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Vitamin E and/or Wheat Germ Oil Supplementation Ameliorate Oxidative Stress Induced by Cadmium Chloride in Pregnant Rats and Their Fetuses

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Abstract

The present study aims to investigate the protective effect of Vit. E and/or WGO against Cd induced toxicity in pregnant rats and their fetuses. Thirty pregnant rats were divided into five groups; Control, CdCl2, CdCl2+Vit. E, CdCl2+WGO and CdCl2+Vit. E+WGO. Oral administration of CdCl2 caused impairment in the hematological parameters as indicated by significant ($P<0.05$) decrease in RBCs, Hb, Hct, PLt s and WBCs in maternal rats and their fetuses. CdCl2 administration caused disturbances in the hepatic and renal functions as reflected by significant ($P<0.05$) increase in ALT, AST, creatinine and urea. Also, CdCl2 administration caused an oxidative stress in the liver and kidney tissues of maternal rats and their fetuses. In addition, CdCl2 induced growth retardations as observed by significant ($P<0.05$) depletion in fetal body weight, length and the number of alive fetuses and significant ($P<0.05$) increase in the number of dead, absorbed and malformed fetuses. The pronounced abnormalities were: exencephaly, exophthalmia, open eyelids, microtia, short tail, short fore and hind limbs, umbilical hernia, and curvature in the vertebral column as compared to control group. The presence of Vit. E and/or WGO with CdCl2 improved the all examined parameters. These natural substances could exhibit a protective effect in preventing physiological alterations and fetal malformations due to their potent antioxidant properties.

Keywords: Cadmium, vitamin E, wheat germ oil, pregnant rats, fetuses.

1. Introduction

Cadmium (Cd) is one of the major occupational and environmental pollutants. Human exposure to Cd occurs chiefly through inhalation or ingestion. Cadmium is considerably toxic with destructive impacts on most organ systems such as respiratory, digestive, reproductive, skeletal and cardiovascular systems and some sensitive organs, including liver and kidney (Jama *et al*., 2013). In addition, Cd induced malformations of the neural tube, craniofacial region, limbs, trunk, viscera, and axial skeleton in fetuses when administered during gestation (EL-Sayed *et al.*, 2013). Cd acts as a stimulator for formation of Reactive Oxygen Species (ROS), hydrogen peroxide also, hydroxyl radicals. These free radicals, enhance lipid peroxidation, DNA damage, altered calcium and sulfhydryl homeostasis (Sevcikova *et al*., 2011).

Antioxidants are substances that protect cells against the adverse effects of xenobiotics, toxicants, drugs and carcinogens. These antioxidants, such as vitamin C, vitamin E, omega-3 fatty acid and wheat germ oil, can be supplemented through diet and have been utilized in a prophylactic manner against toxic substances induced oxidative stress (Aboubakr *et al*., 2014).

Vitamin E (Vit. E) is a fat-soluble antioxidant. It plays an important role in retarding the pathogenesis of different decadence diseases; cancer, inflammatory diseases, neurological disorders and chronic vascular diseases through its function of inhibiting free radical-mediated tissue damage. It is essential for the development of early embryos during implantation as well as for the protection of the fetus against oxidative damage (Wilkinson *et al.*, 2005; Traber & Manor, 2012).

Wheat Germ Oil (WGO) is unique among dietary supplements. It contains some B complex vitamins (B_6, B_{12} and folic acid) that are essential in the formation of red blood cells. It was claimed to be anti-inflammatory and described as a suitable natural antioxidant due to its high content of Vit. E. WGO acts as a protection against oxidative stress appeared to be mediated through decreasing the pro-oxidants and enhancement of cellular antioxidant activities. WGO is rich in unsaturated fatty acids, mainly oleic, α-linoleic and functional phytochemicals mainly flavonoids, sterols, octacosanols and glutathione (Alessandri *et al.*, 2006). It also has a

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number of nutritional and health useful, improving physical fitness and probably retarding effects of aging (Megahed, 2011).

The present work is designed to evaluate the possible protective effects of vitamin E and/or wheat germ oil against cadmium chloride – induced toxicity in pregnant rats and their fetuses.

2. Materials And Methods

2.1. Chemicals

Cadmium chloride anhydrous (98%-Cd pure white powder, 100 g package), vitamin E (α- tocopherol acetate) and wheat germ oil were obtained from Kahira Pharma and Chem. Ind. Co. (Cairo–Egypt) in the form of soft, gelatinous capsules.

2.2. Experimental Animals

Sexually mature female albino rats weighing from 200-210 g were obtained from Faculty of Medicine, Alexandria University, Alexandria, Egypt. Rats were kept on basal diet and tap water *ad libitum*. They were acclimated under controlled environmental conditions at room temperature (25±2ºC) with humidity (50±10%) and a 12h light/dark cycle. For mating purposes, four females were housed overnight with two males starting at 21:00 h. Females were checked by 7:00 h the next morning, and the presence of a vaginal plug was designated as gestational day zero. The experiments and the protocol were carried out according to the guidelines of the National Institutes of Health (NIH).

2.3. Animal Groups

Thirty pregnant rats were divided randomly into five groups, six per each group as follows:

Control group: Each pregnant rat was orally received distilled water and 0.5 ml corn oil as a vehicle.

Cadmium chloride–treated group: Each pregnant rat was orally received cadmium chloride at a dose 5 mg/kg BW/day (1/20 LD_{50}) (ATSDR, 2008).

Cadmium chloride+vitamin E–treated group: Each pregnant rat was orally received cadmium chloride at a dose 5 mg/kg BW/day and intraperitoneally (IP) with vitamin E at a dose 100 mg/kg BW/day (Mahabady and Varzi, 2011).

Cadmium chloride+wheat germ oil–treated group: Each pregnant rat was orally received cadmium chloride at a dose 5 mg/kg BW/day and wheat germ oil at a dose 54 mg/kg BW/day (Reddy *et al*., 2000).

