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Protective Effects of Vitamin E, Selenium and Zinc Supplementation on Hematological and Some Biochemical Parameters in Pregnant Rats Exposed to Cadmium

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Abstract: The aim of this study was to investigate the possible protective role of vitamin E (vit.E), zinc (Zn) and selenium (Se) as antioxidants against cadmium (Cd) toxicity in pregnant rats reflected via assessment of hematological and some biochemical parameters. Ninety pregnant rats were involved and randomly allocated into equally distributed (n=10) nine groups. The first group was kept as control group. While, the other eight groups were treated with 0.3 mg $CdCl_2$/kg b.wt for the first four groups (low dose cadmium) and 1.5 mg $CdCl_2$/kg b.wt for the second four groups (high cadmium dose). Three of the low and 3 of the high doses exposed cadmium groups were treated with one of the following antioxidants: 10 mg vitamin E / animal, 2 mg zinc sulphate ($ZnSO_4$) / kg b.wt. or 1.5 mg sodium selenite ($NaSeO_3$), respectively. Blood samples were collected in heparinized tubes and used for hematological studies and estimation of cadmium level. The plasma was separated to aliquots for evaluation of zinc level and glutathione peroxidase activity (GSH-Px). Exposure to both low and high Cd doses induced significant decrease in RBC's count, hemoglobin concentration and packed cell volume. In addition to significant hypozincemia was recorded. In high dose Cd exposed group glutathione peroxidase activity was significantly decreased. The whole blood of all cadmium exposed mothers rat was higher in cadmium compared to those of control group. Treatment with vit.E, Se and Zn increased significantly the erythrocytic parameters and Zn level and GSH-Px activity. Moreover, reduction of cadmium residual level in blood of mothers rat was evident. It could be concluded that treatment with vit. E, Se and Zn may have a protective effect specially at the low dose Cd exposure which is reflected on improving the blood parameters when compared with Cd treated group.

Key words: Cadmium Toxicity · Hematology · Biochemistry · Antioxidants · Selenium · Zinc · Vit.E. · Glutathione Peroxidase

INTRODUCTION

Cadmium (Cd) is an important industrial and environmental pollutant which is present in soil, water, air and food. It is widely distributed and a highly toxic heavy metal capable of causing damage to various organs with an exact cellular mechanism of toxicity [1]. It has been found that Cd stimulates free radical production, leading to oxidative deterioration of lipids, proteins and DNA and initiating various pathological conditions in humans and animals [2].

After exposure, cadmium enters the blood, where it binds to the erythrocytic membrane and stimulates the formation of metallothioneins and reactive oxygen species (ROS) leading to alterations in the antioxidant system and causing oxidative damage in red bood cells (RBCs) and in various tissues which result in a loss of membrane functions. Cd is known to induce anaemia, reduce the RBCs count, hematocrit value as well as hemoglobin concentration [3-5].

Protection against toxicity of Cd can be accomplished through the antioxidant defense system (AOS). Vit. E, Se and Zn are among the important non enzymatic antioxidant defense system which may modulate its toxicity by an antioxidative mechanism.

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Therefore the aim of the present study is to investigate the possible protective influence of vitamin E, selenium and zinc treatment on blood picture and glutathione peroxidase of pregnant rats intoxicated with cadmium.

MATERIALS AND METHODS

Animals: Mature female rats (Sprague Dowly) were obtained from National Research Center Animal House. Animals were mated using mature males. Animals showed vaginal plugs and/ or sperm in vaginal smear were considered pregnant. A total number of 90 pregnant rats was used in this experiment. The experiment was conducted under normal environmental conditions of nutrition and water supply.

Heavy Metals: Cadmium chloride ($CdCl_2$) was used in two concentrations (0.3 and 1.5 mg/kg b.wt.), which represent 1/200 and 1/40 of LD50 according to El Gindy [6].

Antioxidants:

- 2 mg Zinc sulphate ($ZnSO_4$) / Kg b.wt. was given according to Claverie *et al.* [7].
- Vitamin E (α- tocopherol acetate). It was available in soft gelatinous capsules of 100 mg. Each capsule was dissolved in 10 ml corn oil and each animal was administered one ml orally [8].
- 1.5 mg Sodium Selinite (Na_2SeO_3) / Kg b.wt. was given according to Yiin *et al.* [9].

