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Effect of nickel chloride on reproductive parameters and testicular oxidative stress biomarkers in male guinea pigs

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ABSTRACT: The soil and agricultural products pollution by nickel represents an important public health risk in agrarian areas such as the Dschang in Cameroon. This study was designed to evaluate the reproductive toxicity and oxidative stress potential of nickel chloride in male guinea pig. Four groups of adult male guinea pigs were orally treated with nickel chloride at doses of 0, 17.50, 26.25 and 52.50 mg/kg bw for 90 days. At the end of the experimental period, all animals were sacrificed, and blood samples and vital organs were collected for different analysis. Treatment of male guinea pigs with 52.50 mg/kg nickel chloride resulted in increased kidney weight and volume and decreased weights of the sex accessory glands (seminal vesicle + prostate + coagulating glands), epididymis and vas deferens. The 52.50 mg/kg dose of nickel chloride decreased ($p<0.05$) the animal's sperm mobility, number and viability, while it increased ($p<0.05$) sperm micro and macrocephalies. Assessment of biochemical parameters of toxicity revealed increase ($p<0.05$) of serum creatinine and aminotransferases activities in the nickel chloride-exposed guinea pigs (52.50 mg/kg). The nickel chloride (52.50 mg/kg) also promoted oxidative stress, through decrease ($p<0.05$) of superoxide dismutase and catalase activities, as well as increase ($p<0.05$) in lipid peroxydation. In addition, histology of testis revealed disrupted germ cell arrangement, decreased concentration of sperms in the lumen of the seminiferous tubules and degraded germinal epithelium in the animals exposed to nickel chloride. In conclusion, results obtained in this study revealed that nickel chloride perturbs male reproductive system and induced oxidative stress.

Keywords: Male guinea pig, nickel chloride, oxidative stress, reproductive toxicity.

INTRODUCTION

Pollution of agricultural soils by heavy metals represents a major public health risk because of the various pathologies that those elements can cause. Exposure to heavy metals takes two forms: inhalation of particles, and ingestion, either directly (of dust) or through contaminated food. In both cases, soils are an important mechanical vector for the transfer of metals from the environment to the body (De Miguel et al., 1998; Aelion et al., 2008). Agricultural, industrial and urban developments in developing countries such as Cameroon have raised the possibility of an accumulation of metals in food crops (Lum et al., 2014; Noubissié et al., 2016). In Dschang, western Cameroon, the intensification of market gardening with the continuous and abusive use of organic and inorganic inputs have led to the presence of nickel in soils and agricultural products at levels exceeding WHO values (Temgoua et al., 2012; 2015). Locally, some market garden products such as maize and fodder (*Pennisetum purpureum*) are used as sources of energy and cellulose in the diet of domestic animals such as the guinea pig. This animal is a

herbivorous domestic animal appreciated in the locality for its low cost-meat (Pamo et al., 2003, Emile et al., 2017, Fernand et al., 2020). Thus, the diet represents one of the main sources of exposure to nickel for the mammals including guinea pig in this locality. Scientifically, nickel has associated deprivation with depressed growth, reduced reproductive rates, and alteration of serum lipids and glucose (Das, 2009). The dietary requirement of nickel is 50 to 80 ng/gm of diet (Prasad 1976). On the contrary, high quantity of Ni is injurious for animal and human health (Pandey et al., 1999; Pandey and Srivastava, 2000). In reproduction, it has been shown that nickel negatively affected sperm count, viability and motility in adult male rat and mice (Lu et al., 2014; Hu et al., 2020). One of the main aetiological factors for defective spermatogenesis or male infertility is the disturbance of the antioxidant – pro-oxidant testicular homeostasis in favour of oxidant causing oxidative stress (Pham-Huy et al., 2008). However, to the best of our knowledge, no such study has yet been carried out in guinea pig reproduction, despite the high probability that it has to consume the fodder and maize contaminated by this heavy metal, thus the aim of the present study.

MATERIALS AND METHODS

Materials

Forty (40) adult male guinea pigs (*Cavia porcellus*) with average weight 456.00±47.33 g were used in the experiments. They were produced at the Teaching and Research Farm of the University of Dschang, Cameroon. They were identified at the ear and housed in identical cages of dimensions 100 cm x 80 cm x 60 cm (length, width and height) under 12 hours photoperiod and had free access to water and food. They were handled according to ethical guidelines of the Cameroon National Veterinary Laboratory.