Cadmium chloride+vitamin E+wheat germ oil-treated group: Each pregnant rat was orally received cadmium chloride at a dose 5 mg/kg BW/day and intraperitoneally (IP) with vitamin E at a dose 100 mg/kg BW/day and orally with wheat germ oil at a dose 54 mg/kg BW/day.

All groups were treated with different treatments for 13 days from 6^{th} to 18^{th} day of gestation.

2.4. Maternal and Fetal Endpoints

All of the pregnant rats were sacrificed by ether anesthesia at the 19^{th} day of gestation and fetuses were removed from the uterus. The implantation sites, corpora lutea, living, dead and reabsorbed fetuses were counted. Live fetuses were weighed, and photographed by HD digital camera (Samsung 10x) for evaluating externally visible abnormalities, according to the technique of Wilson's (1978).

2.5. Examination of the Fetus Gross Morphology and the Skeleton

After a brief autopsy one and half of the fetuses were fixed in 10% formalin, examined under stereomicroscope for the occurrence of any malformation. Fetuses were examined for skeletal malformation through two procedures for skeletal staining according to the method of McLeod (1980).

2.6. Collection and Preparation of Blood Samples

Blood samples were collected from anesthetized mother and their fetuses into sterile tubes. The first part of blood was collected in tubes containing EDTA for determination of hematological parameters (RBCs, Hb, Hct, platelet counts and WBCs). The second part allowed to clot and centrifuged at 3000 rpm for 20 min. Serum was separated and stored at -20°C for determination of some biochemical parameters.

2.7. Biochemical Parameters

Determination of serum aspartate aminotransferase (AST; EC 2.6.1.1) and alanine aminotransferase (ALT; EC 2.6.1.2) were estimated using kits from Sentinel Ch. (Via principle Eiagen 5-20155 kit, Milano, Italy) according to Reitman and Frankel (1957) method. Serum creatinine and urea were estimated by using the methods of Bowers and Wong (1980) and Fawcett and Scott (1960), respectively. Liver and kidney MDA were measured as Thiobarbituric Acid Reactive Substance (TBARS) (Ohkawa *et al*., 1979). Also, the levels of reduced GSH (Beulter *et al*., 1963) and the antioxidant enzyme activities, including the catalase (CAT; EC 1.11.1.6) (Aebi, 1984), superoxide dismutase (SOD; EC 1.15.1.1) (Nishikimi *et al*., 1972) and glutathione peroxidase (GPx; EC 1.1.1.9) (Chiu *et al*., 1976) were assayed.

2.8. Statistical Analysis

The results were analyzed using the SPSS computer software package version 19.0 (Chicago, IL, USA). Data were presented as mean±SE. Data were evaluated by one-way ANOVA followed by Least Significant Difference (LSD). Values were considered statistically significant at $P<0.05$.

3. Results

3.1. Effect of Cdcl2, Vit. E, WGO and/or Their Combinations on the Values of RBCs, Hb, Hct, PLts and WBCs in Maternal and Their Fetuses

$CdCl_2$–maternal treated rats and their fetuses showed significant ($P<0.05$) decrease in the values of RBCs, Hb, Hct, PLt s and WBCs as compared to control. While, the administration of Vit. E and/or WGO with $CdCl_2$ showed a significant increase ($P<0.05$) in the measured hematological parameters as compared to $CdCl_2$–maternal treated rats and their fetuses (Table 1).

Table 1: Effect of $CdCl_2$, Vit. E, WGO and/or their combination, on the values of RBCs, Hb, Hct, PLt s and WBCs in maternal and their fetuses

Parameters / Groups	RBCs ($\times10^6$cell/µl)		Hb (g/dl)		Hct (%)		PLt ($\times10^3$ cell /µl)		WBCs ($\times10^3$cell/µl)	
	Mother	Fetus	Mother	Fetus	Mother	Fetus	Mother	Fetus	Mother	Fetus
Control	4.52 ±0.26	4.60 ±0.16	13.70 ±0.22	13.54 ±0.62	45.33 ±1.53	44.80 ±2.86	283.40 ±26.42	408.60 ±10.95	8.72 ±1.02	13.98 ±3.56
$CdCl_2$	2.74 ±0.63^{a}	1.54 ±0.11^{a}	6.44 ±0.66^{a}	4.34 ±0.27^{a}	23.60 ±4.98^{a}	16.80 ±2.17^{a}	222.80 ±16.38^{a}	167.40 ±22.14^{a}	4.60 ±1.14^{a}	5.20 ±0.87^{a}
$CdCl_2$+Vit. E	4.02 ±0.37^{bc}	4.10 ±0.25^{bc}	11.44 ±0.81^{ab}	11.72 ±0.39^{abc}	38.80 ±3.56^{ab}	35.80 ±2.59^{abc}	300.40 ±17.11^{bc}	262.20 ±22.11^{ab}	6.96 ±1.29^{ab}	6.50 ±1.32^{ab}
$CdCl_2$+WGO	3.28 ±0.13^{ab}	3.14 ±0.21^{ab}	9.86 ±0.23^{ab}	9.44 ±0.65^{ab}	30.80 ±1.92^{ab}	25.40 ±3.65^{ab}	288.20 ±13.55^{b}	185.80 ±23.33^{ab}	10.36 ±1.03^{abc}	6.94 ±0.22^{ab}
$CdCl_2$+Vit.E+WGO	4.40 ±0.25^{bc}	3.99 ±0.36^{ab}	12.50 ±0.91^{bc}	11.30 ±0.63^{ab}	40.40 ±4.34^{bc}	35.20 ±3.35^{ab}	303.40 ±17.97^{bc}	337.00 ±27.12^{abc}	8.55 ±0.62^{ab}	8.68 ±1.11^{abc}

Values are expressed as mean ±S.E., n=6 for each group. Mean values within column not sharing common superscript letters (a, b, c) were significantly different (P< 0.05)

3.2. Effect of CdCl2, Vit. E, WGO and/or Their Combinations on the Serum Activities of AST and ALT in Maternal and Their Fetuses

Results presented in Table 2 showed a significant increase in the ALT and AST serum activities, in cadmium-maternal treated group and their fetuses when compared with the control group. While, administration of Vit. E and/or WGO with $CdCl_2$ showed significant (P<0.05) decrease in the activities of AST and ALT as compared to $CdCl_2$-maternal treated rats and their fetuses.

