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PROTECTIVE EFFECTS OF SELENIUM AND ZINC ON NICKEL CHLORIDE INDUCED REPRODUCTIVE TOXICITY IN WISTAR ALBINOS PREIMPLANTED RATS

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Abstract: The aim of this study was to investigate that nickel chloride ($NiCl_2$) induced reproductive toxicity in pre- implanted Wistar Rats and examined the possible protective effect of zinc chloride and selenium on plasma concentration of the hormones of 17 b etradiol (E_2) and progesterone (prog); on the reproductive organ's histology and on development. Experimental results showed the subcutaneous (s.c) administration of $Nicl_2$ to Wistar albino Rats induced a decrease in plasma concentration of E_2 and prog in addition, disturbance in development parameters and structural damages to the histology of the reproductive organs. Conversely, Se and $ZnCl_2$ dues to the antioxidants property, regulate the secretion of E_2 and Prog hormones, prevent alterations in the reproductive organs and in development in preimplanted rates receiving $NiCl_2$.

Keywords: pre-implanted rat; nickel chloride; selenium; zinc chloride; reproductive toxicity.

INTRODUCTION

Nickel is one of the most important industrial and environmental pollutants (Das kk et al., 2007) this toxic heavy metal is accumulated in soils, easily absorbed by plants Thus, they can enter in the food chain and cause deleterious effects on animals (Samal et al., 2011). Nickel is a hemotoxic, immunotoxic, hepatotoxic, nephrotoxic (Chen et al., 2003; Das et al., 2007; Adjroud, 2013; Tikare et al., 2013; Dahdouh et al., 2016), genotoxic (Das et al., 2018) and reprotoxic and also a carcinogenic agent (Das et al., 2008; Forgas et al., 2012). In addition, in fish, the nickel has been reported to cause oxidative stress in various organs of the body (Kubrak et al., 2013; Kubrak et al., 2014; Blewett et al., 2016; Blewett et al., 2017). Nickel is an environmental pollutant that adversely affects the female reproductive system and development. Notably fertility, abortions and malformations (Vaktskjold et al., 2006). During pregnancy, in human and Rat the nickel crosses the placenta (Lin, 1998; Hou et al., 2011), to accumulate in fetal tissues (Hou et al., 2011), and produces teratogenesis and embryotoxicity (Szkmary et al., 1995). Besides, a reducing of implantation sites, number of live fetuses, fetal and placental weights (Saini et al., 2013). Furthermore, studies in male and female shows that nickel also affects the secretion of sex hormones such as follicle stimulating hormone, luteinizing hormone, estradiol serum levels, testosterone and causes histopathological damage to the reproductive organs (Kong et al., 2014). During pregnancy, trace elements such as selenium and zinc are indispensable for life maintenance of pregnant female and fetus (Osada et al., 2012). Selenium and zinc have protective effects against the toxicity of many metals (Eahman et al., 2019) and plays protective role on reproduction function in both male and female (Batra et al., 2004; Pieczyriska et al., 2015). However, new studies indicate that selenium is an essential trace element, antioxidant (Khurana et al., 2019). Protect animals and humans from the risk of various diseases and reduce heavy metals toxicity (Zwolak, 2019). In addition, Selenium is necessary for follicle growth, maturation and dominance in the ovary of both humans and animals (Ceko et al., 2014; Mintziori et al., 2019). In cattle and ewe selenium supplementation is associated with development of placenta, increased in live fetal and their weight (Palmieri et al., 2011). On the other hand, Zinc plays an important role in growth, development and reproduction (Butzow et al., 1975; Maret et al., 2006) and has a vital role in ovarian function, placental and embryo development (Kim et al.,2010). Furthermore, Zinc deficiency could affect oocyte maturation (Branum et al., 2013), reduction of ovarian follicular growth, causes follicular atresia (Taneja et al., 1990), decreasing in placental and fetal development (Tian et al., 2014). Therefore, this study seeks to investigate the possible preventive and ameliorative effects of selenium and zinc against nickel chloride induced reproductive toxicity in pregnant Wistar albinos Rats such as change in development, in sex hormones; 17 b estradiol, progesterone and negative effects on reproductive organs.