The nickel chloride was obtained from Guangdong Guanghua Sci-Tech (China). ELISA kits for determination of testosterone concentration and other biochemical parameters were obtained from Omega Diagnostics (Scotland, United Kingdom) and Chronolab (Barcelona, Spain) respectively.

Essay

Guinea pigs were distributed into 4 groups of 10 animals each (comparable in body weight) and orally treated for 90 days. This duration was selected to fully cover the spermatogenic cycle and maturation of sperm in epididymis which last 90-days in guinea pig (Dadoune and Demoulin, 2001). The control group received 1 mL /kg of body weight (bw) of distilled water, while other groups were treated with the nickel chloride at the doses 17.50, 26.25 and 52.50 mg/kg bw. These doses represent respectively 1/30, 1/20 and 1/10 of the LD50 determined in the preliminary studies (525 mg/kg bw). The animal body weight was recorded weekly and the doses of chemical adjusted accordingly.

Data collection and studied parameters

Twenty-four hours after the last gavage, animals were anesthetized using ether vapour and blood taken by cardiac puncture for serum collection and determination of testosterone and other biochemical parameters concentrations.

Weight and volume of organs

Testes, epididymis, vas deferens, sex accessory glands, liver and kidneys were dissected out, freed of adipose tissue and weighed. The volumes of testes, kidneys and liver were determined by immersing the organs in a 0.9% NaCl solution contained in a graduated cylinder and the displacement of the solution in the cylinder was read.

Concentration of testosterone and biochemical parameters of toxicity

Testosterone and biochemical parameters (aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), creatinine and urea) were quantified in the serum using Omega Diagnostics kit (Scotland, United Kingdom) and Chronolab kits (Barcelona, Spain) respectively. The analyses were conducted according to instructions in the assay kits.

Oxidative stress indicators

The testis homogenate was prepared by dilacerating 15 g of testis in 100 ml of cold 0.9% NaCl followed by centrifugation (3000 rpm, 4ºC, 30 min) and the obtained supernatant used for the measure of oxidative stress indicators concentration. The determination of the concentration of those indicators was done using thiobarbituric acid method as described earlier (Nilsson et al., 1989), while superoxide dismustase (SOD) activity which describes the capacity of this enzyme to inhibit au-oxidation of adrenaline into adrenochrome in basic medium was assessed according to Misra and Fridovich (1972). Total peroxydases (PEROX) and catalase (CAT) activities were determined using Sinha (1972) and Kodjio et al. (2017) methods, respectively.

Quality of cauda epididymal sperm

Animal sperm motility was evaluated by dilacerating epididymal tails in a petri dish containing 0.9% NaCl solution at 37°C and observation under x 400 magnification. The sperm number was determined using a

haemocytometer, while sperm morphological abnormalities (micro and macrocephalies, tails winding) and integrity of the plasma membrane were determined using eosin-nigrosin solution and the hypo-osmotic test, respectively (Kenfack et al., 2018).

Histological structure of the testis

A left testis from each animal was processed for histopathology analysis using standard procedure. Briefly, the entire testis was fixed in 10.00% Bouin solution, then washed, dehydrated in alcohol baths of ascending grades, clarified in xylen, hardened in paraffin and sectioned. The tissue sections (5 µm) were stained with haematoxylin and eosin and observed under a light microscope (400X) for qualitative analysis in seminiferous tubules and intertubular space.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Differences between groups were assessed using one-way ANOVA followed by the Duncan's test. All analyses were performed using the SPSS 20.0 software.

RESULTS

Effects of nickel chloride on the weight and volume of organs

Nickel administration dose dependently decreased the relative weight of the kidney and accessory glands (seminal vesicle + prostate + coagulating glands) and at the highest dose of the chemical significantly ($p<0.05$) decreased the kidney volume and relative weight of the vas deferens (Table 1).

Biochemical markers of nephrotoxicity and hepatotoxicity

Cellular kidney and liver biomarkers of toxicity creatinine and urea concentrations, ALT and AST activities increased in guinea pigs treated with nickel chloride when compared to controls (Table 2). Specifically, Ni administration increased liver biomarkers and blood urea in dose-dependent manner while all doses of the chemical significantly elevated ($p<0.05$) serum creatinine and AST levels.