Experimental Design: Rats were randomly allocated into nine groups (1–9). Animals were exposed to either low or high doses of $CdCl_2$ either alone or in combination with antioxidants $ZnSO_4$, vit. E or $NaSeO_3$ as shown in Table (1) which illustrates treatments of the different groups.

Sampling

Blood Samples: Three blood samples were collected from the retro orbital venous plexus of all animals of the different groups. The first sample was taken during pregnancy (7-10 days), the second and third samples were taken after 15 and 30 days of parturition. The collected blood samples were received into heparinized tubes and used for hematological studies and estimation of cadmium level. A part of heparinized blood was centrifuged at 3000 r.p.m. for 10 minutes and the clear supernatant plasma was kept at – 20°C till used for estimation of zinc level and glutathione peroxidase activity.

Table 1: Different experimental groups of pregnant rats

Group	No.	Treatment*
1	10	Kept as control
2	10	Low dose (L.D.) $CdCl_2$ 0.3 mg / kg b.wt.
3	10	Low dose $CdCl_2$ + 2mg $ZnSO_4$ / kg b wt
4	10	Low dose $CdCl_2$ + 10 mg vitamin E / animal
5	10	Low dose $CdCl_2$ + 1.5 mg Sod. selenite / kg b wt.
6	10	High dose (H.D.) $CdCl_2$ 1.5 mg/ kg b. wt.
7	10	High dose $CdCl_2$ +2mg $ZnSO_4$ / kg b wt.
8	10	High dose $CdCl_2$ + 10 mg vitamin E / animal
9	10	High dose $CdCl_2$ + 1.5 mg Sod. selenite / kg b wt.

*Treatments were dissolved in distilled water except vitamin E who was dissolved in corn oil. Treatments were given in drinking water. Vitamin E was given per os. $CdCl_2$ was given 3 times/ week from conception date till parturition. Antioxidants were given 5 days before mating and continued daily till parturition

Clinicopathological Examinations

Hematological Studies: Erythrocytes count (RBC's), packed cell volume (PCV), hemoglobin concentration (Hb), Total leucocytic count (WBC's) and differential leucocytic count were carried out according to the techniques described by Feldman *et al*. [10].

Biochemical Analysis:

Zinc: Zinc was determined according to Johnsen and Eliasson [11].

Glutathione Peroxidase Activity: This enzyme was assayed adopting the method described by Tamura *et al.* [12].

Estimation of Cadmium Residue in Whole Blood: Cadmium concentrations in samples of whole blood of mothers were determined by Graphite Furnace Atomic Absorption Spectrophotometry. All samples were treated with nitric acid and hydrochloric acid mixture as described by Renard and Tompkins [13].

Statistical Analysis: Data were statistically analyzed using one way analysis of variance (ANOVA) according to the method of Snedecor and Cochran [14] and Statistical Package for Social Science (SPSS) version 11.0.

RESULTS

Erythrogram: Data of the effect of different antioxidants (Se, Vit.E and Zn) on the hemogram (RBCs, Hb and PCV) of Cd-exposed rats revealed variable responses of improvement (Table 2).

Table 2: Effect of different doses of cadmium given with and without antioxidantson the erythrogram of female rats at different intervals of the experiment

