nmol/l), showed a higher significant difference and merely a significant difference respectively in comparison with the combination of Cd + Hg (27.3 ± 1.3 nmol/l) on the 28th day (Fig. 4b).

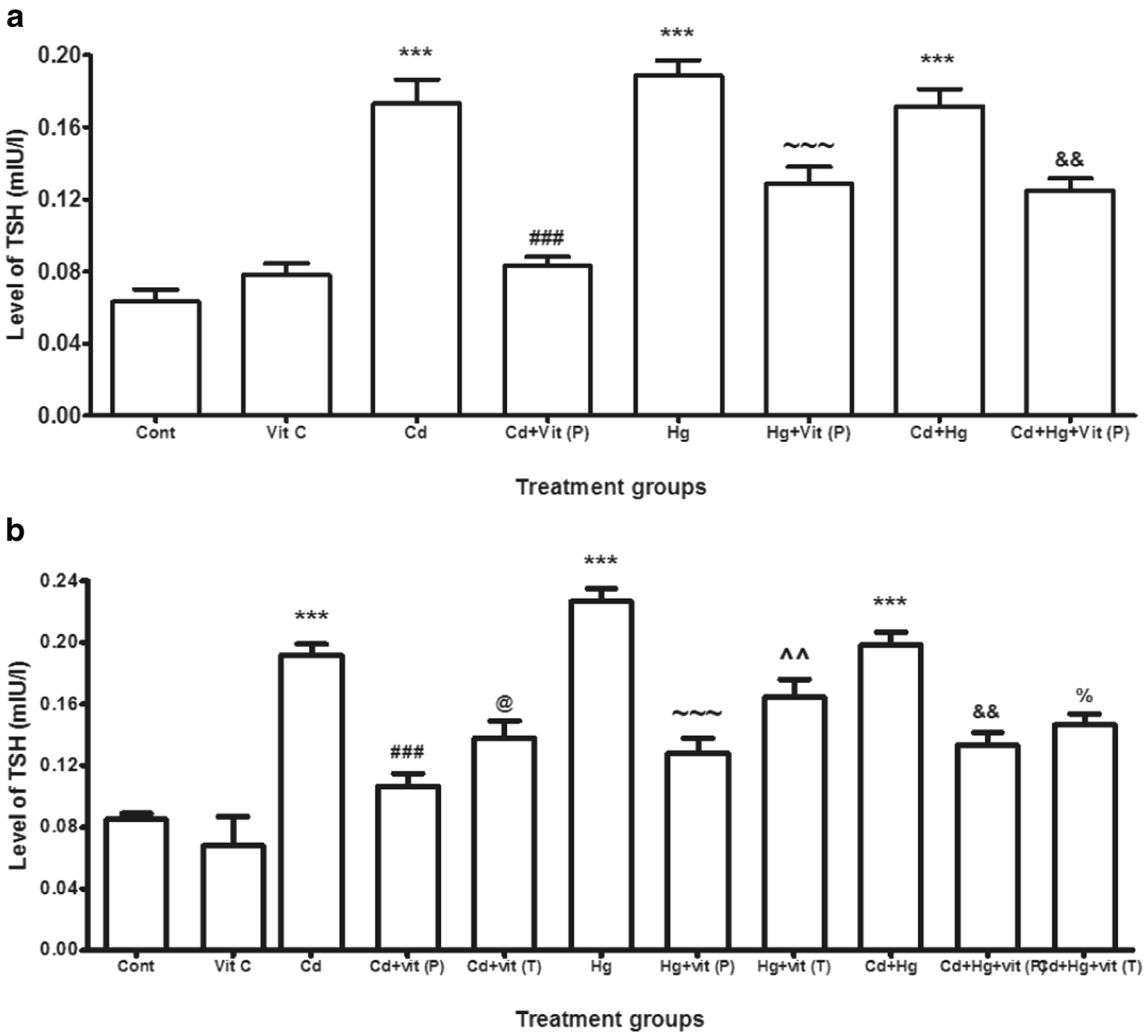

Fig. 4 Analysis of serum TSH in the treatment groups on day 14 (**a**) and day 28 (**b**) of experiments. For abbreviation and keys, see Fig. 2

Effect on triglycerides concentration

Oral administration of cadmium chloride, mercury chloride, and their co-administration for 28 alternative days elevated the level of TG. Level of TG in Cd (3.8 ± 0.1 nmol/l), Hg (3.6 ± 0.2 nmol/l), and co-administration of Cd + Hg (4.3 ± 0.1 nmol/l) showed the highest significant difference as compared with the control group (1.8 ± 0.1 nmol/l) at the 14th day. Prevention of Cd + Vit C (3.0 ± 0.1 nmol/l) and Hg + Vit C (2.8 ± 0.1 nmol/l) showed a higher significant difference as compared with Cd (3.8 ± 0.1 nmol/l) and Hg (3.6 ± 0.2 nmol/l) respectively. Cd + Hg + Vit C (3.5 ± 0.1 nmol/l) showed a significant difference as compared with the combination of Cd + Hg (4.3 ± 0.1 nmol/l) at the 14th day (Fig. 5a).