MATERIALS AND METHODS

Laboratory Animals

All experimental protocols conducted on rats have been carried out in accordance with the standards established by the Institutional Committee of Protection and Use of Animals at the University of Batna. Adult, Wistar albino female rats (Institute Pasteur, Algiers, Algeria), have been housed under a lighting program of 12 hours of light: 12 hours of darkness at 23 ± 1°C, they are fed ad-libitum and watered at will of the water. Females (180-300 g body weight) were caged with

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males overnight. In the morning, female rats with a positive vaginal wash have been considered pregnant and recorded as day 0 of pregnancy. The animals have been tested under experimental conditions on the day 20. The pregnant females have been housed in cages with six rats per cage. The average duration of pregnancy in the reproduction colony was 21 days (Adjroud, 2013). Nickel chloride hexahydrate ($NiCl_2$: $6H_2O$), elemental selenium (Se) and zinc chloride ($ZnCl_2$) have been purchased from Sigma-Aldrich (Chemistry Gmbh., Taufkirchen, Germany). The finely ground crystals of elemental $NiCl_2$, Se and $ZnCl_2$ have been dissolved in sterile saline solution and the pH has been adjusted, if necessary, to 7.5 (Adjroud, 2013).

Experiences

Our study has been performed on 30 pregnant rats Wistar Albino strains are divided into 5 groups, 6 rats in each group. The treatment of the rats had s carried out at the 3rd day of pregnancy by subcutaneous way. Each animal has been weighed before each. In the control group, the 6 rats received 0.9% NaCl (solution composed of 9 g of NaCl dissolved in 1000 ml of water. distilled), the 6 rats are treated by subcutaneous way according to their body weight. A group received 100 mg / kg of nickel chloride alone (40.48 mg / l) dissolved in 100 ml of 0.9% NaCl solution administered by subcutaneous way according to their body weight. A third group that received 0.3 mg / kg of selenium, after one hour we will inject a dose of 100 mg / kg of nickel chloride administered by subcutaneous way according to their body weight. The fourth group received 20 mg / kg of zinc chloride, after one hour we will inject a dose of 100 mg / kg of nickel chloride administered by subcutaneous way according to their body weight and the last group 20 mg / kg of zinc chloride and 0.3 mg / kg of selenium followed by 100 mg / kg of nickel chloride subcutaneously based on their body weight with an interval of one hour of time.

Before blood samples, the rat is anesthetized with diethyl ether for a short time. 5ml of blood are taken from each rat in the jugular vein in hemolyzed heparinized tubes, following blood tests taken on the 6th and 20th days of pregnancy. Then centrifuged at 4000 rpm for 10min at the physiotoxicology research laboratory, cellular pathology and bimolecular molecular. University of Batna2. This plasma is collected in the Eppendorf tubes and then frozen and stored at -20 ° C for the female hormones assays: progesterone and estrogen

At 20 days of pregnancy the maternal body weight has been recorded, before sacrificing the reproductive organs including placenta, ovaries and uterus are removed and rinsed in the 0.9% NaCl at 4 ° C and then stored in formalin for histological studies and developmental setting studies, the uteri have been excised to assess the number of alive fetuses and the number of resorption sites and alive fetuses have been weighed (Adjrou, 2013).

Hormonal Dosages: Estradiol (E_2), Progesterone (Prog)

The dosages of the hormones E_2, prog are performed on the 20th day after treatment, the assay principle combines an enzyme immunoassay competition method with a final fluorescent detection ELFA (Enzyme Linked Fluorescent Assay), using a VIDAS (biomérieux) automaton.

Histology

Uterus and ovary specimens were fixed for 48 h in 10% neutral buffered formalin and dehydrated with graded ethanol, cleaned in xylene, and embedded in paraffin. Five to six lm-thick paraffin wax sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope (Axioskop 20 Carl Zeiss) (Gottingen Germany).

Statistical Analysis

Data for each group of experiments of six rats were analyzed by one-way analysis of variance (ANOVA) and expressed as mean ± SE. Analysis of significance between the mean treated groups and the control groups were performed by Dunnet test. Differences were considered to be significant if $P < 0.05$. Data were analyzed with Graph Pad Prism 7.0.

RESULTS

1-effects of $nicl_2$ administered alone or in combination with Zn and Se on plasma concentrations of sex hormones

1.1 effects of $nicl_2$ administered alone or in combination with Zn and Se on plasma concentrations of 17.β estradiol

Our results show in the rate pre-implanted rate that subcutaneous administered of $NiCl_2$ (100 mg / kg) on day 3 of gestation caused a very significant decrease ($p<0.001$) in the plasma concentration of E_2 on day 20 of gestation compared to the control. (204.4 ± 23.34 vs 661.3 ± 75.66) Fig. 1.On the other hand, the presence of $Zncl_2$ (20 mg / kg, sc) in association with $NiCl_2$ (100 mg / kg, sc) on day 3 of gestation induced a very significant increase ($p<0.001$) in plasma concentration of E2 on days 20 of gestation compared with administration of $Nicl_2$ alone. (604.8 ± 15.65 vs 204.4 ± 23.34) Fig. 1. Moreover, E_2 concentration was very significantly elevated ($p<0.001$) on day 20 of gestation in the combination of Se (0.3 mg / kg, sc) with $NiCl_2$ (100 mg / kg, sc) compared to the $Nicl_2$ alone. (520.3 ± 29.91vs 204.4 ± 23.34) Fig.1. Furthermore, the presence of Se (0.3 mg / kg, sc) and Zncl2 (20 mg / kg, sc) with $NiCl_2$ (100 mg / kg, sc) showed a significant increase ($p<0.01$) in plasma concentration of E_2 on day 20 of gestation compared with $Nicl_2$ alone (651 ± 55.23 vs 204.4 ± 23.34) Fig. 1.This increase reached the plasma E_2 concentration of the control.