Table 2: Effect of $CdCl_2$, Vit. E, WGO and/or their combination on the serum activities of AST and ALT in maternal and their fetuses

Parameters / Groups	AST (U/L)		ALT (U/L)	
	Mother	Fetus	Mother	Fetus
Control	62.92±1.89	43.83±3.54	41.58±4.45	37.50±3.27
$CdCl_2$	378.85±22.11^{a}	72.00±1.90^{a}	286.33±10.05^{a}	78.50±2.59^{a}
$CdCl_2$+ Vit. E	200.03±5.80^{ab}	50.08±2.13^{ab}	161.92±1.88^{ab}	38.87±0.77^{bc}
$CdCl_2$+ WGO	191.17±4.12^{abc}	47.08±4.96^{ab}	116.67±2.07^{ab}	63.00±3.80^{ab}
$CdCl_2$+ Vit. E+ WGO	100.38±4.33^{ab}	38.00±1.10^{abc}	57.92±6.33^{abc}	45.30±4.79^{ab}

Values are expressed as mean±S.E. n=6 for each group. Mean values within column not sharing common superscript letters (a, b, c) were significantly different (P < 0.05).

3.3. Effect of CdCl2, Vit. E, WGO and/or Their Combination on the Serum Contents of Urea and Creatinine in Maternal and Their Fetuses

Table 3 indicates that the levels of serum urea and creatinine in the serum of maternal and their fetuses were significantly (P<0.05) increased in $CdCl_2$– treated group as compared to the control group. In contrast, the administration of Vit.E and/or WGO with $CdCl_2$ caused a significant decline (P<0.05) in the serum concentrations of urea and creatinine in comparison with $CdCl_2$–maternal treated rats and their fetuses.

Table 3: Effect of $CdCl_2$, Vit. E, WGO and/or their combination on the serum concentrations of urea and creatinine in maternal and their fetuses

Parameters / Groups	Urea (mg/dl)		Creatinine (mg/dl)	
	Mother	Fetus	Mother	Fetus
Control	47.07±3.04	46.71±4.45	0.37±0.15	0.42±0.074
$CdCl_2$	78.67±5.47^{a}	65.50±2.81^{a}	0.91±0.12^{a}	0.93±0.08^{a}
$CdCl_2$+ Vit. E	54.67±2.07^{ab}	52.00±1.68^{ab}	0.63±0.18^{ab}	0.50±0.14^{b}
$CdCl_2$+ WGO	55.00±3.52^{ab}	50.00±0.64^{ab}	0.68±0.15^{ab}	0.70±0.14^{ab}
$CdCl_2$+ Vit. E+ WGO	41.72±4.21^{bc}	37.97±2.28^{bc}	0.33±0.16^{bc}	0.32±0.16^{bc}

Values are expressed as mean ±S.E. n =6 for each group. Mean values within column not sharing common superscript letters (a, b, c) were significantly different (p <0.05)

3.4. Effect of CdCl2, Vit. E, WGO and/or Their Combination on the Levels of Liver and Kidney MDA and GSH in Maternal and Their Fetuses

The levels of MDA in the liver and kidney of maternal and their fetuses were significantly incremented (P<0.05) after administration of $CdCl_2$ as compared to the control group (Tables 4&5). While, the levels of hepatic and renal GSH were significantly declined (P<0.05) in $CdCl_2$–maternal treated group and their fetuses compared to control group. Meanwhile, the MDA and GSH levels in $CdCl_2$+Vit. E, $CdCl_2$+WGO and $CdCl_2$+Vit. E+WGO–treated groups were significantly improved in the liver and kidney tissues as compared to $CdCl_2$–maternal treated rats and their fetuses.

Table 4: Effect of $CdCl_2$, Vit. E, WGO and/or their combination on the levels of liver MDA and GSH in maternal and their fetuses

Parameters / Groups	Liver			
	MDA (µmol/ g tissue)		GSH (U/g tissue)	
	Mother	Fetus	Mother	Fetus
Control	36.13±2.89	19.72±1.60	71.50±2.22	53.00±3.35
$CdCl_2$	112.34±10.89^{a}	52.95±3.77^{a}	12.50±0.96^{a}	30.67±1.12^{a}
$CdCl_2$+ Vit. E	67.17±3.82^{ab}	30.00±0.64^{ab}	36.50±3.48^{ab}	57.00±1.90^{bc}
$CdCl_2$+ WGO	70.80±4.21^{ab}	30.33±2.80^{ab}	38.50±1.59^{ab}	42.00±1.90^{ab}
$CdCl_2$+ Vit. E+ WGO	48.00±0.64^{abc}	29.17±0.71^{ab}	72.50±1.59^{bc}	48.30±1.94^{ab}

Values are expressed as mean ±S.E. n=6 for each group. Mean values within column not sharing common superscript letters (a, b, c) were significantly different ($P < 0.05$)

Table 5: Effect of $CdCl_2$, Vit. E, WGO and/or their combination on the levels of kidney MDA and GSH in maternal and their fetuses