Effects of nickel chloride on sperm characteristics

Nickel chloride differentially affected sperm characteristics in the guinea-pigs (Table 3). The mobility, sperm count per tail and per gram of cauda epididymis, sperm with intact plasma membrane were significantly ($p<0.05$) lower in guinea pigs exposed to 52.50 mg/kg bw of nickel chloride compared to controls. The reverse was observed in the level of sperm with increased micro- and macrocephaly. The percentage of sperm tail windings was comparable ($p>0.05$) among treatments.

Effects of nickel chloride on serum testosterone

Serum testosterone level (Figure 1) decreased in dose-dependent manner, and this decrease was significant ($p<0.05$) in guinea pigs treated with 26.25 and 52.50 mg/kg bw when compared to controls.

Effects of nickel chloride on histological structures of testis

The cellular architecture of the seminiferous tubules was normal in control animals with different stages of the developing germ cells and intact lumen of the seminiferous tube containing mature sperms and the interstitial compartment (T_0). Nickel chloride administration dose-dependently disrupted cell arrangement and decreased sperms concentration in seminiferous tubules lumen. Moreover, this chemical at the dose 26.50 mg/kg (T_2) induced undifferentiation of germ cells in seminiferous tubule lumen. Reduced numbers of germ cells were also observed in the lumen of seminiferous tubules in the animals treated with the highest dose of nickel chloride 52.50 mg/kg (T_3) (Figure 2).

Effects of nickel chloride on the concentration of oxidative stress indicators

The administration of nickel chloride to guinea pigs significantly ($p<0.05$) increased the activity of enzymatic antioxidants (SOD and catalase) and dose-dependently elevated cellular lipid peroxidation (MDA levels) compared to the control animals (Table 4).

DISCUSSION

The evaluation of detoxifying organs and body weights is of a great importance for the appreciation of the toxic potential of a substance (Oloyede et al., 2011). Kidney plays a principal role in the toxicokinetics of Ni, since it serves as a major organ of Ni excretion and as a site of accumulation, as well as target organ of Ni toxicity (Saroj and Chengjiang, 1999). The increase of the kidney volume and weight in nickel chloride-treated guinea pigs in this study is similar to that reported by Amudha and Pari. (2011) in rat treated with Nickel sulfate (20 mg/kg. bw). The change in kidney weight/size suggests histological

Table 1. Effects of nickel chloride on the weights and volumes of kidneys and liver in male guinea pig.

Weights and volumes of kidneys and liver	Dose of nickel chloride (mg/kg bw)				P
	0.00 (n = 10)	17.50 (n = 10)	26.25 (n = 10)	52.50 (n = 10)	
Weights (g/100 g bw)					
Testis	0.51±0.08	0.50±0.07	0.54±0.06	0.53±0.09	0.68
Epididymis	0.10±0.01^{ab}	0.10±0.03^{ab}	0.11±0.02^{a}	0.09±0.01^{b}	0.04
Vas deferens	0.05±0.01^{a}	0.05±0.01^{a}	0.05±0.01^{a}	0.04±0.01^{b}	0.04
accessory glands	0.61±0.13^{a}	0.54±0.08^{ab}	0.48±0.11^{b}	0.46±0.08^{b}	0.03
Liver	2.77±0.29	2.79±0.35	3.05±0.45	2.77±0.48	0,02
Kidneys	0.71±0.04^{a}	0.67±0.05^{ab}	0.65±0.06^{b}	0.64±0.05^{b}	0,04
Volume (ml)					
Testis	2.98±0.62	2.92±1.06	2.65±0.75	2.61±0.61	0.80
Liver	14.00±3.05	14.00±1.41	12.60±2.70	13.50±4.96	0.86
Kidneys	3.78±0.30^{ab}	4.09±0.24^{a}	3.56±0.28^{bc}	3.28±0.59^{c}	0.00

Data are in mean ± standard deviation of 10 observations. a,b: Within the same line, values with the same letters are not significantly (p>0.05) different. bw: body weight.

Table 2. Effects of the nickel chloride on biochemical parameters of toxicity.