Parameters		RBC'S ($x10^6/\mu l$)			HB (g/dl)			PCV (%)		
Periods	Groups	During* pregnancy	After birth (15 days)	After birth (30 days)	During pregnancy	After birth (15 days)	After birth (30 days)	During pregnancy	After birth (15 days)	After birth (30 days)
Control		$4.71^{a}\pm0.46$	$4.74^{ab}\pm0.20$	$4.62^{ac}\pm0.21$	$12.35^{a}\pm0.22$	$12.91^{ab}\pm0.58$	$11.96^{ac}\pm0.25$	$42.20^{a}\pm1.46$	$46.60^{ab}\pm1.03$	$47.00^{ac}\pm0.84$
L.D.	Cd	$4.14^{a}\pm0.31$	$3.92^{ab}\pm0.02$	$4.71^{c}\pm0.06$	$10.39^{a}\pm0.17$	$10.59^{ab}\pm0.32$	$10.68^{c}\pm0.77$	$37.40^{a}\pm1.50$	$42.60^{b}\pm0.32$	$44.60^{c}\pm0.32$
	Cd + Zn	$3.76^{a}\pm0.08$	$3.75^{ab}\pm0.09$	$4.35^{ac}\pm0.25$	$10.26^{a}\pm0.03$	$11.45^{b}\pm0.46$	$12.53^{c}\pm0.22$	$41.20^{a}\pm1.20$	$49.60^{b}\pm0.24$	$47.00^{c}\pm0.32$
	Cd + Vit.E	$3.83^{a}\pm0.08$	$3.80^{ab}\pm0.04$	$3.87^{ac}\pm0.05$	$11.73^{a}\pm0.27$	$12.22^{ab}\pm0.31$	$11.69^{ac}\pm0.33$	$46.00^{a}\pm1.18$	$46.00^{ab}\pm0.51$	$47.40^{ac}\pm0.51$
	Cd + Se	$3.80^{a}\pm0.10$	$4.17^{b}\pm0.21$	$4.11^{c}\pm0.15$	$13.31^{a}\pm0.85$	$12.10^{b}\pm0.15$	$11.80^{c}\pm0.24$	$48.20^{a}\pm0.37$	$46.60^{b}\pm0.81$	$45.60^{c}\pm0.60$
H.D.	Cd	$3.86^{a}\pm0.11$	$3.74^{ab}\pm0.59$	$4.09^{ac}\pm0.28$	$10.83^{a}\pm0.39$	$12.24^{b}\pm0.34$	$11.10^{ac}\pm0.45$	$40.20^{a}\pm1.74$	$47.80^{b}\pm1.46$	$45.20^{c}\pm1.46$
	Cd + Zn	$3.79^{a}\pm0.59$	$3.70^{ab}\pm0.59$	$3.79^{ac}\pm0.13$	$10.47^{a}\pm0.89$	$10.97^{ab}\pm0.59$	$12.19^{ac}\pm0.26$	$42.80^{a}\pm1.20$	$47.00^{b}\pm0.84$	$47.00^{c}\pm0.84$
	Cd + Vit.E	$4.25^{a}\pm0.14$	$3.85^{ab}\pm0.18$	$3.92^{ac}\pm0.47$	$12.70^{a}\pm0.11$	$12.88^{ab}\pm0.30$	$11.99^{ac}\pm0.39$	$44.20^{a}\pm0.49$	$47.40^{b}\pm0.98$	$47.40^{c}\pm0.98$
	Cd + Se	$3.85^{a}\pm0.15$	$4.19^{ab}\pm0.19$	$4.07^{ac}\pm0.11$	$12.90^{a}\pm0.39$	$11.45^{ab}\pm0.29$	$12.42^{ac}\pm0.32$	$48.00^{a}\pm1.58$	$45.60^{ab}\pm0.51$	$46.60^{ac}\pm1.12$
	LSD	0.47	0.35	0.43	1.41	1.13	1.28	3.61	2.05	2.48

*Samples were taken 7 to 14 days during pregnancy. Values represent means ±standard error. Cd =cadmium, L.D. =low dose, H.D. =high dose, Zn =zinc, Se =selenium. Means with different superscripts (a, b, c) within row are significantly different at $p<0.05$

Difference between means in the same column higher than LSD indicates significance.

Table 3: Effect of different doses of cadmium given with and without antioxidants on the red cell indices of female rats at different intervals of the experiment.