Parameters / Groups	Kidney			
	MDA (µmol/g tissue)		GSH (U/g tissue)	
	Mother	Fetus	Mother	Fetus
Control	36.00±2.53	28.30±1.94	61.00±1.27	65.51±0.35
$CdCl_2$	200.42±6.01^{a}	72.25±2.88^{a}	13.00±1.68^{a}	24.08±2.64^{a}
$CdCl_2$+ Vit. E	94.67±5.90^{ab}	49.00±1.27^{ab}	37.50±2.85^{ab}	44.50±2.85^{ab}
$CdCl_2$+ WGO	72.50±1.59^{ab}	37.67±3.01^{ab}	49.50±1.59^{ab}	50.75±1.14^{ab}
$CdCl_2$+ Vit. E+ WGO	51.00±1.90^{abc}	28.17±0.76^{bc}	59.00±3.16^{bc}	61.33±3.67^{bc}

Values are expressed as mean ±S.E. n =6 for each group. Mean values within column not sharing common superscript letters (a, b, c) were significantly different ($P < 0.05$)

3.5. Effect of CdCl2, Vit. E, WGO and/or Their Combination on the Activities of Liver and Kidney SOD, CAT and GPx in Maternal and Their Fetuses

The activities of SOD, CAT and GPx in the liver and kidney of $CdCl_2$-maternal treated group and their fetuses were significantly declined ($P<0.05$) in comparison with the control group (Tables 6&7). Interestingly, the activities of SOD, CAT and GPx were significantly elevated ($P<0.05$) in $CdCl_2$+Vit. E, $CdCl_2$+WGO and $CdCl_2$+Vit. E+WGO–treated groups, when compared with the $CdCl_2$–maternally treated rats and their fetuses.

Table 6: Effect of $CdCl_2$, Vit. E, WGO and/or their combination on the activities of liver SOD, CAT and GPx in maternal and their fetuses.

Parameters / Groups	Liver					
	SOD (U/mg protein)		CAT (U/mg protein)		GPx (U/mg protein)	
	Mother	Fetus	Mother	Fetus	Mother	Fetus
Control	83.63±5.53	80.30±3.49	55.03±5.18	49.30±3.71	61.00±1.27	38.63±2.99
$CdCl_2$	20.00±1.38^{a}	31.67±3.01^{a}	22.63±3.49^{a}	25.92±3.18^{a}	6.53±1.00^{a}	10.33±0.81^{a}
$CdCl_2$+Vit. E	49.08±1.72^{ab}	46.00±3.80^{ab}	50.25±2.30^{bc}	44.58±4.14^{bc}	27.20±2.17^{ab}	30.50±0.34^{ab}
$CdCl_2$+WGO	35.33±2.66^{ab}	40.00±2.53^{ab}	46.33±2.66^{ab}	35.00±2.53^{ab}	30.17±2.41^{ab}	33.50±3.48^{ab}
$CdCl_2$+Vit.E+WGO	65.50±2.85^{abc}	72.42±1.60^{abc}	58.87±3.90^{bc}	43.30±4.12^{ab}	49.50±3.42^{abc}	51.03±5.18^{abc}

Values are expressed as mean ±S.E. n =6 for each group. Mean values within column not sharing common superscript letters (a,b,c) were significantly different ($P<0.05$)

Table 7: Effect of $CdCl_2$, Vit. E, WGO and/or their combination on the activities of kidney SOD, CAT and GPx in maternal and their fetuses

Parameters / Groups	Kidney					
	SOD (U/mg protein)		CAT (U/mg protein)		GPx (U/mg protein)	
	Mothers	Fetuses	Mothers	Fetuses	Mothers	Fetuses
Control	88.63±4.83	49.00±2.53	52.80±2.54	47.53±4.58	40.80±1.69	48.63±2.23
$CdCl_2$	17.30±1.94^{a}	22.83±3.56^{a}	29.28±5.38^{a}	39.83±2.45^{a}	6.50±0.96^{a}	7.50±0.34^{a}
$CdCl_2$+ Vit. E	49.00±1.42^{ab}	48.00±1.90^{bc}	42.00±4.15^{ab}	40.50±0.96^{ab}	38.08±1.89^{b}	46.33±2.96^{bc}
$CdCl_2$+ WGO	39.58±0.40^{ab}	39.50±0.34^{ab}	38.00±1.27^{ab}	44.42±4.14^{b}	35.32±3.12^{ab}	28.30±2.86^{ab}
$CdCl_2$+Vit. E+WGO	89.58±3.63^{bc}	50.00±3.80^{bc}	59.58±0.40^{bc}	50.67±1.60^{bc}	45.83±3.43^{abc}	44.50±2.85^{bc}

Values are expressed as mean ±S.E. n =6 for each group. Mean values within column not sharing common superscript letters (a, b, c) were significantly different ($P<0.05$)

3.6. Effect of CdCl2, Vit. E, WGO and/or Their Combinations on the Number and Size of Implanted Fetuses

The uterus of the pregnant rats treated with $CdCl_2$ showed diminution in the number and size of implanted fetuses in addition to fetal absorption sites as compared to the uteri of control group (Figure 1: A & B, Table 8). In contrast, the uterus of the pregnant rats treated with Vit E and/or WGO showed the nearly normal appearance (Figure 1: C, D & E). The fetus body weight and length significantly (P<0.05) decreased compared to those of the control group. While, the body weight and length significantly increased (P< 0.05) in $CdCl_2$+Vit. E, $CdCl_2$ +WGO and $CdCl_2$+Vit.E+WGO-treated groups as compared to those of the fetus of $CdCl_2$- maternal treated rats (Table 8).