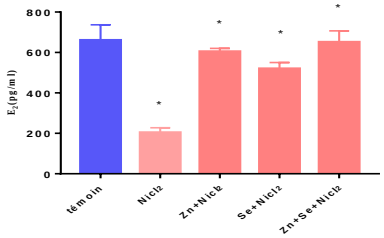

Fig. 1. Effects of nickel chloride administered alone or in combination with zinc chloride and selenium on plasma concentrations of 17.β estradiol.

1.2 effects of $nicl_2$ administered alone or in combination with Zn and Se on plasma concentrations progesterone

Our study shows in the pre-implanted rate that subcutaneous administered of $NiCl_2$ (100 mg / kg) on day 3 of gestation caused a slight decrease in plasma concentration of progesterone on day 20 of gestation compared with control (52.87 ± 10.71 vs 65.15 ± 11.37) Fig. 2. In contrast, the presence of $Zncl_2$ (20 mg / kg, sc) in combination with $Nicl_2$ (100 mg / kg) at day 3 of pregnancy caused a slight increase in plasma concentration in progesterone at day 21st of comparatively pregnancy with $NiCl_2$ alone (62.98 ± 8.55 vs 52.87 ± 10.71) Fig. 2. In addition, progesterone concentration was a slight elevated on day 20 of gestation in the association of Se (0.3 mg / kg, sc) with $NiCl_2$ (100 mg / kg, sc) compared to $NiCl_2$ alone (49.17 ± 3.027vs 52.87 ± 10.71) Fig. 2. Furthermore, the presence of Se (0.3 mg / kg, sc) and Zncl2 (20 mg / kg, sc) with $NiCl_2$ (100 mg / kg, sc) on day 3 of gestation showed a slight increase in plasma concentration of progesterone on day 20 of gestation compared to $NiCl_2$ alone 62.59 ± 7.83vs 52.87 ± 10.71) Fig. 2.

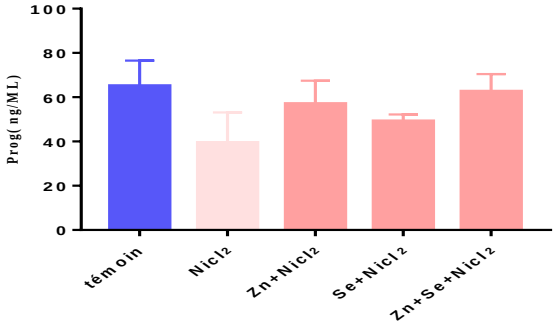

Fig. 2. Effects of nickel chloride administered alone or in combination with zinc chloride and selenium on plasma concentrations of progesterone.

2. effects of $nicl_2$ administered alone or in combination with Zn and Se on developmental parameters

2.1. effects of $nicl_2$ administered alone or in combination with Zn and Se on the fetus number

Our results showed in the pre-implantated rate that administrated of $NiCl_2$ (100 mg / kg, sc) on day 3 of gestation provoked a very significant decrease in the number of living fetus ($p<0.001$) on day 20 of gestation compared to controls, Table1. On the other hand, the presence of $ZnCl_2$ (20 mg / kg, sc) in combination with $NiCl_2$ (100 mg / kg, sc) on day 3 of gestation caused a very significant increase in the number of living fetus ($p<0.001$) on day 20 of gestation compared with $NiCl_2$ alone.Table 1. Similarly, the combination of Se (0.3 mg / kg, sc) with Nicl2 (100 mg / kg, sc) on day 3 of gestation showed a very significant increase in the number of living fetus on day 20 of gestation compared with $NiCl_2$ alone.Table 1. Moreover, the number of living fetus was very significantly high ($p<0.001$) on day 20 of gestation in the presence of Se (0.3 mg / kg, sc) and Zncl2 (20 mg / kg, sc) combined with $NiCl_2$ (100 mg / kg, sc) compared with $NiCl_2$ alone. Table 1.