Parameters		MCV (fl)			MCH (pg)			MCHC (g %)		
Periods	Groups	During* pregnancy	After birth (15 days)	After birth (30 days)	During pregnancy	After birth (15 days)	After birth (30 days)	During pregnancy	After birth (15 days)	After birth (30 days)
Control		$99.85^{a}\pm7.75$	$98.33^{ab}\pm4.13$	$101.95^{ac}\pm6.52$	$26.39^{a}\pm1.22$	$27.28^{ab}\pm1.14$	$26.02^{ac}\pm2.44$	$26.77^{a}\pm1.97$	$27.78^{ab}\pm0.78$	$25.42^{ac}\pm1.09$
L.D.	Cd	$88.76^{a}\pm6.19$	$108.48^{b}\pm2.89$	$94.97^{ac}\pm3.01$	$25.49^{a}\pm1.87$	$27.02^{ab}\pm0.85$	$23.19^{ac}\pm2.19$	$28.73^{a}\pm0.65$	$24.94^{b}\pm0.83$	$23.87^{c}\pm1.67$
	Cd + Zn	$110.69^{a}\pm4.87$	$132.33^{b}\pm3.29$	$125.74^{c}\pm3.04$	$27.36^{a}\pm0.59$	$30.86^{ab}\pm0.38$	$33.31^{c}\pm0.38$	$24.81^{a}\pm0.83$	$23.24^{ab}\pm1.20$	$26.52^{c}\pm0.38$
	Cd + Vit.E	$121.13^{a}\pm5.23$	$121.18^{ab}\pm4.94$	$122.68^{ac}\pm2.88$	$30.69^{a}\pm0.84$	$32.16^{ab}\pm0.69$	$30.18^{ac}\pm0.85$	$25.49^{a}\pm1.34$	$26.68^{ab}\pm1.32$	$24.62^{ac}\pm0.61$
	Cd + Se	$127.88^{a}\pm3.15$	$113.29^{b}\pm5.27$	$111.66^{c}\pm3.62$	$35.07^{a}\pm1.22$	$29.21^{b}\pm1.56$	$28.82^{c}\pm1.10$	$27.43^{a}\pm0.67$	$25.76^{ab}\pm0.38$	$25.83^{ac}\pm0.86$
H.D.	Cd	$104.74^{a}\pm6.59$	$119.31^{ab}\pm1.52$	$114.08^{ac}\pm5.98$	$27.59^{a}\pm1.50$	$32.73^{ab}\pm0.83$	$27.62^{ac}\pm2.19$	$27.04^{a}\pm0.91$	$27.46a^{b}\pm0.91$	$23.94^{c}\pm1.32$
	Cd + Zn	$112.34^{a}\pm4.65$	$129.17^{b}\pm5.29$	$126.49^{c}\pm5.29$	$27.67^{a}\pm2.39$	$29.62^{ab}\pm1.30$	$32.69^{ac}\pm1.84$	$24.59^{a}\pm1.60$	23.03ab±1.53	$25.81^{ac}\pm0.56$
	Cd + Vit.E	$105.14^{a}\pm4.35$	$124.97^{b}\pm6.71$	$119.97^{c}\pm4.28$	$30.01^{a}\pm1.09$	$33.76^{ab}\pm2.16$	$30.56^{ac}\pm1.01$	$28.55^{a}\pm0.24$	$26.99^{ab}\pm0.63$	$25.56^{c}\pm1.22$
	Cd + Se	$126.02^{a}\pm7.23$	$110.35^{ab}\pm4.06$	$113.75^{ac}\pm2.32$	$33.73^{a}\pm2.70$	$27.57^{ab}\pm1.74$	$30.59^{ac}\pm1.04$	$26.88^{a}\pm2.24$	$24.91^{ab}\pm0.71$	$26.96^{ac}\pm1.30$
	LSD	14.89	10.79	11.91	4.26	3.88	4.02	3.42	2.56	2.80

*Samples were taken 7 to 14 days during pregnancy

Values represent means ±standard error. Cd =cadmium, L.D. =low dose, H.D. =high dose, Zn =zinc, Se =selenium.

Means with different superscripts (a, b, c) within row are significantly different at $p<0.05$

Difference between means in the same column higher than LSD indicates significance.