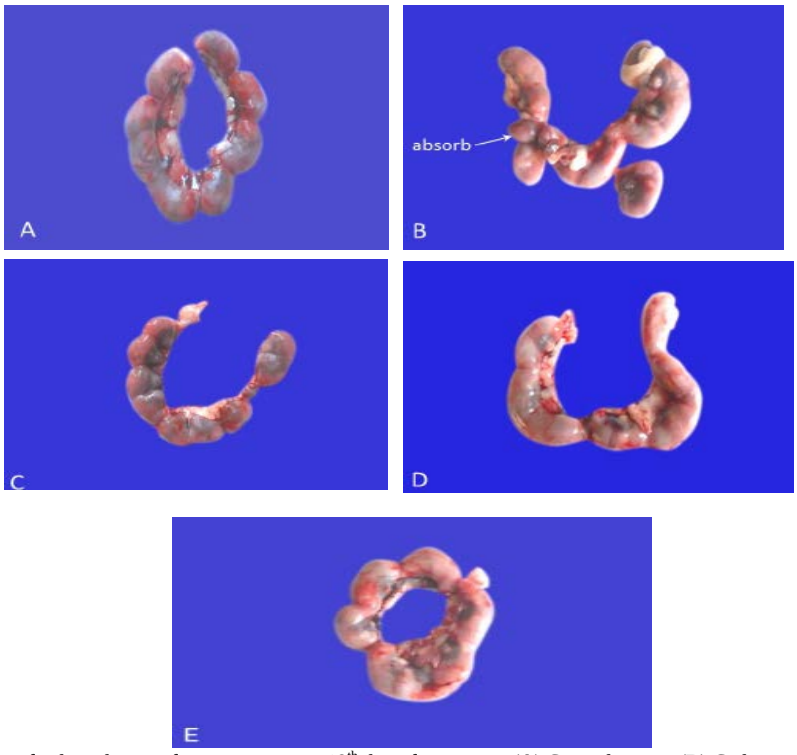


Figure (1): Photographs of selected uteri of pregnant rats on 19th day of gestation**: (A)** Control group. **(B)** Cadmium chloride $CdCl_2$ – treated group, showed uterine malformation and absorbed fetus (white arrow) **(C)** $CdCl_2$ + Vit.E - treated group. **(D)** $CdCl_2$+ WGO - treated group. **(E)** $CdCl_2$+Vit.E + WGO - treated group. Notice: C, D & E showed more or less normal appearance of the fetuses in the uterus

Table 8: Effect of $CdCl_2$, Vit. E, WGO and/or their combination on the number of alive, dead, absorbed, malformed, body weight and body length of fetuses on the 19th day of gestation

Parameters / Groups	Fetuses on the 19th day of gestation						
	No. of alive fetuses	No. of Dead fetuses	No. of absorbed fetuses	Malformed fetuses No.	%	Body weight (g)	Body length (Cm)
Control	46	-	-	-	-	4.60±0.40	5.10±0.20
$CdCl_2$	31	8	5	18	56. 25	2.20±0.40^{a}	3.10±0.20^{a}
$CdCl_2$+Vit. E	45	-	-	-	-	3.80±0.30^{ab}	3.90±0.30^{ab}
$CdCl_2$+WGO	43	-	-	-	-	4.40±0.50^{abc}	4.40±0.30^{abc}
$CdCl_2$+Vit. E+WGO	44	-	-	-	-	4.20±0.50^{abc}	4.90±0.50^{abc}

Values are expressed as mean ±S.E. n =6 for each group. Mean values within column not sharing common superscript letters (a, b, c) were significantly different (P<0.05)

3.7. Effect of CdCl2, Vit. E, WGO and/or Their Combinations on the Morphological Characters of Fetuses

On the 19th day of gestation, the normal fetus slightly appeared with straight body in both back and neck regions (Figure 2: A). The head appeared straight and small in size in relation to the whole body. The eyes were closed with upper and lower eyelids and the external auditory canal was completely invisible, being covered by the well developed ear pinna. In those fetuses, different regions of both fore and hind limbs appeared with well-developed structures and their extremities showed the distinctive number of digits with clear demarcated phalanges. The abdominal region of normal fetuses displayed a cylindrical shape ending with the tail (Figure 2: A).

While, the external malformations in fetuses maternally treated with cadmium chloride were observed in figures 2 (B & B1) 3 (A & B) and 4 (A, B, C & D). The pronounced abnormalities were: exencephaly, exophthalmia, open eyelids, microtia, short tail, short fore and hind limbs, umbilical hernia, and curvature in the vertebral column as compared to control group. In contrast, administration of Vit. E, WGO and/or their combination with $CdCl_2$ showed amelioration in such external abnormalities compared to $CdCl_2$- treated group.

