Biokemistri
Vol. 32, No. 1, March 31, 2020, pages 1 - 10
Printed in Nigeria

0795-8080/2020 $10.00 + 0.00

http://www.niseb.org.ng/journals
Also available online at http://www.bioline.org.br/bk

BKR 2020003/32101

Heavy metals correlate with cellular adenosine triphosphate and fructose levels in municipal dumpsite exposure

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(Received January 16, 2020; Accepted March 9, 2020)

ABSTRACT: This study assessed the influence of direct exposure of rodents to Awotan Solid Waste Dumpsite (ASWD) in Ibadan, Nigeria. Reproductive energy content was evaluated by assessing testicular ATP activity, testicular polychlorinated biphenyls (PCBs), testicular heavy metal and fructose levels in seminal vesicle and coagulating glands. The exposure of the male rats was from postnatal day 22 and for a period of 10 weeks. The direct municipal dumpsite exposure resulted in testicular heavy metal accumulation viz: mercury > iron > zinc > nickel > cadmium > arsenic. There was a decrease in serum lactate dehydrogenase (LDH) concentration, an increase in testicular LDH concentration (markers of cellular ATP) and a decrease in the fructose level in the seminal vesicle of the dumpsite exposed (DSE) animals compared with the control. The PCBs were not detected in the testis of DSE animals but there was significant correlation between testicular LDH and some metals. It is concluded that some possible mechanisms by which direct exposure to a dumpsite elicits energy depletion in the male rat testes could be through the inhibition of fructose level and LDH activity.

Keywords: Heavy metal, ATP, Fructose, Dumpsite, Polychlorinated biphenyls

Introduction

Reproductive disorders, high infertility rate, increased birth defects, repressed immunological function, and increased frequency of cancers have been linked to exposure to landfill sites (1,2). Landfill which refers to the deposition of waste in an area specially designed for such is a major method in waste management hierarchy in developing countries (1). Landfills may contain household or municipal wastes, industrial, biomedical or infectious wastes (3). The landfills thus can contain toxic substances such as heavy metals, dissolved organic matter, polychlorinated biphenyls and inorganic macro compounds. Some of these xenobiotics have been classified by The United States Environmental Protection Agency to be of concern to the male reproductive health (4).

Heavy metal environmental pollution even at low levels and their subsequent long-term collective health effects are among the leading causes of health concerns the world over (5). There have been studies suggesting that heavy metals are a major source of oxidative stress in the cell and that they play an important role in the aetiology of diverse human pathologies like carcinogenesis (6). Research has also revealed that heavy metals directly modify and /or damage the DNA structure (7). There have also been adverse effects of these metals on male reproduction (8). The adverse effects include altering the morphology and functional systems, fluctuating secretory functions as well as altering energy balance resulting in infertility or impotence (9). Exposure to cadmium for instance has been reported to affect the prostate function and serum testosterone levels (10). It was reported that workers exposed to chromium (VI) had significantly higher serum follicle stimulating hormone (FSH) concentration and lower seminal plasma zinc levels, sperm concentration, motility and lactate dehydrogenase (LDH) levels (11) and significantly higher percentage of abnormal sperm than control workers (12).

Lactate dehydrogenase is involved in metabolic processes of sperm in the seminal plasma and is closely related to reproductive performance of the males (13). Lactate dehydrogenase (LDH-C4) is known as a major isoenzyme of sperm synthesized in the testes during spermatogenesis. Lactate dehydrogenase and its isoenzyme fulfils a specialized function for the metabolic development requirements of adult male (13) and it is related to metabolic processes by which the stem cells obtain their energy (14). Lactate dehydrogenase and its isoenzyme are important markers of seminiferous epithelium activity in the diagnostics and treatment of male infertility (15). An *in vitro* study revealed that LDH-C4 is also involved in sperm capacitation (16). Lactate dehydrogenase activity is a marker of cellular adenosine triphosphate (ATP) thus the level of cellular ATP during anaerobic conditions has been widely assessed using LDH activity. The role of adenosine triphosphate (ATP) as the energy metabolism of the mitochondria is a major factor supporting multiple functions of the sperm. They harbour significant metabolic pathways during germ cell development and fertilization, energy maintenance and fertility (17). Testicular energy metabolism is desired to maintain spermatogenesis and research reveals that the germ cells depend on lactate for energy. Lactate dehydrogenase is associated with survival and maturation of germ cells and adenosine triphosphate production.