At the 28th day, the level of serum TG in the Cd (4.42 ± 0.18 nmol/l), Hg (3.90 ± 0.10 nmol/l), and Cd + Hg group (4.77 ± 0.16 nmol/l) showed the highest significant increase as compared with the control group (1.74 ± 0.08 nmol/l). Chemo-prevention with cadmium, i.e., Cd + Vit C (3.05 ± 0.21 nmol/l), showed the highest significant difference while chemo-treatment with cadmium, i.e., Cd + Vit C (3.52 ± 0.14 ± 1.2 nmol/l), showed a higher significant difference as compared with Cd (4.42 ± 0.18 nmol/l). Chemo-prevention of mercury, i.e., Hg + Vit C (2.87 ± 0.16 nmol/l), showed a higher significant difference and chemo-treatment of mercury, i.e., Hg + Vit C (3.02 ± 0.16 nmol/l), showed merely a significant difference as compared with Hg (3.90 ± 0.10 nmol/l) at the 28th day. Chemo-prevention and chemo-treatment of metal combination, i.e., Cd + Hg + Vit C (3.70 ± 0.08 nmol/l), showed the highest significant difference and Cd + Hg + Vit C (4.00 ± 0.09 nmol/l) showed a simply significant difference in comparison with the combination of Cd + Hg (4.77 ± 0.16 nmol/l) on the 28th day (Fig. 5b).

Effect on hemoglobin

Oral administration of cadmium chloride, mercury chloride, and their co-administration for 28 alternative days decreased the level of Hb. The level of Hb in Cd (10.6 ± 0.6 g/dl) and Hg (9.3 ± 0.6 g/dl) showed the highest significant difference, and

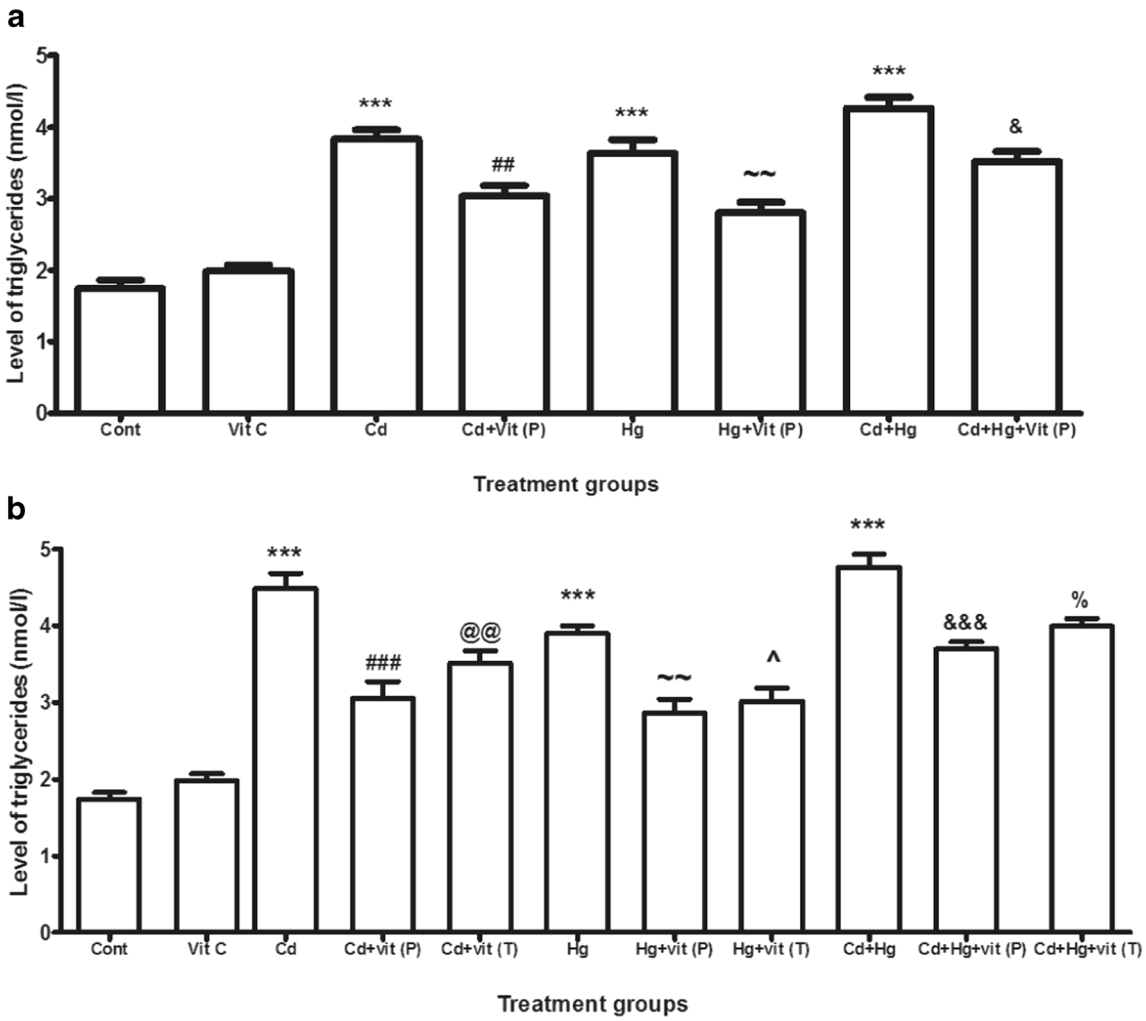

Fig. 5 Analysis of serum tryglycerides in the treatment groups on day 14 (**a**) and day 28 (**b**) of experiments. For abbreviation and keys, see Fig. 2. Effects of cadmium and mercury chloride and therapeutic role of vitamin C on hematological parameters