Table 4: Effect of different doses of cadmium given with and without antioxidants on the leucogram of female rats at different intervals of the experiment

Parameters		TLC ($x10^3/\mu l$)			Lymph ($x10^3/\mu l$)			Neutro ($x10^3/\mu l$)			Mono ($x10^3/\mu l$)			Eosino ($x10^3/\mu l$)		
Periods	Groups	During pregn-ancy*	After birth (15 days)	After birth (30 days)	During pregn-ancy	After birth (15 days)	After birth (30 days)	During pregn-ancy	After birth (15 days)	After birth (30 days)	During pregn-ancy	After birth (15 days)	After birth (30 days)	During pregn-ancy	After birth (15 days)	After birth (30 days)
Control		35.67^{a}	40.17^{ab}	36.60^{ac}	26.28^{a}	29.91^{ab}	28.41^{ac}	6.38^{a}	6.06^{ab}	5.74^{ac}	2.54^{a}	3.24^{ab}	1.95^{ac}	0.46^{a}	0.95^{ab}	0.49^{ac}
		±1.86	±2.49	±1.44	±2.01	±1.28	±0.98	±1.03	±0.73	±0.37	±0.65	±0.43	±0.19	±0.09	±0.19	±0.13
L.D.	Cd	36.67^{a}	34.27^{ab}	41.67^{c}	26.02^{a}	25.59^{ab}	31.84^{c}	6.85^{a}	5.83^{ab}	7.36^{c}	3.18^{a}	2.16^{ab}	2.19^{ac}	0.26^{a}	0.68^{ab}	0.78^{ac}
		±0.88	±0.37	±1.46	±0.53	±0.98	±1.59	±0.39	±0.37	±0.28	±0.35	±0.44	±0.44	±0.13	±0.19	±0.14
	Cd + Zn	32.35^{a}	37.73^{ab}	35.73^{ac}	25.19^{a}	29.33^{b}	27.26^{ac}	5.32^{a}	5.82^{ab}	5.97^{ac}	1.29^{a}	1.52^{ab}	2.16^{ac}	0.54^{a}	0.72^{ab}	0.35^{ac}
		±0.59	±2.60	±1.29	±0.66	±1.48	±0.66	±1.11	±1.08	±0.45	±0.20	±0.30	±0.27	±0.11	±0.17	±0.21
	Cd + Vit. E	39.69^{a}	36.33^{ab}	37.13^{ac}	30.24^{a}	26.64^{ab}	28.45^{ac}	6.17^{a}	6.15^{ab}	5.91^{ac}	2.49^{a}	3.05^{ab}	2.39^{ac}	0.49^{a}	0.49^{ab}	0.39^{ac}
		±2.26	±0.88	±1.34	±2.29	±0.72	±0.73	±0.18	±0.79	±0.17	±0.03	±0.65	±0.63	±0.35	±0.34	±0.23
	Cd + Se	37.67^{a}	36.67^{ab}	38.13^{ac}	29.27^{a}	28.37^{ab}	28.74^{ac}	5.38^{a}	5.38^{ab}	6.74^{ac}	2.39^{a}	2.56^{ab}	2.02^{ac}	0.63^{a}	0.36^{ab}	0.63^{ac}
		±0.88	±0.41	±1.39	±1.36	±0.88	±1.27	±0.55	±0.14	±0.45	±0.36	±0.53	±0.05	±0.26	±0.34	±0.34
H.D.	Cd	43.40^{a}	34.33^{b}	38.33^{ac}	30.94^{a}	26.21^{ab}	28.77^{ab}	9.56^{a}	5.60^{b}	6.46^{c}	2.61^{a}	2.06^{ab}	2.33^{ac}	0.58^{a}	0.46^{ab}	0.77^{ac}
		±0.31	±0.18	±2.84	±1.11	±0.87	±2.35	±0.97	±0.27	±0.27	±0.75	±0.39	±0.39	±0.14	±0.11	±0.22
	Cd + Zn	36.07^{a}	34.53^{ab}	37.33^{ac}	27.87^{a}	26.00^{ab}	28.15^{ac}	6.24^{a}	5.67^{ab}	6.51^{ac}	1.47^{a}	2.49^{ab}	2.20^{ac}	0.49^{a}	0.37^{ab}	0.47^{ac}
		±1.44	±2.02	±1.20	±1.20	±1.44	±1.30	±0.60	±0.77	±1.04	±0.42	±0.29	±0.57	±0.11	±021	±0.31
	Cd + Vit. E	38.33^{a}	36.33^{ab}	36.00^{ac}	28.93^{a}	27.03^{ab}	27.24^{ac}	6.31^{a}	6.39^{ab}	7.10^{ac}	2.01^{a}	2.29^{ab}	2.02^{ac}	0.90^{a}	0.61^{ab}	0.63^{ac}
		±1.40	±0.88	±0.58	±1.41	±1.13	±0.45	±0.51	±0.38	±0.35	±0.66	±0.47	±0.09	±0.16	±0.33	±0.13
	Cd + Se	41.67^{a}	40.00^{ab}	37.91^{ac}	30.15^{a}	30.39^{ab}	28.98^{ac}	7.35^{a}	6.29^{ab}	6.61^{ac}	3.46^{a}	2.53^{ab}	2.16^{ac}	0.70^{a}	0.79^{ab}	0.52^{ac}
		±1.20	±1.15	±0.52	±1.21	±0.79	±1.37	±0.08	±0.64	±0.31	±0.32	±0.54	±0.09	±0.15	±0.21	±0.14
	LSD.	3.97	4.46	4.49	4.20	3.26	3.89	2.07	1.91	1.48	1.40	1.37	1.09	0.48	0.69	0.64