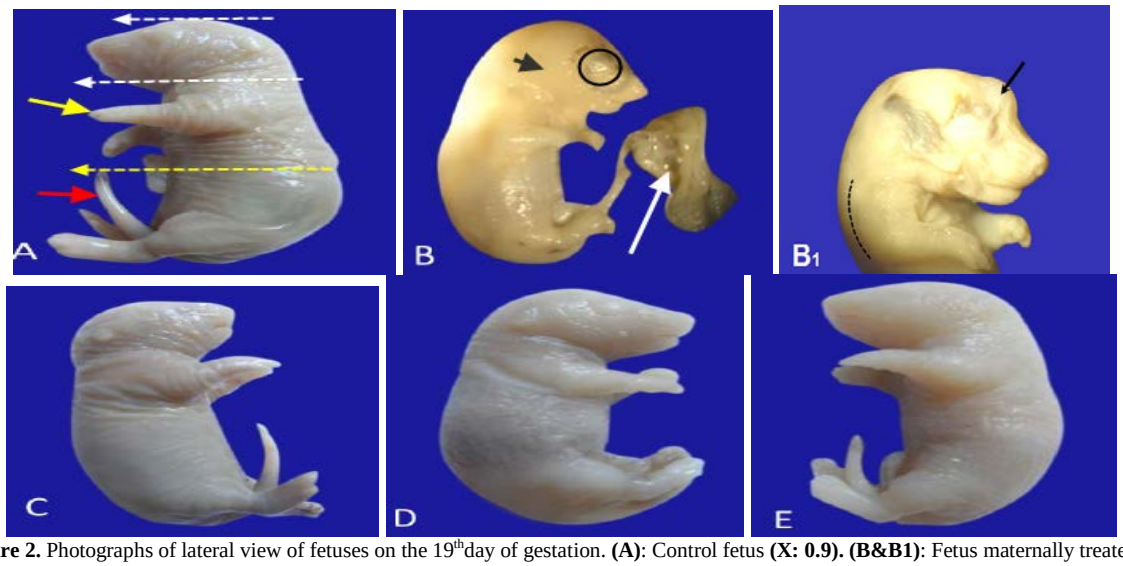
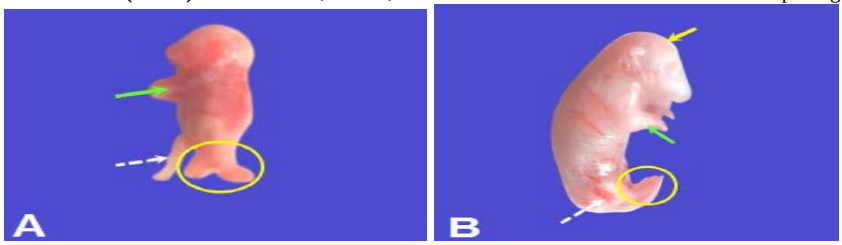
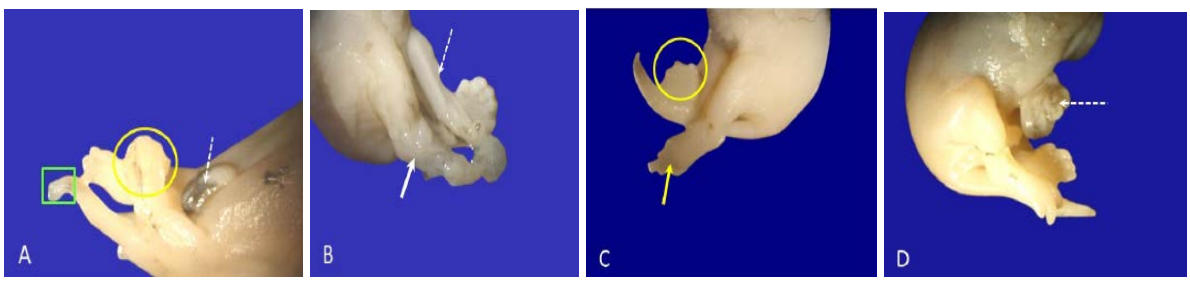


Figure 2. Photographs of lateral view of fetuses on the 19thday of gestation. **(A)**: Control fetus **(X: 0.9). (B&B1)**: Fetus maternally treated with CdCl2 showed growth retardation, exophthalmia and open eyelid (black circle) microtia (arrow head), short fore and hind limbs and umbilical hernia (white arrow), exencephaly (black arrow) and abnormal bending of the body (dotted black line) **(X: 1.4&1.6)** respectively**. (C):** Fetus maternally treated with CdCl2 + Vit. E **(X: 0.9). (D):** Fetus maternally treated with CdCl2+ WGO **(X: 0.9). (E):** Fetus maternally treated with CdCl2+ WGO +Vit. E **(X: 0.9).** Notice: In C, D & E; the fetuses more or less have a normal morphological appearance.



Figures (3): Photographs of fetuses maternally treated with cadmium chloride on the 19thday of gestation showing gross morphology. **(A)** Fetus shows atrophy in the forelimb (green arrow), umbilical hernia (dotted white arrow) and syndactayly (yellow circle) **(X:1.2). (B)** Fetus shows exencephaly (yellow arrow), short fore limbs (green arrow), atrophy in left hind limb (dotted white arrow), right hind limb club foot (yellow circle) and slight bending of the body **(X:1.3)**



Figures (4): Photographs of fetuses maternally treated with CdCl2 on the 19th day of gestation, showing different types of malformations in the posterior region **(X:1.5). (A)** Umbilical hernia (dotted white arrow), syndactayly (yellow circle), kinky tail (green square). **(B)** Abnormal hind limb without distinct toes (white arrow). **(C)** Abnormality of the foot region (yellow arrow). **(D)** Umbilical hernia (dotted white arrow)

4. Discussion

Cadmium is one of the most dangerous occupations and environmental toxins. It promotes an early oxidative stress and contributes to the development of serious pathological conditions (Jama *et al.*, 2013). Furthermore, cadmium has been shown to be both embryotoxic and teratogenic in different animal species, take place as a consequence of cadmium given in early pregnancy (EL-Sayed *et al.*, 2013).

The present anemic status could be attributed to a reduction in the rate of the erythrocyte formation through hypo-induction of erythropoietin in the kidneys after long-term of cadmium exposure (Onwuka *et al*., 2010). Furthermore, cadmium intoxication occurred by loss of cell membrane integrity, shortened life span of erythrocytes and occurrence of anemia (Attia *et al*., 2013). These findings were consistent with a previous study by Goncalves *et al.* (2009) who reported that treatment with cadmium chloride caused a reduction in hematological parameters as a result of erythrocyte destruction. Exposure to Cd Cl_2 induced oxidative damage of erythrocytes leading to an observation of normocytic normochromatic anemia and lymphopenia (Hassan *et al.*, 2012). Al-Asgah *et al.* (2015) reported that the decrease in WBCs count might be the consequence of Cd-induced lipid peroxidation and damage of their cell membrane. The liver, spleen and bone marrow are the major hematopoietic organs which are considered as targets of Cd exposure (Amara *et al.*, 2008). Moreover, cadmium may inhibit heme synthesis by decreasing the absorption of iron from the gastrointestinal tract (Elsharkawy and El-Nisr, 2012).