the co-administration of Cd + Hg (13.1 ± 0.4 g/dl) showed a higher significant difference as compared with the control group (14.4 ± 0.5 g/dl) at the 14th day. Prevention groups, i.e., Cd + Vit C (13.4 ± 0.6 g/dl), Hg + Vit C (12.1 ± 0.5 g/dl), and Cd + Hg + Vit C (13.9 ± 0.4 g/dl), showed a significant difference as compared with Cd (10.6 ± 0.6 g/dl) and Hg (9.3 ± 0.6 g/dl), and the combination of Cd + Hg (13.1 ± 0.4 g/dl) showed a significant difference as compared with control at the 14th day (Fig. 6a).

At the 28th day, the level of hemoglobin in Cd (9.9 ± 0.3 g/dl) and Hg (10.2 ± 0.5 g/dl) showed the highest while the Cd + Hg group (11.1 ± 0.4 g/dl) showed a higher significant decrease as compared with the control group (14.0 ± 0.4 g/dl). Chemo-prevention with cadmium, i.e., Cd + Vit C (13.4 ± 0.6 g/dl), showed the highest significant difference while chemo-treatment with cadmium, i.e., Cd + Vit C (12.4 ± 0.5 nmol/l), showed a higher significant difference as compared with Cd (9.9 ± 0.3 g/dl). Chemo-prevention of mercury, i.e., Hg + Vit C (13.9 ± 0.4 g/dl), showed the highest significant difference and chemo-treatment of mercury, i.e., Hg + Vit C (12.9 ± 0.2 g/dl), showed a higher significant difference as compared with Hg (10.2 ± 0.5 g/dl) at the 28th day. Chemo-prevention and chemo-treatment of metal combination, i.e., Cd + Hg + Vit C (13.4 ± 0.3 g/dl) and Cd + Hg + Vit C (13.5 ± 0.3 g/dl), showed simply a significant difference in comparison with the combination of Cd + Hg (11.1 ± 0.4 g/dl) on the 28th day (Fig. 6b).

Effect on mean corpuscular hemoglobin concentration

Oral administration of cadmium chloride, mercury chloride, and their co-administration for 28 alternative days decreased the level of MCHC. Level of MCHC in Cd (20.4 ± 0.9%) and Hg (15.6 ± 0.6%) showed the highest significant difference as compared with the control group, whereas co-administration of Cd + Hg (13.6 ± 0.7%) showed a higher significant difference compared with the control group (31.1 ± 0.9%) at the 14th day. Prevention of Cd + Vit C (26.4 ± 1.0%) that of Hg