*Samples were taken 7 to 14 days during pregnancy. Values represent means ± standard error. Cd =cadmium, L.D. =low dose, H.D. =high dose, Zn =zinc, Se =selenium. Means with different superscripts (a, b, c) within row are significantly different at $p<0.05$

Difference between means in the same column higher than LSD indicates significance.

Table 5: Effect of different doses of cadmium given with and without antioxidants on plasma zinc concentrationin female rats at different intervals of the experiment

Parameters		Zinc (μ/dl)		
Periods Groups		During pregnancy	After birth (15 days)	After birth (30 days)
Control		248.56^{a}± 24.69	239.06^{ab}± 13.37	239.99^{ac}± 24.34
L.D.	Cd	204.69^{a}± 9.16	183.69^{ab}± 13.44	238.59^{ac}± 21.60
	Cd + Zn	242.19^{a}± 24.56	226.39^{ab}± 14.57	231.55^{ac}± 8.49
	Cd + Vit. E	250.44^{a}± 24.68	200.35^{ab}± 14.55	289.36^{ac}± 25.29
	Cd + Se	232.37^{a}± 14.29	199.77^{ab}± 8.18	233.19^{ac}± 10.22
H.D.	Cd	179.46^{a}± 10.05	175.13^{ab}± 3.88	183.11^{ac}± 7.56
	Cd + Zn	281.64^{a}± 34.08	213.96^{ab}± 14.44	219.35^{ac}± 26.56
	Cd + Vit. E	197.37^{a}± 8.82	187.68^{ab}± 6.70	183.58^{ac}± 5.29
	Cd + Se	233.67^{a}± 23.32	230.73^{ab}± 17.95	209.97^{ac}± 7.26
	LSD	6.47	36.32	49.90

*Samples were taken 7 to 14 days during pregnancy

Values represent means ± standard error. Cd =cadmium, L.D. =low dose, H.D. =high dose, Zn =zinc, Se =selenium.

Means with different superscripts (a, b, c) within row are significantly different at $p<0.05$

Difference between means in the same column higher than LSD indicates significance.

Table 6: Effect of different doses of cadmium given with and without selenium on the glutathione peroxidase level (u/l) 15 days after parturition in female rats as correlated with control

Groups	Control	L. D. Cd	L .D. Cd + Se	H. D. Cd	H. D. Cd + Se
Glutathione activity	2053.58^{a}± 46.52	1931.39^{a}± 20.79	2050.84^{a}± 69.34	1750.03^{b}±62.01	2061.61^{a}±43.74

Values represent means ± standard error (M ± S.E.) Cd =cadmium, L.D. =low dose, H.D. =high dose, Se =selenium.

Means with different superscripts (a, b) are significantly different at $p<0.05$.

Table 7: Effect of different doses of cadmium given with and without antioxidants on the cadmium level (ppm) in whole blood of female rats at different intervals of the experiments

		Periods		
Treatments		During pregnancy*	After birth (15 days)	After birth (30 days)
	Control	2	2.1	2.3
L.D.	Cd	2.9	3.3	3.4
	Cd +Zn	3	3.1	3.7
	Cd + Vit E	2.9	2.9	4.1
	Cd + Se	2	2.4	3
H.D.	Cd	3.1	3.9	4.2
	Cd + Zn	2.9	3.5	3.7
	Cd + Vit. E	3	3.7	4.1
	Cd + Se	2.8	2.9	3.3

Cd=cadmium, L.D. =low dose, H.D. =high dose, Zn =zinc, Se =selenium.