Biochemical marker	Dose of nickel chloride (mg/kg bw)				P
	0.00 (n = 10)	17.50 (n = 10)	26.25 (n = 10)	52.50 (n = 10)	
Creatinine (mg/dL)	6.52±0.54^{b}	10.32±1.18^{a}	9.20±0.81^{a}	9.55±0.71^{a}	0.00
Urea (mg/dL)	36.30±1.19^{b}	42.54±6.66^{ab}	43.30±6.11^{ab}	47.36±5.86^{a}	0.02
ALT (UI)	44.19±8,79^{b}	47.13±9.33^{b}	68.83±14.54^{a}	72.80±16.83^{a}	0.01
AST (UI)	56.70±7.21^{b}	92.75±15.54^{a}	99.75±14.85^{a}	102.90±17.64^{a}	0.00

Data are in mean ± standard deviation of 10 observations. a,b: Within the same line, numbers with the same letters are not significantly (p>0.05) different; for the same parameter different letters mean significant difference (p>0.05). bw: body weight.

Table 3. Effects of nickel chloride on sperm characteristics.

Sperm characteristics	Dose of nickel chloride (mg/kg bw)				P
	0.00 (n = 10)	17.50 (n = 10)	26.25 (n = 10)	52.50 (n = 10)	
Mobility (%)	87.50±5.00^{a}	75.00±8.37^{a}	75.00±12.91^{a}	55.00±12.91^{b}	0.00
Sperm number /cauda (x10^{6})	163.75±26.26^{a}	95.50±30.79^{b}	109.00±46.22^{b}	44.38±13.90^{c}	0.00
Sperm number/g of epididymis (x10^{6})	524.17±53.19^{a}	375.07±192.12^{ab}	379.88± 143.44^{ab}	199.29± 50.82^{b}	0.02
Spermatozoa with IPM (%)	52.25±11.84^{a}	45.00±10.97^{ab}	42.83±12.02^{ab}	34.50±7.42^{b}	0.04
Micro and macro-cephalies (%)	11.50±1.87^{b}	16.20±3.49^{a}	12.40±0.89^{b}	16.60±2.41^{a}	0.00

a,b,c: Within the same line, numbers with the same letters are not significantly (P >0.05) different; P: probability; IPM: integral plasma membrane; n: number of guinea pigs; bw: body weight.

modifications in the organ. This hypothesis was investigated through measurement of biochemical markers of nephrotoxoxicity such as creatinine and urea levels, which were all increased in the animals exposed to the nickel chloride. Likewise, previous investigations on nickel revealed necrotic changes of renal tubules in males (Amudha and Pari, 2011; Kadiand and Dahdouh, 2016). Nickel chloride has also exhibited hepatotoxicity potential as illustrated by increased serum levels of AST and ALT activities upon exposure to the heavy metal in this study. The increased levels of liver markers after nickel treatment reflects its interaction with the cell membrane, leading to altered cell membrane permeability and increased enzyme leakage (Djemli Samir et al., 2012; Oluyomi et al., 2017).

Heavy metals have been shown to affect various end-points on male reproductive function including hormone, germ cells and weights of the reproductive organs (Manfo et al., 2014). A significant decrease serum testosterone levels was recorded in nickel-exposed guinea pigs. Testosterone synthesis involves several enzymes which