The improvement of hematological parameters in Vit. E and/or WGO treated groups might be due to increase the coronary and peripheral blood circulation also, vitamin E is a highly effective fat-soluble vitamin with a variety of cellular membrane stabilizing-antioxidant functions. Vitamin E has been suggested to prevent the oxidation of polyunsaturated fatty acids in Red Blood Cell (RBC) membrane, thus inhibiting the premature erythrocytelysis. Animal studies have shown that treatment with vitamin E enhanced erythropoiesis and improved blood hemoglobin levels in these animals (Jilani and Iqbal, 2011) Moreover, wheat germ oil contains some B complex vitamins (B_6, B_{12} and folic acid) which are essential for the formation of red blood cells and acts as anti-inflammatory (Abdel-Fattah *et al.*, 2011).

The increase in serum AST and ALT activities of maternal and their fetuses reflected the disturbances in hepatic function after cadmium administration during the gestation period. These results were in agreement with the result of Heydamejad *et al*. (2013). The main mechanism involved in Cd hepatotoxicity, its binding to sulfhydryl group in mitochondria and the initiation of inflammation. Also, oxidative stress, due to decrease in antioxidative capacity, plays a role in chronic Cd hepatotoxicity. Khalifa *et al*. (2011) stated that the results of the his study indicated that Wheat Germ Oil (WGO) significantly reduced the toxic effects of chlorpyrifos by altering the hepatic enzyme activities and thus they can be considered a potential hepatoprotective agent in conditions of organophosphate poisoning.

The elevation in serum concentrations of urea and creatinine in maternal and their fetuses of Cd –treated group could be considered as a reflection of deteriorating renal performance. Chronic Cd exposure can cause renal proximal tubular dysfunction resulting from the release of Cd Metallothionein (MT) from the liver and its accumulation and degradation in the renal tubular epithelial cells, inducing proximal apoptosis in different cell types (Tarasub *et al*., 2011). Buha *et al*. (2012) reported that cadmium was highly accumulated in kidney of animals exposed to it via oral routes. These results were in the same line with Wang *et al.* (2010) who suggested that the elevation of urea and creatinine concentrations could be attributed to the degenerative changes in the lining epithelial cells of renal tubules.

This indicated that vitamin E and wheat germ oil significantly reduce the toxic effect of cadmium by improving the hepatic enzyme activities in serum and thus can be considered a potential hepatoprotective agent (Ahmed *et al*., 2013) These results were in agreement with Megahed (2011) who demonstrated that the supplementation of vitamin E and wheat germ oil caused an improvement in the levels of creatinine and urea as well as AST and ALT activities.

The elevation in MDA and decline in GSH levels in liver and kidney of the $CdCl_2$ maternal treatment and their fetuses might be attributed to cadmium induced oxidative stress in tissues by increasing lipid peroxidation and altering the antioxidant status in liver and kidney tissues (Rajasekaran and Periasamy, 2012). The depletion of cellular glutathione could be explained through the exhaustive use of GSH in conjugation to cadmium catalyzed by Glutathione-S-Transferases (GST) (Sarkar *et al*., 2013).

In general, mechanisms by which Cd can induce oxidative stress through free radicals over production and the disruption of the mitochondrial membrane which appear to be the primary target of its cellular effect (Thompson and Bannigan, 2008). These results came accordance with previous studies, which reported that Cd exposure resulted in GSH depletion and increased MDA level in kidney and liver cells in maternal and their fetuses (Al-Attar, 2011).

The present depletion in liver and kidney antioxidant enzyme activities of $CdCl_2$ maternal treated group and their fetuses might be due to cadmium induced cell membrane damage and alterations in dynamic permeability of membranes, which was followed by the release of intracellular enzymes to the blood stream or might be attributed to their utilization by the enhanced production of ROS (Ho *et al*., 2013). These results were in agreement with the study of Lakshmi *et al.* (2012). They showed a decrease in the activity of hepatic catalase and glutathione peroxidase in cadmium-maternal treated animals.

Pregnancy itself is a stressful condition in which many physiological and metabolic functions can be altered to a considerable extent against the increase in reactive oxygen species (ROS) during pregnancy and protect the fetus. The presence of heavy metals in the placenta may be detrimental for placental SOD and GPx activities and, as a result, the fetus is subject to some degree of oxidative stress which may result in potential damage. In addition, oxidative stress influences both implantation and early

development which decides a successful pregnancy (Lee *et al.*, 2009).

Vitamin E and wheat germ oil supplements have significantly minimized the severity of lipid peroxidation, and enhanced the activities of antioxidant enzymes as well as reduced GSH level in hepatic and renal tissues of maternal and their fetuses. These results were in agreement with Layachi and Kechrid (2012). This effect could be due to vitamin E, a strong lipid soluble antioxidant present in the cell, naturally accumulates in the membranes of mitochondria, endoplasmic reticulum and protects liver and kidney cells from lipid peroxidation. Furthermore, Abdul-Hammid *et al.* (2004) reported that co-administration of wheat germ oil caused amelioration in the antioxidant enzymes and reduced peroxidative process. This may be due to vitamin E in wheat germ oil which is a potent peroxyl radical scavenger that prevents the propagation of free radical damage in biological cell membranes.

Cd administration could be teratogenic or fetotoxic depending on the dose, chemical species and administration during gestational period (Salvatori *et al.*, 2004). The reduction in the number of viable fetuses might be explained on the basis of incomplete formation of the placenta and degeneration of the trophoblast and decidual cells, which play an important role in the transmission of nutrients to the embryo (Aboubakr *et al.*, 2014). Also, they added that the fetotoxicity, high resorption ratio and fetal loss and malformations could be attributed to the inhibition of DNA transcription in the rapidly divided fetal cells.

It has been observed that the placenta is a natural defense against Cd toxicity during pregnancy, because it acts as a barrier for Cd transfer from mother to fetus by sequestering its excess from the blood and minimizing its transfer to the fetus (Sorkun *et al.*, 2007). In addition, it has been reported that cadmium is bound to MT in the placenta, while the placenta functions as a partial barrier for cadmium between maternal and fetal blood. Also, García and González (2010) stated that cadmium reaches the placenta or embryo at organogenetically sensitive time (9^{th} day of gestation) in *Wistar* rats.