*Samples were taken at 7 to 14 days during pregnancy.

Values represent pooled samples

Data of red blood cells indices in Cd exposed rats revealed different types of apparent anaemia (Table 3).

Leucogram: Mean values of leucogram of pregnant rats received doses of cadmium chloride with or without antioxidants are illustrated in Table (4).

Results showed variable improvement concerning the different treatments with antioxidants (Table 4).

Plasma Biochemical Parameters

Zinc Concentration: As illustrated in Table (5), results revealed significant hypozincemia during pregnancy and 15 days after birth in the low dose Cd exposed group and allover the experimental intervals in the high dose group.

Assessment of the antioxidants effect in the low dose Cd exposed pregnant rats showed significant elevation of zinc during pregnancy, 15 and 30 days after birth in zinc and vit. E treated groups. Also, significant elevation of zinc during pregnancy was observed with selenium administration. In the high dose Cd groups, significant elevations were noticed in zinc and selenium treated groups during pregnancy and 15 days after birth. Also, significant increase was noticed with vit. E during pregnancy.

Glutathione Peroxidase Activity: The effect of cadmium exposure on glutathione peroxidase activity revealed significant decrease at 15 days after birth in the high dose Cd exposed group compared to control as shown in Table (6).

Assessment of the effect of selenium as an antioxidant revealed significant increase in the low and high dose Cd exposed groups.

Estimation of cadmium residue in whole blood of pregnant rats: As shown in Table (7), all the Cd-exposed groups were higher in cadmium level in whole blood of pregnant rats compared to control group. The results revealed that the high dose Cd treated group had the highest level in all groups whereas, low and high dose Cd groups that received selenium showed reduction in the cadmium level in blood.

DISCUSSION

In the present work, results of erythrogram showed significant decrease in the values of RBC's count, hemoglobin concentration and packed cell volume in low and high dose cadmium exposed groups. These findings are supported by observations of Horignchi *et al*. [15], Nemmiche *et al.* [16], Kanter *et al.* [5] and Cinar *et al.* [17]. In this respect, it was reported that cadmium intoxication induced oxidative stress and altered the antioxidant system, that may result in oxidative damage by inhibition of erythrocyte Na^+- K^+ATP ase leading to loss of cell membrane integrity and functions, shortened life span of erythrocyte and occurrence of anemia [18, 19]. Additionally, several studies suggested that Cd could may cause impairment of iron absorption leading to the decrease the haemoglobin synthesis and would contribute to anaemia [3].

Assessment of the effect of different antioxidants on the hemogram, Vitamin E and selenium behaved rather similar in their effect in low and high dose Cd exposed rats where significant improvement in RBC's count, packed cell volume and hemoglobin concentration were noticed. These findings are in agreement with many previous studies on different species recorded by Karmakar *et al.* [18], Ognjanovic *et al.* [4], El Demerdash [19], Nemmiche *et al.* [16] and Kanter *et al.* [5] in rats indicating that free radical scavengers and antioxidants such as vitamins C, A, ß carotene and E. are useful in protecting against cadmium toxicity. They added that presence of antioxidant with $CdCl_2$ alleviated harmful and toxic effects on hematological parameters and had a protective role in anaemia induced by Cd.

Ersteniuk [20] found that selenium counteracts the effect of cadmium poisoning by its protective effect on stabilization of metabolic processes in erythrocytes that prevent the development of oxidation stress and hypoxia. Results of leucogram of pregnant rats showed significant leucopenia due to lymphopenia followed by significant leucocytosis due to neutrophilia in low dose exposed group. In high dose, vice versa was occurred. The increase of leukocytic count may be due to inflammatory response to cadmium [21] and mainly as a result of neutrophilia [22, 23]. Moreover, El-Demerdash et al. [19] added that leucocytosis indicates an activation of the animal's immune system.