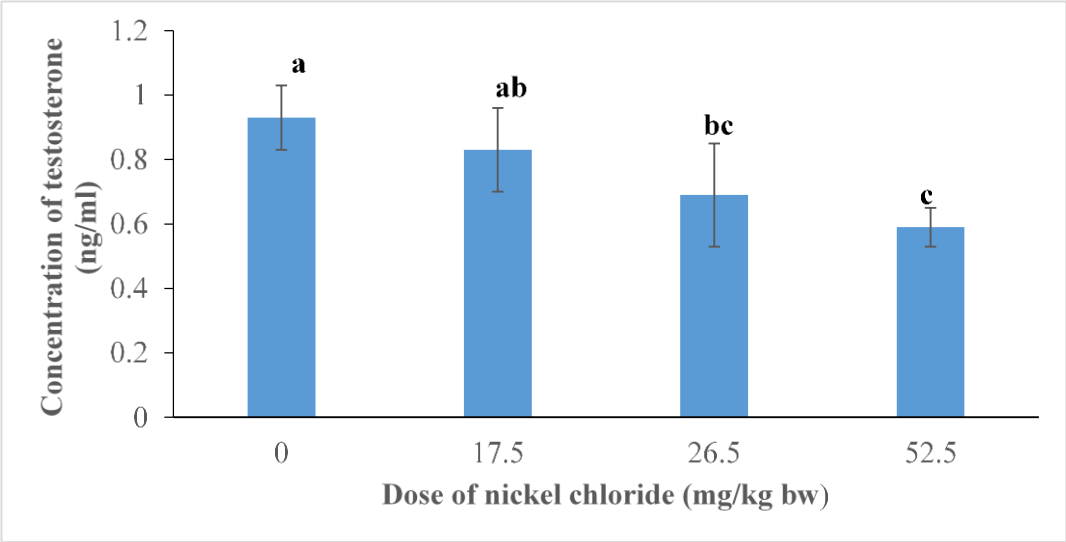

Figure 1. Effect of nickel chloride on the concentration of testosterone. a,b,c: bars with the same letters are not significantly (p>0.05) different.

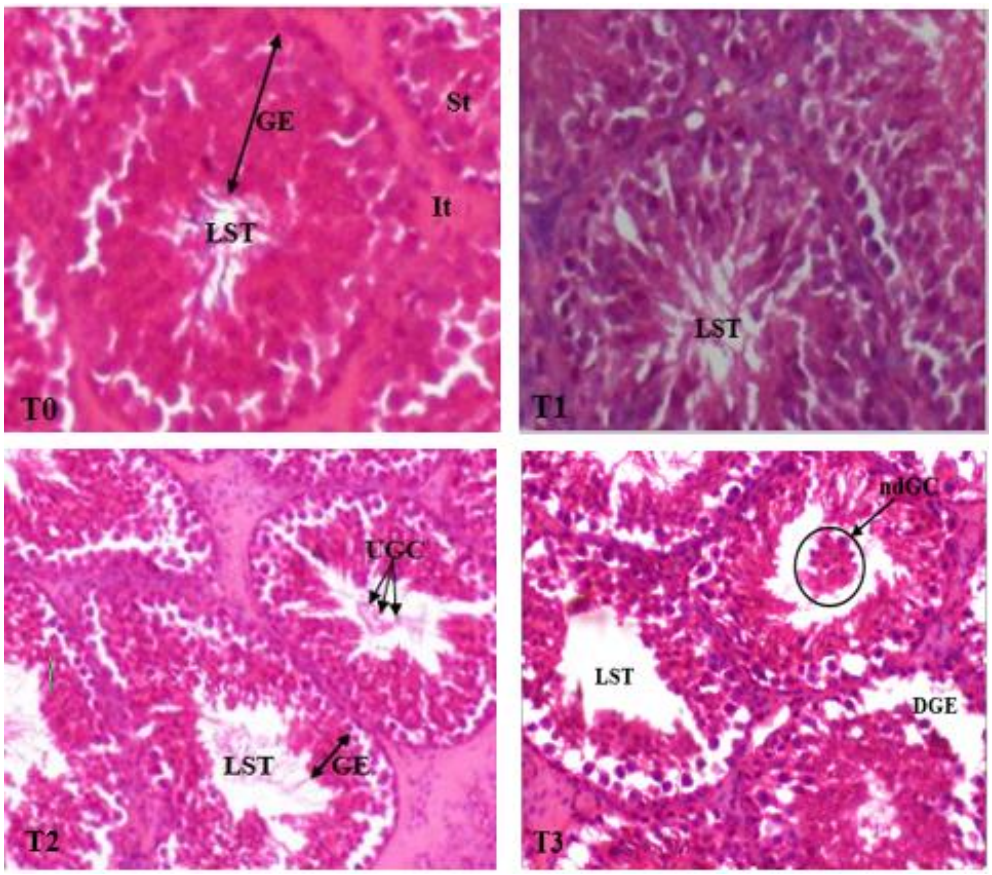

Figure 2. Testis histological sections of guinea pigs exposed to nickel chloride (HE x 400). **ndGC**: non differentiated germinal cells; **Is**: interstitial space; **It**: interstitial tissue; **LST**: seminiferous tubule lumen; **St**: seminiferous tubule; **GE**: germinal epithelium; **DGE**: degradation of the germinal epithelium; **UCG**: undifferentiated germ cells; **T0**: control; **T1**: guinea pigs treated with 17.50 mg/kg; **T2**: guinea pigs gavaged with 26.25 mg/kg; **T3**: guinea pigs treated with 52.50 mg/kg body weight.