2.2. effects of $nicl_2$ alone or in combination with Zn and Se on fetal body weight

Fetal body weight was very significantly decreased ($p<0.001$) on day 20 of gestation in the $NiCl_2$ (100 mg / kg, sc) treated group compared to the control. Table 2. On the other hand, the presence of $ZnCl_2$ (20 mg / kg, sc) or Se (0.3 mg / kg, s.c) in association with $NiCl_2$ (100 mg / kg, sc) on day3 of gestation showed a very significant increase in the fetal weight ($p<0.01$) on day 20 of gestation compared to $NiCl_2$ alone. Table 2. Moreover, the presence of Se (0.3 mg / kg, sc) and $ZnCl_2$ (20 mg / kg, sc) with $NiCl_2$ (100 mg / kg, sc) on day3 of gestation caused a very significant increase in the fetal body weight ($p<0.001$) on day 20 of gestation compared with $NiCl_2$ alone. Table 1.

Table 1

Effect of $NiCl_2$ alone or in combination with Zn and Se on developmental parameters in *Wistar albino* preimplanted rats

	Control	$NiCl_2$	Zn+ $NiCl_2$	Se+$NiCl_2$	Zn+Se+ $NiCl_2$
Parameters	(0.3ml/rat s.c)	100 mg/kg (s.c.)	Zn(20mg/kg)+ $NiCl_2$ (100 mg/kg)	Se (0.3mg/kg) + $NiCl_2$ (100 mg/kg)	Zn (20 mg/kg) + Se (0.3mg/kg + $NiCl_2$(100mg/kg)
Number of Live Fetuses	9.667±0.7149	4.333±1.202*	9.167±1.202*	8.833±0.307*	10.00±0.683*
Number of fetal loss	0.00±0.00	1.167±0.494**	0.166±0.166*	0.333±0.210*	0.166±0.166*
Fetal body weight (g)	3.683±0.2342	2.27±0.1367***	3.616±0.1867***	3.2530±0.0847**	3.661±0.1459***

Value are mean ±SEM, n=6. *p<0.05; **p<0.01; ***p<0.001. Control vs $Nicl_2$; Zn+$Nicl_2$ vs $Nicl_2$; Se+ $Nicl_2$ vs $Nicl_2$; Se+ $Nicl_2$ vs $Nicl_2$; Zn+Se+$Nicl_2$ vs $Nicl_2$. (Dunnett test).

2.3. effects of $nicl_2$ alone or in combination with Zn and Se on fetal loss

Administration of $NiCl_2$ (100 mg / kg, sc) on day 3 of gestation caused a very significant elevation of the fetal loss ($p<0.001$) on day 20 of gestation compared to the control. Table1. On the other hand, the presence of $ZnCl_2$ (20 mg / kg, sc) in combination with $NiCl_2$ (100 mg / kg) on day 3 of gestation caused a very significant reduction in the fetal loss ($p<0.01$) on day 20 of gestation with $NiCl_2$ alone. Table 2. Similarly, the combination of Se (0.3 mg / kg, sc) with $NiCl_2$ (100 mg / kg, sc) on day3of gestation induced a very significant decrease in the fetal loss ($p<0.01$) on day 20 of gestation compared to the $NiCl_2$ alone. Table1. Likewise, the fetal loss was a very significantly decreased ($p<0.01$) in the association of Se (0.3 mg / kg, sc) and Zncl2 (20 mg / kg, sc) with $NiCl_2$ (100 mg / kg, sc) on day 20 of gestation compared with $NiCl_2$ alone. Table1.

3-effects of $nicl_2$ administered alone or in combination with Zn and Se on the reproductive organs histology

3.1 effects of $nicl_2$ alone or in combination with Zn and Se on the uterine histology

The uterine histology for the control group on day 20 of gestation showed a uterine cavity and endometrial glands of normal structure Fig. 3. In contrast, the uterine histology of the $NiCl_2$ (100 mg / kg, sc) treated group showed on day 20 of gestation structural damages, glandulo–cystic hyperplasia characterized by the multiple formation small cysts which are inflammatory cell scattered among the endometrial gland ,these cysts resulted the dilatation of some glands compared to the control. Fig. 3. On the other hand, a low dilatation with absence of inflammatory cells was observed on day 20 of gestation in the pre-implantation rates treated with Zncl2 (20 mg / kg, sc) in combination with $Nicl_2$ (100 mg / kg, sc) on day 3 of gestation compared to $NiCl_2$ Fig. 3. However, when Se (0.3 mg / kg, sc) administered in combination with Nicl2 (100 mg / kg, sc) on day 3 of gestation, a low dilatation with decrease in inflammatory cells were observed on day 20 of gestation compared with the $NiCl_2$ alone Fig. 4. In contrast, endometrial glands of the normal structure and complete absence of inflammatory cells were observed on day 20 of gestation in the presence of Se (0.3 mg / kg, sc) and $ZnCl_2$ (20 mg / kg, sc) with $NiCl_2$ (100 mg / kg, sc) in pre-implanted rates compared with the $NiCl_2$alone. This observation is very comparable to that of the normal structure of the control Fig. 3.