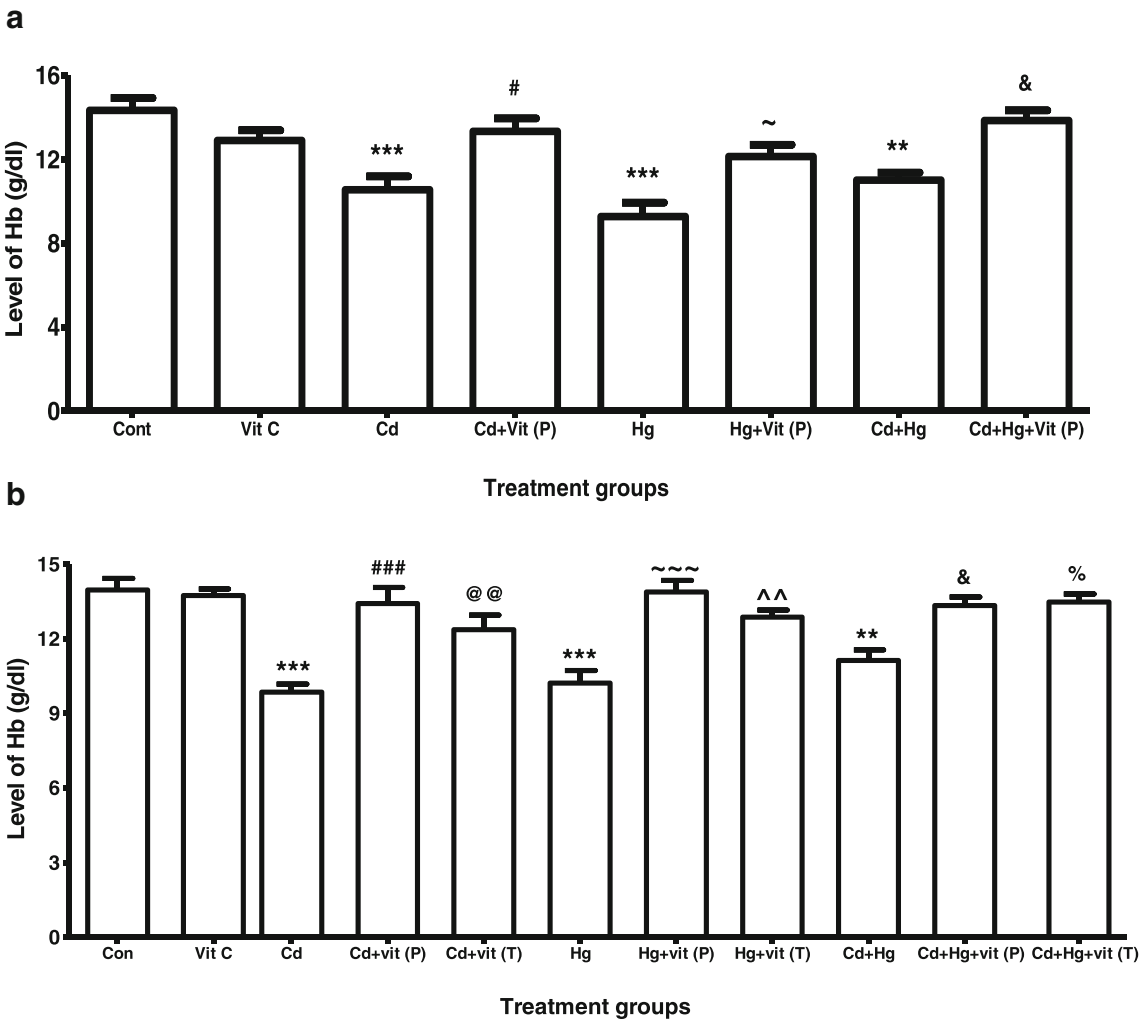

Fig. 6 Analysis of serum Hb in the treatment groups on day 14 (**a**) and day 28 (**b**) of experiments. For abbreviation and keys, see Fig. 2

+ Vit C (21.8 ± 1.3%) showed a higher significant difference as compared with Cd (20.4 ± 0.9%) and Hg (15.6 ± 0.6%) respectively. Cd + Hg + Vit C (20.3 ± 1.4%) showed a higher significant difference as compared with the combination of Cd + Hg (13.6 ± 0.7%) at the 14th day (Fig. 7a).

At the 28th day, the level of MCHC in the Cd (20.0 ± 1.2%), Hg (14.6 ± 1.1%), and Cd + Hg group (16.4 ± 0.9 nmol/l) showed the highest significant decrease as compared with the control group (30.0 ± 0.9%). Chemo-prevention with cadmium, i.e., Cd + Vit C (28.2 ± 0.9%), showed the highest significant difference while chemo-treatment with cadmium, i.e., Cd + Vit C (27.7 ± 1.8%), showed a higher significant difference as compared with Cd (20.0 ± 1.2%). Chemo-prevention of mercury, i.e., Hg + Vit C (24.1 ± 1.2%), showed a higher significant difference and chemo-treatment of mercury, i.e., Hg + Vit C (22.7 ± 1.1%), showed merely a significant difference as compared with Hg (14.6 ± 1.1%) at the 28th day. Chemo-prevention and chemo-treatment of metal combination, i.e., Cd + Hg + Vit C (23.1 ± 1.1%), showed the highest significant difference and Cd + Hg + Vit C (22.9 ± 1.0%) showed simply a significant difference in comparison with the combination of Cd + Hg (16.4 ± 0.9%) on the 28th day(Fig. 7b).

Effect on packed cell volume

Oral administration of cadmium chloride, mercury chloride, and their co-administration for 28 alternative days declined the level of PCV. The level of PCV in Cd (19.4 ± 1.2%) and Hg (16.2 ± 0.7%) showed the highest significant difference compared with the control group, whereas co-administration of Cd + Hg (20.8 ± 1.0%) showed a higher significant difference compared with the control group (29.1 ± 1.1%) at the 14th day. Prevention of Cd + Vit C (26.6 ± 0.8%) and that of Hg + Vit C (23.3 ± 1.0%) showed a higher significant difference as compared with Cd (19.4 ± 1.2%) and Hg (16.2 ± 0.7%) respectively. Cd + Hg + Vit C (26.6 ± 0.9%) showed a higher significant difference as compared with the combination of Cd + Hg (20.8 ± 1.0%) at the 14th day (Fig. 8a).

At the 28th day, the level of PCV in the Cd (19.6 ± 2.3%), Hg (19.6 ± 2.3%), and Cd + Hg group (19.1 ± 0.9%) showed