The occurrence of health symptoms with solid waste management workers, as well as people living near municipal dumpsites is increasing (18,19). Previous studies have linked municipal dumpsites to the adverse effects of leachates on sperm and testicular activities (20-24). It remains to be determined if there is a link between the direct exposure to municipal landfill and its effects on the male reproductive activity.

The fact that the Awotan Solid Waste Dumpsite is located not too far from a residential settlement and the possibility of using the solid waste dumpsite mixture for agricultural soil enrichment, considered this study of importance, to evaluate the reproductive energy content in the male rats. The energy content was evaluated by assessing adenosine triphosphate energy production, testicular polychlorinated biphenyl content, metal content on testicular function. Also, fructose levels in the seminal vesicle and coagulating glands, of directly exposed rats to the municipal dumpsite were assessed.

Materials and Methods

Study area

The study area, Awotan Solid Waste Dumpsite (ASWD) is one of the three government approved open dumpsites in the city of Ibadan, Nigeria. It is located within 07’27.719’-07’27.811’ North and 003’51.003’- 003’50.999’East, Ibadan. The ASWD receives 36000 tonnes of municipal wastes in an area of 14 hectares and it has been active since 1998 (25). Residential settlements, block industries, markets, schools and churches are located within thirty meters radius to the dumpsite.

Animal Model and Experimental Protocol

All procedures of animal handlings and experimental protocols conformed with the internationally accepted guideline for laboratory animal use and care (NIH publication No.85-23, revised 1985), as approved by the Institutional Animal Care and Use Ethics Committee of the College of Medicine, University of Lagos. Brown rats (*Rattus norvegicus*) of 22 days old were housed in the vicinity of the Awotan Solid Waste Dumpsite for 10 weeks and they fed on dumpsite waste. The rats were exposed to the municipal waste according to Luvizutto *et al.*, (26) with slight modifications. The municipal solid waste which is a mixture of complex materials (food wastes, market wastes, microorganisms, plants, chemical contaminants and minerals) was given to the rats at 5000ppm *ad libitum.* This served as the dumpsite exposed (DSE) group (n=12). Daily monitoring of the rats was done to observe any clinical signs. The laboratory rats (*Rattus norvegicus domesticus*) of 22 days old were bred in the Institution's laboratory animal facility and they served as the control (n=12). The rats were provided with rat pellets and water *ad libitum*. All animals were subjected to a natural 12:12h light-to- dark photoperiod. Rats were maintained under the controlled conditions of temperature (35 ± 2˚C) and humidity (50 ± 5%). At the end of the 10 weeks' period, DSE animals were returned to the laboratory animal facility. Twenty-four hours after the DSE animals were brought to the laboratory facility, the blood from the animals was drawn from retro-orbital venous plexus for serum lactate dehydrogenase (LDH) assay. The animals were then killed by cervical dislocation. The testes, epididymis, seminal vesicles/coagulating glands were removed rapidly and weighed. The testes, seminal vesicle and coagulating glands were processed for further assays.

Lactate Dehydrogenase (LDH) Assay

The serum and testicular homogenates were assayed for LDH activity using a commercially available kit (Randox Laboratories, UK). The LDH assay was performed and according to the manufacturer's instructions.

Fructose Assay

Fructose level was estimated from the seminal vesicles and coagulating gland homogenate by means of Fructose test kit (FertiPro N.V. Industriepak Noord 32, 8730 Bernem, Belgium). The assays were carried out according to the manufacturer's instructions. The seminal fructose was done photometrically.

Testicular Heavy Metal Analysis

The right testis of animals from each group were washed in ice-cold 1.15% KCl solution, then blotted and weighed. They were subsequently homogenized in 4 volumes of homogenizing buffer (50mM Tris-HCL mixed with 1.15% KCL [pH adjusted to 7.4]) using a homogenizer. The testes supernatants were used to determine heavy metal levels (27). The levels of lead, copper, nickel, zinc, iron, cadmium, arsenic, and mercury in the filtrates of the digested samples were determined using an atomic absorption spectrophotometer (Perkin Elmer A Analyzer 200, USA).