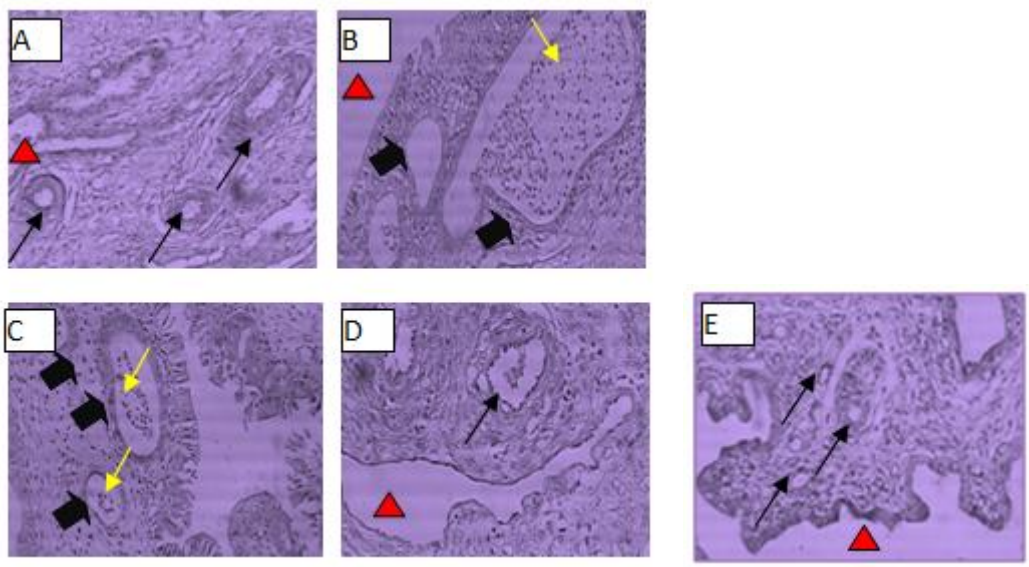

Fig. 3. Effect of $Nicl_2$ alone or in combination with Zn and Se on uterine histology on day 20 of pregnancy treated on day 3 during preimplanted period of Wistar albinos rat Uteri were stained with H&E and photographs taken a magnification of 10 ×.A: control; B: Nicl2; C : $Nicl_2$+Se; D : $Nicl_2$+$Zncl_2$; E : $Nicl_2$+Se + $Zncl_2$. The small black arrows indicate endometrial dilated endometrial gland, the red triangle indicate uterine cavity, yellow arrows indicate inflammatory cells gland, the big

black arrows indicate dilated endometrial gland, the red triangle indicate uterine cavity, yellow arrows indicate inflammatory cells.

3.3 effects of $nicl_2$ alone or in combination with Zn and se on the histology of the ovary

Ovarian histology of the control rates showed on day 20 of gestation a normal structure with follicles in a different stage of development, primordial, primary, secondary and tertiary respectively and presence of luteal cells of preserved morphology. Fig.4. in contrast in the pre-implanted rates treated with $NiCl_2$ (100 mg / kg, sc), on day 3 of gestation we observed a decrease in the number of follicles with a preponderance of primordial follicles compared and the presence of malformed dystrophic follicles compared of the control. Fig. 4. On the other hand, the $ZnCl_2$ (20 mg / kg, sc) combined with $NiCl_2$ (100 mg / kg, sc) on day 3 of gestation in the pre –implanted rates showed an increase in the number of follicles of a normal structure, a presence of primordial follicles, secondary, and a graafian follicle respectively also the presence of luteal cells of preserved morphology compared to $NiCl_2$ alone . Fig. 4. Likewise, in the pre-implanted rates, the Se (0.3 mg / kg, sc) associated with $NiCl_2$ (100 mg / kg, sc) administered on day 3 of gestation showed an increase in the number of follicles of normal structure, a presence of primordial follicles, primary and secondary and the presence of luteal cells of preserved morphology compared to $NiCl_2$ alone. Fig.4. Additionally, the combination of Se (0.3mg / kg, sc) and $ZnCl_2$ (20mg / kg, sc) with $NiCl_2$ (100mg / kg, sc) on day 3 of gestation caused an increase in follicles number of a normal structures in the different development stages, primordial, primary, secondary, and tertiary respectively with the presence of luteal cells of preserved morphology compared to $NiCl_2$. This structure of the ovary is very similar to that of the histology of the control rate. Fig. 4.