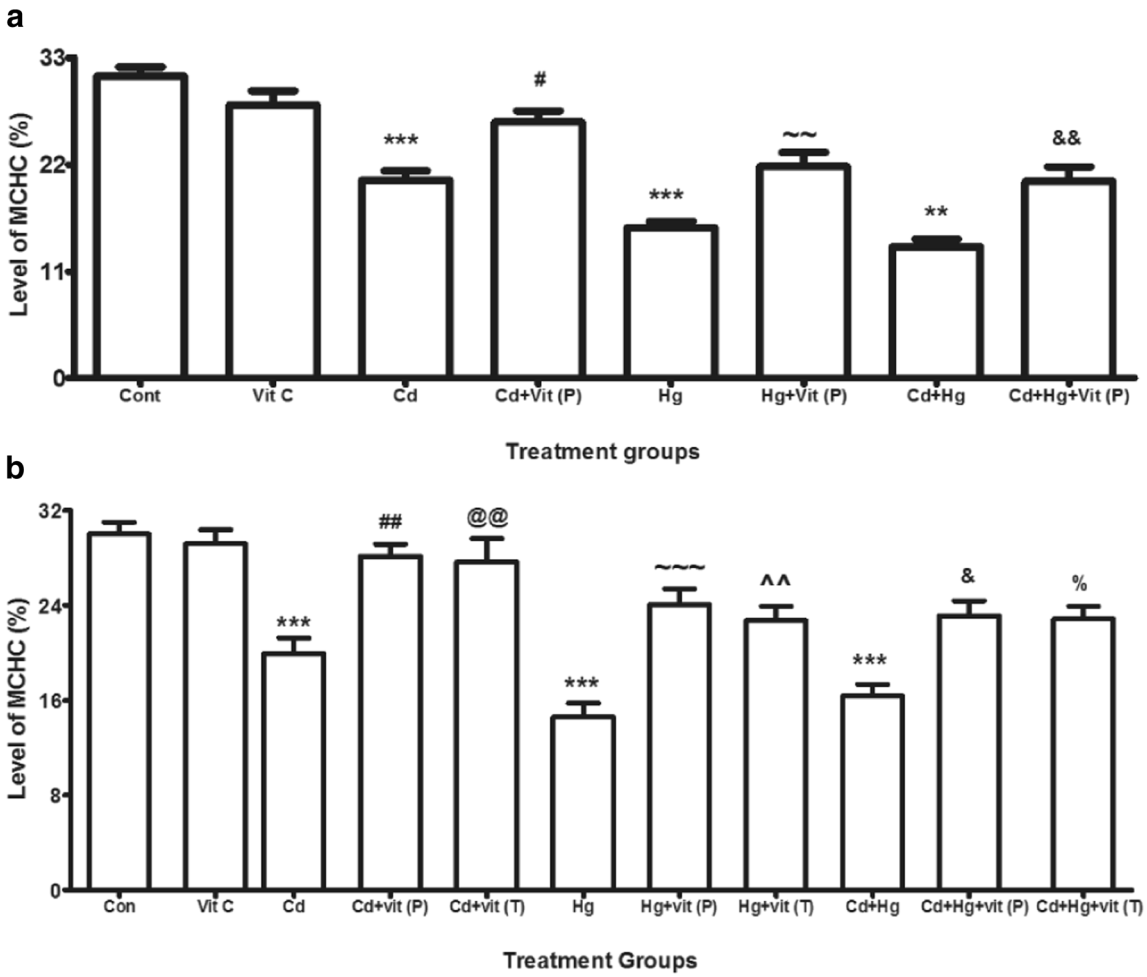

Fig. 7 Analysis of serum mean corpuscular hemoglobin in the treatment groups on day 14 (**a**) and day 28 (**b**) of experiments. For abbreviation and keys, see Fig. 2

the highest significant decrease as compared with the control group (30.0 ± 0.9%). Chemo-prevention with cadmium, i.e., Cd + Vit C (28.2 ± 0.9%), showed the highest significant difference while chemo-treatment with cadmium, i.e., Cd + Vit C (27.1 ± 0.8%), showed a higher significant difference as compared with Cd (19.6 ± 2.3%). Chemo-prevention of mercury, i.e., Hg + Vit C (24.2 ± 0.8%), showed a higher significant difference and chemo-treatment of mercury, i.e., Hg + Vit C (23.6 ± 1.4%), showed merely a significant difference as compared with Hg (19.6 ± 2.3%) at the 28th day. Chemo-prevention and chemo-treatment of metal combination, i.e., Cd + Hg + Vit C (31.0 ± 1.4%), showed the highest significant difference and Cd + Hg + Vit C (25.6 ± 0.7%) showed simply a significant difference in comparison with the combination of Cd + Hg (19.1 ± 0.9%) on the 28th day (Fig. 8b).

The concentration of cadmium in body organs

In comparison with the control group (0 ± 0 mg/kg body weight), the kidney, heart, liver, and lungs treated with Cd (164.40 ± 8.25 mg/kg, 14.00 ± 1.15 mg/kg, 42.33 ± 1.86 mg/kg, 10.00 ± 0.58 mg/kg) and Cd + Hg (146.07 ± 8.05 mg/kg, 11.67 ± 1.20 mg/kg, 30.33 ± 2.03 mg/kg, and 11.33 ± 0.88 mg/kg body weight) showed the highest significant difference. Comparing the Cd group (164.40 ± 8.25 mg/kg, 14.00 ± 1.15 mg/kg, 42.33 ± 1.86 mg/kg, 10.00 ± 0.58 mg/kg) with the prevention group of Cd + Vit C (158.20 ± 5.95 mg/kg, 5.67 ± 0.88 mg/kg, 18.00 ± 1.15 mg/kg, 5.00 ± 0.58 mg/kg) showed the highest significant difference while the treatment group of Cd + Vit C showed a higher significant difference in the heart (8.00 ± 1.15 mg/kg) and kidney (169.10 ± 5.92 mg/kg) while a significant difference in the lungs (6.67 ± 0.33 mg/kg) and the highest significant difference in the liver (27.00 ± 1.73 mg/kg body weight). In the kidney and liver, the prevention group Cd + Vit C (158.20 ± 5.95 mg/kg and 18.00 ± 1.15 mg/kg body weight respectively) showed a significant difference as compared with the treatment group Cd + Vit C (27.00 ± 1.73 mg/kg and 169.10 ± 5.92 mg/kg body weight). The comparison of the Cd + Hg group (146.07 ± 8.05 mg/kg, 11.67 ± 1.20 mg/kg, 30.33 ± 2.03 mg/kg, 11.33 ± 0.88 mg/kg) with the prevention group Cd + Hg + Vit C showed the highest significant difference in