Resorption and fetuses lethality might be attributed to the inhibitory action of $CdCl_2$ on the protein synthesis, placental dysfunction and/or Cd intoxication to heart, kidney and liver (Ji *et al.*, 2011). Cd is an endocrine disrupter with detrimental effects on mammalian reproduction the hypothesized that Cd disrupts the proliferative growth and physiological function of placental trophoblasts cells in rat, depending on the studies which showed that cadmium has potent estrogen-and androgen receptors (Sekhon *et al.*, 2010). The reduction in the body weight and length of fetus maternally treated with $CdCl_2$ might be due to an impairment of blood flow to the placenta and reduced uterine transfer of nutrients and oxygen to the fetal circulation. So, these results coincide with the previous studies of Shirai *et al.* (2010). Llanos and Ronco (2009) reported that the fetal growth restriction could be related to impaired placental function due to toxic metals, thus inhibiting the appropriate transfer of essential nutrients to the fetus, which are indispensable for life maintenance and normal development and growing.

Furthermore, this reduction could be explained by the fact that cadmium may devastate the placental function through its congestive effect and hyaline degeneration as well as thrombus formation of its vessels, thus interfering with the transport of amino acids necessary for normal growth of the fetus. Also, the direct cytotoxic effect of cadmium on the fetal tissue may lead to decline in fetuses's size (Ji *et al.*, 2011).

The obtained results revealed that cadmium toxicity produced multiple external deformities in the fetus like exencephaly. This may be due to neural tube defects during neurulation and/or due to $CdCl_2$ exposure before neurulation caused an opening in the anterior neural pore, anophthalamia, microphthalmia may be due to reduced thickness of the neuroblastic layer, neurosis or pyknosis of retinal cells (Yang *et al*., 2006).

Fetuses maternally treated with $CdCl_2$ showed deformities in limbs such as clubfoot. Club foot formation might be due to: 1- indirect action of metal, 2- alteration of maternal physiology, which disturbs the hormonal balance in mother or 3- direct effect on the tissue primordial of foot. Moreover, syndactayly and amelia might be via reduction in cell proliferation of the distal margin and inhibition of chronic genesis (Behbahani *et al*., 2014).

Also, the occurrence of open eyelids in the present study after $CdCl_2$ mother administration might be due to partial ossification of dermal bones that may have affected the diameter of eye orbit. This alteration could result in changes in the attachment of eye muscles, thereby leading to the condition of open eyes.

In addition, umbilical hernia, abnormal bending of the body, short and absent tail; microtia in fetuses maternally treated with $CdCl_2$ were observed. These results were in agreement with the results of El-Sayed *et al.* (2013) who reported that Cd toxicity produced multiple external deformities in the fetus like exencephaly, micrognathia, ablephary, microphthalmia, short and kinky tail and clubfoot.These malformations could be due to the genotoxic effects of cadmium that produced breakdown of the DNA and DNA protein cross link, thus interfering with normal formation of different parts of the body and/or transport of even smaller quantities of the metal into the embryo during early gestation could cause severe malformations (Velázquez *et al*., 2013). The increase in ROS also involved in defective embryo development and the retardation of embryo growth, which is attributed to cell membrane damage, DNA damage and apoptosis (Ronco *et al.*, 2011). The results of Dı´az *et al*. (2014) revealed a clear embryotoxic and a teratogenic effect of Cd, the former as a significant increase in the number of resorptions, and the latter as a significant decrease of the gestational sac weight, and the size and weight of foetuses of Cd-treated dams as well as induced malformations in skull bones, vertebrae and thoracic, and pelvian limbs.

In the present study, supplementation of vitamin E, wheat germ oil or their combination with cadmium chloride more or less prevented fetal malformations and fetal resorption also, improved body growth and bone formation. These results were in agreement with Delashoub and Khojasteh (2012) who reported that co-administration of Vit. E reduced oxidative stress induced Intrauterine Growth Retardation (IUGR) and reversed metal induced growth retardation. Vitamin E has a protective effect against source of free radical in pregnant rats. Also, vitamin E has increased the status of fertility

and percentage of normal pups born from the metal exposed rats. Vitamin E supplementation may also play a role in fetal growth, as shown previously that body length was found to be positively associated with maternal-tocopherol concentration which indicated that maternal vitamin E helped in fetal growth (Ammar *et al*., 2009). Also, those stated that vitamin E may be beneficial in preventing fetal malformation and fetal resorption due to its antioxidant potency (Ahmed *et al*., 2013).

The present results are in the same line with the results of Abd El- Aziem *et al.* (2005) who suggested that wheat germ oil contains high levels of essential fatty acids, which the body does not naturally produce. So, for this reason, these fatty acids need to be ingested in order to stimulate cell regeneration and growth also, to maintain a healthy immune system, reproductive system, nervous system, and cardiovascular system. It has been proven that using wheat germ oil is more effective in delivering results than using synthetic varieties of vitamin E. Moreover, the presence of different types of fatty acids in wheat germ oil caused increasing the stability of the genetic protein P53, which is a critical factor in the reduction of cell mutation as regulates tumor necrosis factor, regulation of both normal embryonic development and prevention of developmental defects after teratogenic exposure (Omima *et al.*, 2011).

5. Conclusion

The present results revealed that the supplementation with vitamin E, wheat germ oil and their combination during $CdCl_2$ exposure, showed an antioxidant activity and a protective effect against $CdCl_2$ induced hematological, hepatic and renal toxicities, fetal growth retardations and malformations. However, future studies may be able to ensure many mechanisms involved in the beneficial effect of vitamin E and wheat germ oil against $CdCl_2$ induced deleterious effects.

6. Conflict of interest

The authors declare that they have no conflict of interest.

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