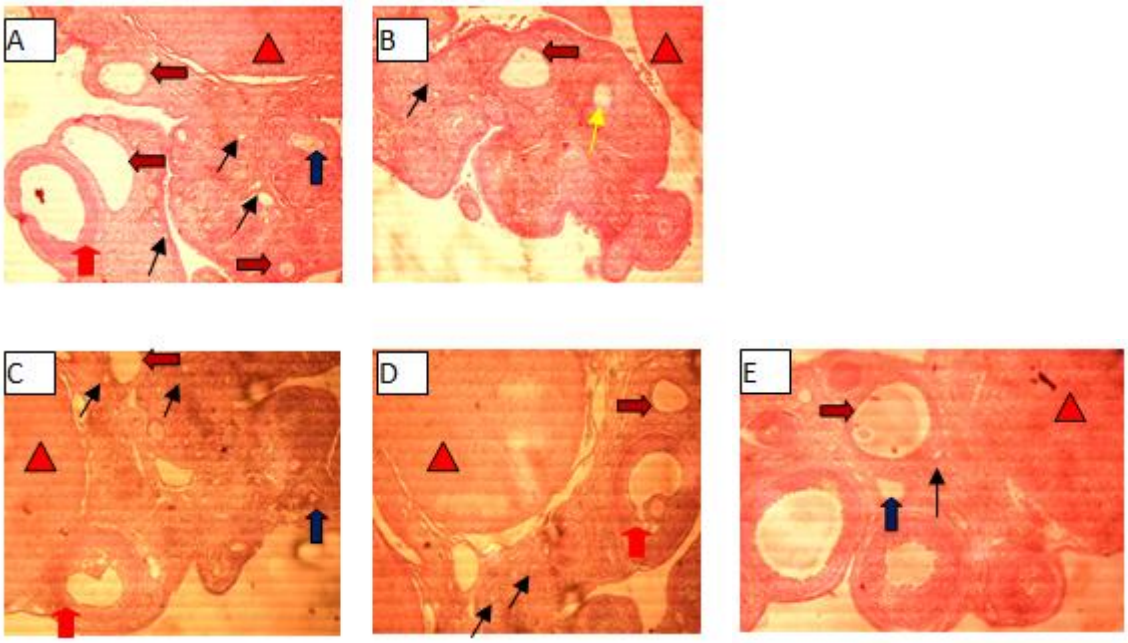

Fig. 4. Effect of $Nicl_2$ alone or in combination with $Zncl_2$ and Se on ovarian histology on day 20 of pregnancy treated on day 3 during preimplantation period of Wistar albinos rats. The ovaries were stained with H&E and photographs taken a magnification of 10 ×.A: control; B: $Nicl_2$; C: $Nicl_2$+$Zncl_2$; D: $Nicl_2$+Se; E: $Nicl_2$+$Zncl_2$+Se.the red triangle indicate corpus luteum, the black arrows indicate primordial follicle, the blue arrows indicate primary follicle, the brown arrows indicate secondary follicle, the red arrows indicate tertiary follicle, yellow arrows indicate distorted follicle.

DISCUSSION

Reproductive toxicology is defined by the study of xenobiotic agents' effects on the development and reproductive capacity of individuals. Female reproductive functions may be affected by exposure to occupational and environmental toxins (Hoyer ,2018). The hypothalamus and pituitary play key roles in reproductive function. Studies indicate that environmental toxicants such as nickel can alter female reproductive function by disrupting hypothalamic control of the pituitary and consequently, the endocrine control of ovarian function (Farmeret al., 2018). The gonadotropins (FSH and LH) secreted by the anterior pituitary gland are necessary for the synthesis of Estrogen and progesterone (Priya et al., 2004). On the other hand, estrogen and progesterone are both essential for the embryonic development, differentiation of reproductive tissue (Da Silva Faria et al., 2010), maintenance of pregnancy and implantation (Pepe et al., 1995; Spencer et al., 2004).

Our results showed that nickel chloride 100 mg/kg administered subcutaneously in the pre –implanted rate induced a very significant decrease in the plasma concentration of E_2 on day 20 of gestation. This result agrees with those observed in female fish (Drienssnacket al., 2017). On the other hand, this decrease in plasma E_2 may be due to the modification in the plasma gonadotropins. In fact, (Kong et al., 2014) reported that nickel nanoparticle increased follicle stimulating hormone (FSH) and luteinizing