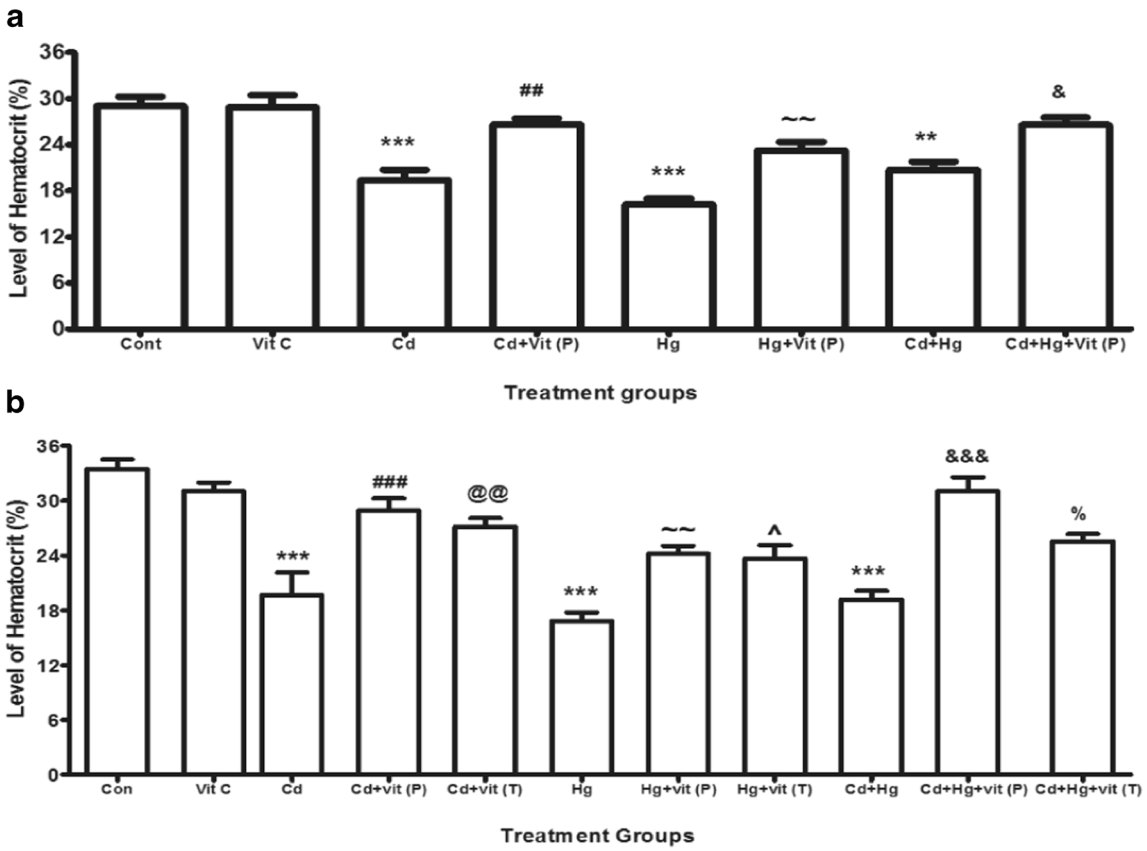

Fig. 8 Analysis of serum packed cell volume in the treatment groups on day 14 (**a**) and day 28 (**b**) of experiments. For abbreviation and keys, see Fig. 2

the kidney (119.17 ± 4.40 mg/kg), liver (11.33 ± 1.45 mg/kg), and lungs (4.33 ± 0.33 mg/kg) and a higher significant difference in the heart (4.33 ± 0.33 mg/kg) while the treatment group Cd + Hg + Vit C showed a significant difference in the kidney (131.27 ± 7.65 mg/kg) and heart (6.67 ± 0.88 mg/kg body weight) while a higher significant difference in the liver (19.33 ± 1.20 mg/kg) and lungs (6.67 ± 0.88 mg/kg body weight). In the liver, the prevention group Cd + Hg + Vit C (11.33 ± 1.45 mg/kg) showed a significant difference as compared with the treatment group Cd + Hg + Vit C (19.33 ± 1.20 mg/kg body weight) shown in (Tables 1 and 2).