Polychlorinated biphenyls (PCBs) concentration determination

The testis sample (2g) each, was weighed into 250ml conical flask and 10ml of dichloromethane was added to the sample. The mixture was shaken vigorously on the shaker for about 45minutes. It was then transferred to the ultrasonic bath for ultrasonic extraction for 2hours. The isolation of Polychlorinated biphenyls (PCBs) from the lipid matrix was done by solid phase extraction in a normal phase mode. Activated silica gel was loaded onto a glass chromatographic column (i.d 20 mm, height 400 mm) and conditioned with dichloromethane. The effluents were then concentrated using a rotary evaporator and the samples were thereafter dissolved in 1 ml acetone and transferred to viral bottles for Gas Chromatography Mass Spectroscopy (GCMS) analysis. The analyses were performed with Agilent Tech model 7890A gas chromatograph system coupled with an MS Agilent Tech 5975C VL MSD (USA). The identification of the found components was confirmed by comparison of the retention indices with those of authentic compounds and with the NIST 02 library.

Statistical Analysis

The results were presented as mean $\pm$ standard error of mean (SEM) and were subjected to post hoc test using Duncan's multiple range tests using GraphPad Prism-5 statistical software (San Diego, California, U.S.A.). The differences were considered significant at $p < 0.05$.

Results

Body Weight and Absolute Reproductive Organ Weights

There was no significant difference in the final body weights of the control and the DSE animals. However, the absolute weights of the epididymis, testis and seminal vesicle and coagulating glands were significantly increased in the DSE animals compared with the control group (Table 1).

Table 1: Body weight and Reproductive organ weights after DSE for 10 weeks in rats

Variables	Control	DSE
Body weight initial (g)	40.01 ± 0.01	40.01 ± 0.01
Body weight final (g)	202.40 ± 4.17	185.30 ± 7.65
Epidydimal weight (g)	0.39 ± 0.01	0.58 ± 0.06*
Testicular weight (g)	0.48 ± 0.01	0.72 ± 0.03*
Seminal vesicle and CG weight (g)	0.39 ± 0.01	2.25 ± 0.07*

Data are expressed as mean ± SEM for 12 rats/ group. *p <0.05 compared with the control

Testicular Heavy Metal Concentrations

The exposure of the rats to the dumpsite resulted in a significant increase in the levels of nickel, zinc, iron, cadmium, arsenic and mercury accumulation in the testis of the animals compared with the control (Figs 1 and 2). However, there was a significant decrease in the level of lead in the DSE animals compared with the control (Fig 2).

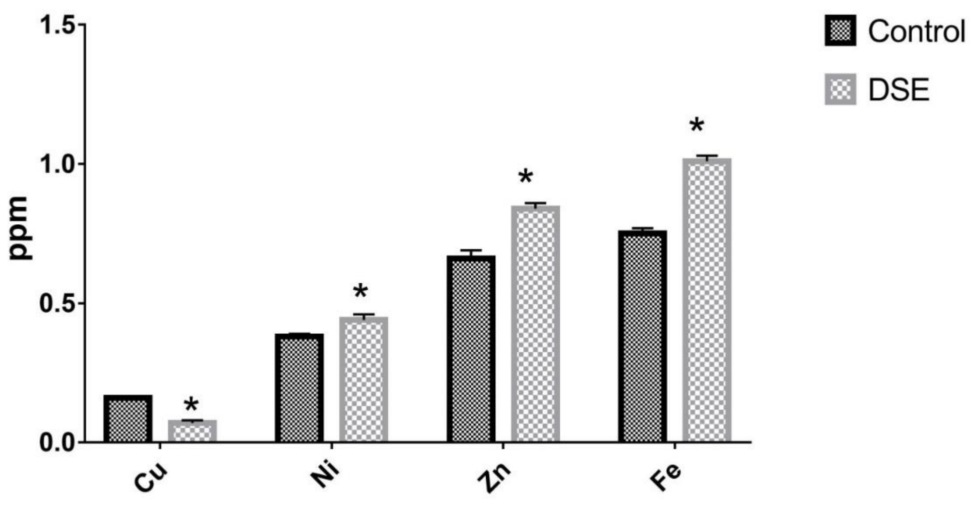

Fig. 1 Levels of Cu, Ni, Zn, and Fe in testes of rats after 10 weeks of dumpsite exposure (DSE). Each bar represents mean ± SEM of 12 rats. *Values differ significantly from the control (p<0.05).