hormone (LH) lowered estradiol E_2 serum levels in rats. Moreover, studies in female rats reported that cadmium or sodium fluoride decreased serum concentration of estradiol, progesterone, follicle stimulating hormone and luteinizing hormone. (Lieneschet al., 2000; Munga et al., 2013; Zhou et al., 2013; Nna et al., 2017). In zebra fish ,cadmium act as a potent anti estrogen in vivo et in vitro ,effects on transcriptional activation of the estrogen receptor (ERs),decreased Aro-B and molecular expression of related genes (esr1,esr2a,esr2b,cyp19a1b,ef1) (Chouchene et al., 2016). On the other hand, (Das et al., 2013) reported that the exposure of fish with cadmium chloride attenuated serum and ovarian 17 β-estradiol used by inhibiting of aromatase and cytochrome p 450 arom gene expression in carp ovarian follicles and ovarian SF1.Furthermore, in the pregnant rats or in hen ,the cadmium chloride decreased serum 17 β-estradiol levels and this change was accompanied with a dysfunction of oxidative stress (Samuel et al., 2011; Yang et al., 2012) . Our study showed a slight decrease in plasma concentration of progesterone on day 20 of gestation. in pregnant rats treated with nickel chloride 100 mg/k compared to the control. This study was agreeing with those observed in female rat (Nna et al., 2017) but by another heavy metal including cadmium chloride. On the other hand, this decrease in the plasma progesterone which would be due to oxidative damage of hen's ovary tissue (Yang et al., 2012). Furthermore, the administration of sodium fluoride and cadmium in female rats shows a reduction of the sex hormone such as progesterone, this reduction due to an inhibition of the secretion of FSH and LH (Munga et al., 2013; Zhou et al., 2013; Monsefi et al., 2013). An earlier study using male rat showed that nickel accumulated in the hypothalamus (Clemons et al., 1981) causing diminutions in luteinizing hormone and follicle-stimulating hormone (Adedara et al., 2019). Moreover, Hormonal effects may play an important role in the reproductive toxicology of nickel both at the neuroendocrine and gonadal level in the hypothalamic –pituitary –gonadal (HPG) axis (Forgacs et al., 2012). This may partly responsible for the decreased plasma concentration of estrogen and progesterone in pre implanted rat.

In this study Our results showed that selenium and zinc improved circulating levels of 17 β estradiol and progesterone levels in pre implanted rats treated with Nickel chloride, This important improvement characterized by a very significant increase for 17 β estradiol and slight significant increase for progesterone was observed in both treatment groups (Selenium + Nickel chloride) and (Zinc chloride + Nickel chloride) with better protection in the group that treated with (Zinc chloride + Selenium + Nickel chloride).Would probably be due to an increase in the rate of follicle-stimulating hormone and luteinizing hormone. A recent study (Adedara et al., 2019) showed that exposure to zinc abated nickel-mediated diminutions in luteinizing hormone, follicle-stimulating hormone in male rat. Other study, reported in Wistar female rats that quercetin exerts preventive and ameliorating effects on the toxicity of cadmium chloride characterized by a significant increase in serum concentration of estrogen, progesterone, follicle-stimulating hormone and luteinizing hormone (Nnaet al., 2017). Indeed, Curcumin is a powerful antioxidant which causes protective effects against the toxicity of cadmium chloride, and increases the secretion of serum estrogen concentration (Kamfiruzi et al., 2016). In addition, zinc sulphate has some significant positive effects on male sex hormones such as progesterone, estradiol, Luteinizing hormone and follicle stimulating hormone (Egwurugwu et al., 2013).

In our study, we reported that nickel chloride decreased fetal body weight and also caused fetal loss and decreased the number of fetal live. This result suggested that Nickel chloride May affect embryo development by inducing resorption and death of embryo. A morphological study of uterine alteration due to exposure to nickel chloride may be responsible of both fetal loss and the decrease in the number of fetal live. Indeed, placenta is known to have multifunctional role during gestation and serves as interface between maternal circulation and mediates exchange of gases, transport of nutrients and waste products and was regarded as an indicator organ widely when exposed to metals (Iyengar et al., 2001; Guo et al., 2010). The placenta was shown to have a high affinity for nickel (Jasim et al.1986) and therefore placental barrier does not protect the fetus from nickel exposure(Sunderman et al., 1978) Studies have shown that toxic and foreign chemicals may interfere with placental hormone secretion and further result in abortion ,stunted fetal growth and intrauterine fetal death(Spencer et al.,2004). During pregnancy, exposure to nickel in mice and rats induces a reduction in the number of fetuses, body weight of fetuses and offspring (Sunderman et al., 1978; Storeng et al., 1981). .in fact heavy metals concentration including nickel ,cadmium ,mercury ,arsenic and lead in umbilical cord blood were found significantly higher in the adverse pregnancy outcome (Klopov, 1998; Zheng et al., 2014). On the other hand, in animals and humans, heavy metals can cross the placenta and result maternal fetal susceptibility(Caserta et al., 2013), in pregnant rats the placenta has a high affinity for nickel and its barrier does not protect the fetus from nickel exposure, nickel actively transferred across the blood-placenta-barrier into blood and fetal organs (Hou et al., 2011) . In humans nickel is found higher in umbilical cord blood than in maternal blood (Klopov, 1998). Moreover, the reduction in fetal growth and development found in maternal residential exposure to air pollution or cadmium was associated with placental gene expression (Kingsley et al., 2017; Everson et al., 2018).