The concentration of mercury in body organs

In comparison with the control group (0 ± 0 mg/kg), the kidney, heart, liver, and lungs treated with Hg (114.73 ± 7.02 mg/kg, 10.33 ± 0.88 mg/kg, 27.33 ± 2.33 mg/kg, 8.33 ± 0.33 mg/kg) and Cd + Hg (94.10 ± 7.16 mg/kg, 9.67 ± 1.45 mg/kg, 24.33 ± 1.45 mg/kg, 8.00 ± 0.58 mg/kg body weight) showed the highest significant difference. Comparing the Hg group (114.73 ± 7.02 mg/kg, 10.33 ± 0.88 mg/kg, 27.33 ± 2.33 mg/kg, 8.33 ± 0.33 mg/kg body weight) with the prevention group, Cd + Vit C showed the highest significant difference in the kidney (118.87 ± 6.40 mg/kg), heart (4.00 ± 0.58 mg/kg), and lungs (4.00 ± 0.58 mg/kg) and a higher significant difference (14.00 ± 2.89 mg/kg) in the liver, while the treatment group Cd + Vit C showed a significant difference in the kidney, heart, liver, and lungs (128.03 ± 7.25 mg/kg, 5.67 ± 0.33 mg/kg, 17.67 ± 1.20 mg/kg, 5.67 ± 0.33 mg/kg body weight respectively). In the kidney, the prevention group Cd + Vit C (118.87 ± 6.40 mg/kg body weight) showed a significant difference as compared with the treatment group Cd + Vit C (128.03 ± 7.25 mg/kg body weight). The comparison of the Cd + Hg group (94.10 ± 7.16 mg/kg, 9.67 ± 1.45 mg/kg, 24.33 ± 1.45 mg/kg, 8.00 ± 0.58 mg/kg) with the prevention group Cd + Hg + Vit C showed a higher significant difference in the kidney (69.03 ± 4.33 mg/kg) and heart (3.67 ± 0.33 mg/kg), and the highest significant difference in the liver (8.33 ± 0.67 mg/kg) and lungs (3.67 ± 0.33 mg/kg body weight), while the treatment group Cd + Hg + Vit C showed a significant difference in the kidney (80.50 ± 8.33 mg/kg), heart (5.00 ± 0.88 mg/kg body weight), and liver (14.33 ± 1.48 mg/kg body weight) with no significant difference in the lungs (5.67 ± 0.88 mg/kg body weight) as shown in Tables 1 and 2.

Discussion

An increased use of metals in anthropogenic activities has increased the exposure to several environmental contaminants

and their role has been identified in disrupting many physiologic functions in the body. In the present study, it is demonstrated that a chronic exposure of toxic metals had adverse effects on thyroid function parameters in exposed rabbits. As indicated by serum level endpoints, thyroid homeostasis was significantly altered with the exposure of both toxic metals either alone or in combination. The results from thyroid function tests revealed that cadmium, mercury, and their co-administration had significantly decreased the average values of T_3 and T_4 as compared with that of control, whereas TSH increased in both cadmium- and mercury-treated rabbits. However, the chemo-prevention with vitamin C had showed a significant elevation in the level of both T_3 and T_4 that draws persuasive support from the work of Gupta and Kar (1999) that the administration of Cd to Swiss male mice induced thyroid malfunctioning and lipid peroxidation leading to a change in T_3, T_4, and hepatic type I iodothyronine 5-Monpdeiodinase (5 D-I) activity in the serum. As far as we know, the effect of chronic exposure to $CdCl_2$ and $HgCl_2$ on thyroid has not been reported yet. Many studies showed that the mechanisms interfering with liver thyroid hormone metabolism via diminished "outer ring de-iodine enzyme ORD" activity that converts thyroid hormone T_4 in biologically active form (T_3); defects in signaling pathway of T_3 also result in decreased T_4 conversion. In addition, cadmium and mercury cause thyroid iodine uptake to fall by disrupting the follicular cells of the thyroid (Gupta and Kar 1999). Long-term exposure to cadmium and mercury can induce activity in liver microsomal enzymes, especially Uridine DiPhosphate Glucournyl Tranferase (UDP-GT) (Wade et al. 2003) and phenol Sulftransferase that result in T_3 and T_4 evacuation. Cadmium accumulation might depress oxidative phosphorylation in mitochondrial thyroid follicle epithelial cells with consistent energy loss that inhibits synthesis and release of thyroid hormones (Yoshizuko et al. 1991). Cadmium treatment can lead to histological changes, initiation of the fibrotic process, inflammatory reaction, and over-conversion of the growth factor B thus playing important role in thyroid morphology (Ruze et al. 1999).

The metal reduced hepatic 5D-I activity but the serum T_3 level was touch up by supplementation of ascorbic acid. The thyroid may accumulate mercury in follicular cells at exposure (WHO 1991; Falnoga et al. 2000). It has been shown that moderate occupational exposure affects a seleno-enzyme called deiodinase that is accountable for the deiodination of T_4 to T_3 (Barregård et al. 1994; Ellingsen et al. 2000). This causes T_4 level to increase by reversing T_3 levels and raise the T_4/T_3 ratio (Barregård et al. 1994; Ellingsen et al. 2000).