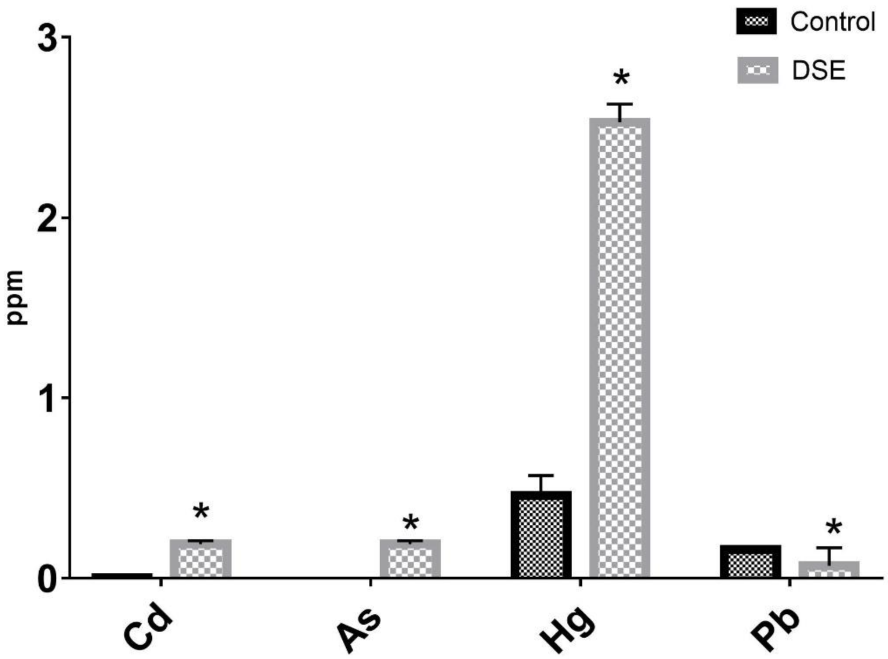

Fig. 2 Levels of Cd, As, Hg, and Pb in testes of rats after 10 weeks of dumpsite exposure (DSE). Each bar represents mean ± SEM of 12 rats. *Values differ significantly from the control ($p<0.05$).

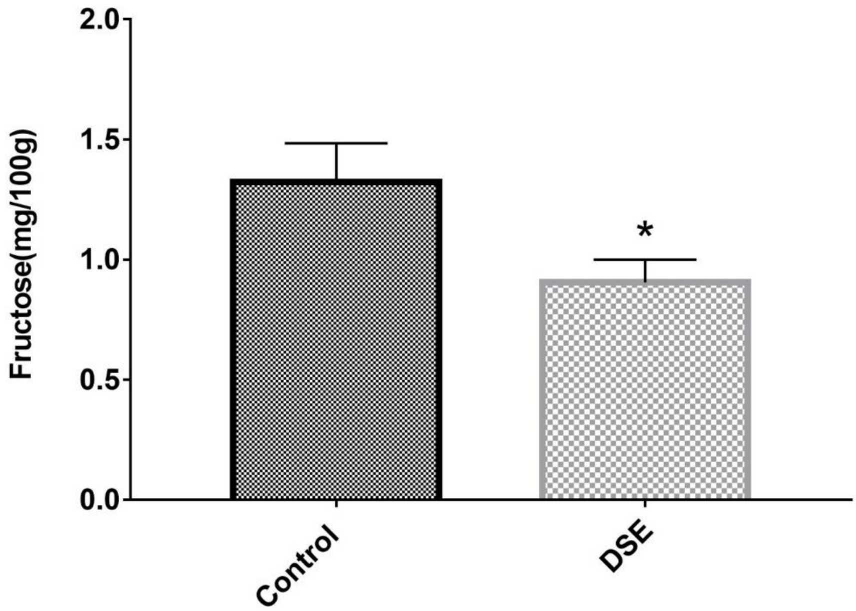

Fig. 3 Fructose level in seminal vesicle and coagulating glands of rats after 10 weeks of dumpsite exposure (DSE). Each bar represents mean ± SEM of 12 rats. *Values differ significantly from the control ($p<0.05$).