In this study, fetus number and fetal weight were very significantly increased but very significantly decreased in the fetal loss in the (zinc chloride +nickel chloride), (selenium + nickel chloride) and (zinc chloride + selenium + nickel chloride) treated groups.

In pregnant rat, zinc and selenium have a very effective protection against cadmium-induced

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 30, issue 3, 2020, pp. 126 - 135

developmental toxicity (Wlodarczyk et al., 2000; El-Sayed et al.,2013). Zinc Reducing the percentage of abortion and embryonic resorption (El-Sayed et al.,2013) and selenium decreased post implantation losses and increased fetal weight (Wlodarczyk et al., 2000. In mice the zinc ameliorates dysmorphology and postnatal mortality caused by ethanol exposure in early pregnancy (Summers et al.,2009). Selenium may act as antagonist to cadmium and as a protective factor in fetal growth, decreased of intrauterine growth restriction (Everson et al., 2018). Moreover, during gestation, the zinc is necessary for growth of fetus and plays a protective role against cadmium induced skeletal abnormalities (Boughammoura et al., 2016). In rat the zinc plays a protective role against lead induced toxicity in fetal development (Ali et al.,2016). In female rats and during gestation, zinc shows a beneficial and protective role against the damage caused by the mercury toxicity, notably a reduction in fetal development (Weng et al., 2017). In addition, in pregnancy mice, the iron exerts preventive effect on cadmium induced growth fetal retardation but the zinc and selenium were ineffective (Webster, 1979).

In the present study, in the pre implanted rat the uterine histology of the Nickel chloride treated group showed structural damages, glandulo–cystic hyperplasia characterized by the multiple formation small cysts which are inflammatory cell scattered among the endometrial gland, these cysts cause the dilatation of some glands .This result may be due to a decrease in progesterone and estrogen hormones. Indeed, the placenta can synthesize a number of hormones, such as progesterone and estrogen, which play important roles in implantation, pregnancy maintenance and embryo development (Pepe et al., 1995; Spencer et al., 2004). On the other hand, in rats, exposure to nickel decreased the implantation sites in embryogenesis, increasing in the frequency of embryonic resorptions, and causes histopathological changes in reproductive organs (Forgacs et al., 2012). In animal, cadmium and nicotine provoked a stress oxidative, vacuolation, congetion and necrosis in the uterus (Massanyi et al., 2007; Nasiadek et al., 2014). histopathological examination of the ovaries of pre implanted rats treated with Nickel chloride showed an important decrease in the number of follicular cells in a different stage of development and presence of malformed dystrophic follicles. This decrease may be due to the decrease in ovaries weights (Hfaiedh et al.,2004; Kong et al., 2014) and similar findings were found but in rats exposed to amodiaquine hydrochloride which causes follicular atresia (Gbotolorun et al., 2011). Moreover, other studies demonstrated in hen, rat and fish that cadmium exposure caused histopathological changes in the ovary associated with ovarian lesions, severe necrosis and degeneration of the ovarian follicle (Samuel et al., 2011; Yang et al., 2012; Munga et al., 2013;chouchene et al.,2016). In addition, administration of 1-(N-methyl-N-nitrosamino)-1-(3-pyridinyl)-4-butanal in female mice reduced ovary weight and follicle (Liu et al., 2019). In female albino rats, Chlorpyrifos altered uteri structure and induced follicular atresia in the ovary (Nishi et al., 2013). On the other hand, in preimplanted rats treated with nickel chloride in combination with selenium and zinc we observed the uterus histology that endometrial glands were of normal structure, total absence of inflammatory cells and increased number of follicles of normal structure at different stages of development in the ovary. This may be due to a decrease in the fetal loss. These results are consistent with the results of (Nna et al., 2017) which showed that in female Wistar Rats, Quercetin is an antioxidant, anti apoptotic and exert a preventive effect in the uterus and ovary against the cadmium toxicity. Other study suggests that in fish, Zinc and Selenium play a protective role in ovary against oxidative stress induced by cadmium (Banni et al., 2011).

CONCLUSION

In the present study, we demonstrated in preimplanted Wistar Albinos Rats, that nickel chloride exposure by subcutaneous decreased plasma concentration of 17 b estradiol, progesterone, human chorionic gonadotropin; disrupt the developmental parameters and causes histopathological damages level of the reproductive organs. These negative effects were attenuated by selenium or zinc chloride in the pretreated model before nickel chloride administration. We concluded that selenium and zinc chloride exert multi-mechanistic effect, including increasing in plasma concentration of sex hormones; beneficial effects on developmental parameters and reducing ovarian, placental and uterine histopathological damages.

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