According to the data reported by Nishida et al. (1986), thyroidal secretion of T_4 was subdued by mercury while Kawada et al. (1980) suggested that both organic and inorganic Hg disrupted thyroid function by interfering both with the production of thyroidal hormones and the conversion of T_4 to T_3. Experimental exposure of methyl mercury to monkeys indicated high concentrations of inorganic Hg in the pituitary (Vahter et al. 1995). Thyroid dysfunction in our study can be explained by the probable accretion of Hg in the thyroid gland. Since, T_4 synthesis is associated with the thyroid gland, so its decline in the serum level of Hg-treated rabbits could suggest that Hg-induced thyroid dysfunction is due to affected production and/or secretion of T_4 by the follicular cells of the thyroid gland. A significant increase in TSH levels in the Hg-treated rabbits could also affect regulatory enzymes associated with the hypothalamic-pituitary-thyroid (HPT) axis. A significant increase in serum TSH level in both heavy metal exposed rabbits may be explained because of feedback control mechanisms as previously reported by different researchers (Hammouda et al. 2008; Mohamed et al. 2015). Metabolism of thyroid hormones in the peripheral tissues by deiodination, conjugation, deamination, and decarboxylation enzymatic reactions may influence thyroid role at the cellular level (Badiei et al. 2010; Paier et al. 1993; Chaurasia et al. 1996; Kelly 2000; Pilat-Marcinkiewicz et al. 2003).

The present study revealed that oral administration of $CdCl_2$, $HgCl_2$, and their combined usage significantly decreases the level of Hb and PCV in all the cadmium chloride– and mercury chloride–exposed groups with respect to the control group. The supplementation of vitamin C significantly restored the level of Hb and hematocrit at the 28th day of treatment. These results can be interpreted from the observations of Hounkpatin et al. (2012) who debated about the causes that affect the common stipulations of the animals such as fluid balance and nutritional condition influence hematocrit pathology.

Previous studies showed that individual stimulation of $CdCl_2$ in chicks causes the elevation of MCHC level at the beginning; however, due to increased concentration of dose, MCHC levels were declined (Gabol et al. 2014). Current study showed that the level of MCHC significantly declined at the 28th day of treatment in cadmium chloride and mercury chloride groups, however. Chemo-treatment with vitamin C stabilizes the level of MCHC in intoxicated rabbits.

The endocrine system like thyroid hormones plays an important role in homeostasis. Thyroid hormone is involved in hemoglobin synthesis. Hypothyroidism also causes anemia probably through decreasing the oxygen, resultantly also perturbs the RBCs indices like PCV, MCH, and MCHC (Dorgalaleh et al. 2013; Iddah et al. 2013). These findings suggest exploring the molecular mechanisms altering hematopoiesis that may provide the targets for therapeutic interventions.

Our findings are in line with the Grosicki (2004) and Jiraungkoorskul et al. (2007). They demonstrated that vitamin C reduced the metal-induced toxicity. In current research, it was observed that co-administration of both metals with vitamin C exhibited more protection than the group supplemented with vitamin C separately. The restoration of liver functions

from $CdCl_2$ and $HgCl_2$ toxicity was observed in the prevention groups due to vitamin C which indicated the free radical scavenging mechanism and detoxification effect (Suzuki 1990).

The metals tend to accumulate in different organs such as the lungs, kidney, liver, and heart (Siraj et al. 2016). The amount of accumulation depends on stage, prolonged, and short-term exposures both revealed the diverse outcomes (Ali et al. 2016). The liver is a target organ for the accumulation of $CdCl_2$ in rabbits, afterwards it accumulates in renal system and finally in tissues as well as in skeletal muscles (Josthna et al. 2012). The present study revealed that vital organs such as the liver and kidneys had significant accumulation of toxic metals in all metals' exposed groups. However, accumulation was less in the $CdCl_2$ + $HgCl_2$–treated group than individual cadmium chloride– and mercury chloride–exposed group. Pretreatment and chemo-treatment with vitamin C significantly decline the concentration in studied vital organs.

In the set-up of our experiment, more cadmium and mercury compounds are accumulated in the organs of rabbits and had altered the biochemical profile of serum when administered orally. Changed levels of these biomarkers resulted in thyroid injury. However, the intensity of these alterations of biochemical profile was encountered in the presence of vitamin C co-administration. These findings may indicate a protective mechanism of ascorbic acid in thyroid toxicity in toxic metals-intoxicated rabbits. Ameliorating potential of vitamin C in cadmium- and mercury-administered rabbits was observed on the 14th and 28th day of experiment. Vitamin C treatment exhibit a reduction in the adverse effects of cadmium compounds by restoring hematological and biochemical changes as reported earlier (Abdelaziz et al. 2013). Co-administration of ascorbic acid has significantly decreased the entry of chromium into the cells (Lin et al. 2018; Rana et al. 2018). In present research, more concentration of cadmium and mercury was found to be in the thyroid gland of rabbit, when exposed to these metals, which depicts that the thyroid gland might be capable of accumulating these metals in higher concentration. While supplementation of vitamin C can prevent the production of free radicals and maintain the hematological parameters, these findings may indicate the therapeutic mechanism of vitamin C against toxic metal–induced thyroid toxicity and biochemical alterations in intoxicated rabbits.

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