Effect of zinc and vitamin E on both low and high dose cadmium exposed groups showed significant leucopenia due to lymphopenia and neutropenia during pregnancy. While there was significant leucocytosis due to lymphocytosis in high dose cadmium treated with selenium. These results are in agreement with the findings. of El Demerdash *et al*. [19] in rats. This indicated that the antioxidants were positively effective in reducing the hazardous effects of $CdCl_2$ on the immune system through equilibrating the increase or decrease, into a stable normal range of total leucocytic count (TLC). In this respect, the most apparently effective treatment on cadmium was vitamin E.

Zinc concentration showed significant decrease in low and high dose cadmium exposed groups. These findings agree with Sowa and Steibert [24]. The administration of Cd could induce changes in the metabolism of essential minerals and hypozincemia could be attributed to degenerative changes in the liver [25]. On the other hand, significant hyperzincemia was evident in low dose cadmium exposed rats treated with zinc and vit.E. In this respect, zinc exhibits antioxidative properties during exposure to Cd through its ability to interact with essential elements such as Cu and Fe decreasing their content in tissues and retarding the oxidative processes [26]. This trace element prevents reactive oxygen species (ROS) production and is involved in cell membrane stabilization, metallothionin (Mt) synthesis and superoxide dismutase (Cu/Zn SOD) structure [25].

In the present study, glutathione peroxidase activity showed significant decrease in the high dose cadmium exposed group. Similar results were obtained in blood of rats [27, 28] liver and kidney [16, 29-31] of rats after Cd administration . The decreased activity of the GSH-Px may result from a direct depletion of selenium by Cd or from insufficient incorporation of sulphur containing amino acids into GSH-Px because of competition with the

Cd –metallothioniens synthesis [2, 17, 31]. Moreover, Cd inhibits the activities of many enzymes by binding to their sulfhydryl groups or by inhibiting the protein synthesis [32] On the other hand, Jemai *et al*. [26] found that Cd had no effect on GSH-Px activity. Meanwhile, Ognjanovic *et al*. [4] and Kanter *et al*. [5] observed significant increase of RBCs GSH-Px in Cd treated rats. It is known that Cd induces the formation of superoxide anion radical in RBCs and it is reasonable to expect an increased activity of SOD [3, 28]. Cd induced an increase in CAT and GSH-Px activities which may be explained by their influence on hydrogen peroxide as substrate which is formed in the process of dismutation of superoxide anion radical [2, 32].

GSH-Px activity showed significant increase in high dose cadmium with selenium-exposed rats. Similar observations were reported by Ognjanovic *et al.* [31] who mentioned that treatment with selenium eliminates the toxic effects of cadmium on the activity of GSH-Px and prevents lipid peroxidation and oxidative damage induced by Cd. This protection includes the capability of Se to alter the distribution of Cd in tissues and to induce binding of Cd-Se complexes to proteins which are similar to metallothioneins. Also, it has been reported that selenium supplementation increases the activities of selenium-dependent antioxidant enzymes such as GSH-Px and TrxR that could be due to increased incorporation of selenocysteine which is essential for their activities [30].

Concerning cadmium residue, all the Cd exposed groups were higher in cadmium level in whole blood of mothers compared to control. These results are in accordance with Oishi *et al.* [33] and Shata *et al.* [34]. It has been reported that pregnancy could mobilize the hepatic cadmium, which can be transferred to the placenta through the blood plasma Chan and Cherian [35]. These findings simulate that described by Tandon *et al.* [36] who found that the accumulation of Cd in blood was decreased significantly upon co-exposure to vitamin E. The antioxidant property of vitamin E seems to be responsible for the observed protection of Cd intoxication. Shaikh *et al.* [32] reported that zinc antagonizes cellular cadmium transport and accumulation by competing for binding to sulfhydryl ligands. Pretreatment with zinc can decrease cadmium concentrations in dams' blood [34, 37-39]. In addition, it reduces cadmium in blood through its excretion in the urine.

It could be concluded that from the present results that cadmium induced damage in erythrocytic cells (had a toxic effect on the erythrocytic parameters) resulting in occurence of anaemia .Treatment with antioxidants vit E. selenium and zinc expressed (exerted) protective role against the harmful effect of Cd through improving the blood parameters, zinc level glutathion peroxidase activity. Selenium administration appeared more efficient against Cd exposure.

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