Table 4. Effects of nickel chloride on testicular oxidative stress parameters.

Oxidative stress markers	Dose of nickel chloride (mg/kg bw)				P
	0.00 (n = 10)	17.50 (n = 10)	26.25 (n = 10)	52.50 (n = 10)	
MDA (nM/g of testis)	2.58±0.51^{d}	8.39±1.38^{c}	13.29±1.56^{b}	18±1.45^{a}	0.01
CAT (µmole/min/g of proteins)	9.42±2.45^{c}	13.36±1.98^{ab}	11.85±1.12^{bc}	15.72±4.25^{a}	0.04
SOD (U/g of proteins)	0.22±4.46^{b}	0.36±4.28^{b}	0.33±1.55^{b}	0.81±2.51^{a}	0.02
PEROX (µmole/min/g of proteins)	14.37±0.43	14.16±0.25	14.24± 0.20	13.75±0.20	0.32

Data are in mean ± standard deviation of 10 observations. a,b: Within the same line, numbers with the same letters are not significantly (p>0.05) different; for the same parameter different letters mean significant difference (p < 0.05). bw: body weight. MDA: Malondialdehyde, SOD: superoxide dismustase, PEROX: total peroxydases, CAT: catalase.

are likely to be affected by heavy metal, though the detailed biosynthetic pathway has not been investigated herein (Payne and Hales, 2014). Das and Dasgupta (2000) found that nickel exert an inhibitory effect on the activity of two testicular steroidogenic enzymes, 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD), which plays an indispensable role in testosterone synthesis. This detrimental effect of nickel on testosterone secretion has been previously reported in rats (Pandey et al., 1999; Das and Dasgupta, 2002).

Testosterone is the primary sex hormone with potent anabolic properties in various animal systems. In male, testosterone plays a key role in sexual arousal, libido and development and maintenance of reproductive organs (Motofei and Rowland, 2005; Jordan and Don Carlos, 2008). Therefore, any disturbance in testosterone production and/or action may result in serious consequences on the reproductive function. The decrease in reproductive organ weight and deterioration in spermatozoa quality and quantity may thus be related at least partly to reduction of testosterone levels following exposure to nickel chloride in the current study. The results in this study are similar to those of Pandey et al. (1999) who demonstrated that in Nickel-exposed rats that spermatozoa damage was associated with a decrease in testosterone.

One of the main aetiological factors for defective spermatogenesis or male infertility is the disturbance of the antioxidant – pro-oxidant testicular homeostasis in favour of oxidant causing oxidative stress (Pham-Huy et al., 2008). In this study, nickel chloride increased catalase and SOD activities and testicular lipid peroxidation levels. Catalase and SOD are antixodant enzymes that metabolise reactive oxygen species (ROS) (Pham-Huy et al., 2008), and nickel has been show to induce oxidative stress in the testicular environment. The decrease in the activity of these antioxidant enzymes has resulted into induction of oxidative stress in the testis, as illustrated by elevated MDA levels. The oxidative stress caused by chloride nickel in the testis could therefore affect sperm cells. Such a hypothesis could explain the decrease in low sperm characteristics in animals treated with chloride nickel. Sperm cells contain high levels of polyunsaturated fatty acids that may be subjected to peroxidation by ROS (lipid peroxidation) causing a permeabilization of cell membranes, which produce a loss of sperm viability and mobility. Moreover, sperm cells have a limited store of antioxidant molecules/enzymes which could protect against oxidative stress (Garrido et al., 2004; Mathur et al., 2011), thereby rendering the cells more vulnerable to ROS. This could explain the decreased sperm mobility, sperm number and increased sperm malformation observed in the animals exposed to nickel chloride. Moreover, treatment with this heavy metal resulted in disturbance of cellular architecture of the seminiferous tubules, as shown by histological analyses (Figure 2). Literatures also support alteration of antioxidant status, sperm parameters in animals exposed to heavy metal (Pandey et al., 1999; Das and Dasgupta, 2002; Knazicka et al., 2015).

Conclusion

Administration of nickel chloride for 90 days in male guinea pigs perturbs guinea pig male reproductive parameters and induced testicular oxidative stress. The use of nickel rich agricultural entrants should therefore be discouraged within the vicinity of guinea pigs breeding.

CONFLICTING INTERESTS

The authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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