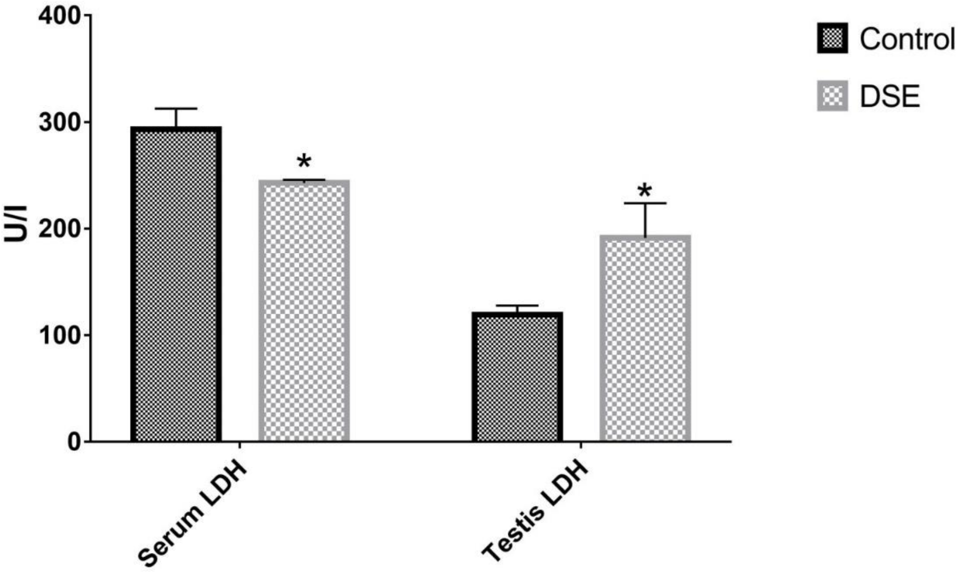

Fig. 4 Serum and testis LDH levels of rats after 10 weeks of dumpsite exposure (DSE). Each bar represents mean ± SEM of 12 rats. *Values differ significantly from the control (p<0.05).

Seminal vesicle and coagulating gland Fructose level

There was a significant decrease in the level of fructose found in the seminal vesicle and coagulating glands compared with the control (Fig 3).

Activity of Serum and Testicular Lactate Dehydrogenase (LDH) (Functional marker of ATP).

The activity of the serum LDH was significantly inhibited in the rats from the dumpsite compared with the control rats however, the dumpsite exposure was associated with a significant increase in the testicular activities of LDH compared with the control (Fig 4). Testicular LDH significantly correlated with mercury and copper (Table 2).

Polychlorinated biphenyls (PCBs) concentration

Polychlorinated biphenyls (PCBs) were absent in the testis of the dumpsite-exposed animals.

Table 2: Correlation matrix between Heavy metals and Testicular LDH, Serum LDH, Fructose in control animals

		Cu	Ni	As	Zn	Hg	Fe	Cd	Pb
	Testicular LDH	0.66*	0.30	0.47	-0.36	0.70	0.67	0.72	0.72
Control	Serum LDH	-0.1	0.10	0.01	-0.31	0.18	0.47	-0.36	-0.36
	Fructose	-0.43	0.06	0.02	-0.58	-0.24	-0.49	-0.95	-0.95**

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

Table 3: Correlation matrix between Heavy metals and Testicular LDH, Serum LDH, Fructose in dumpsite exposed (DSE) animals.

		Cu	Ni	As	Zn	Hg	Fe	Cd	Pb
	Testicular LDH	0.77*	-0.19	0.04	-0.60	0.83*	0.12	-0.23	0.54
DSE	Serum LDH	-0.10	-0.08	0.38	-0.22	-0.14	0.27	0.27	0.41
	Fructose	-0.55	0.01	-0.04	0.30	-0.62	-0.62	-0.62	-0.03

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

Discussion

There was an accumulation of mercury, arsenic, cadmium, iron, zinc and nickel in the testes of the dumpsite exposed (DSE) animals. A previous study from our laboratory (28) reported that heavy metal contents found in the soil of Awotan Solid Waste Dumpsite (ASWD) which is the study area for this research were above the USEPA permissible limits which would have resulted in the accumulation of these toxic heavy metals signifying their roles in testicular toxicity observed in the DSE animals. Although the levels of lead and copper were above the USEPA permissible limits in the soil of ASWD, they were not accumulated in the testis of the animals exposed to the dumpsite. A probable reason is that metals interact additively, synergistically as well as antagonistically and affect each other's absorption, distribution and excretion. This interference with metabolism can reduce the concentration in the organism or decrease the bioavailability. The competition between lead and/or cadmium and zinc for the same binding sites in enzymes, proteins, and transporters can affect structure and/or function of cell membranes, change enzyme activity, induce oxidative stress and apoptosis which all have grave consequences on cell growth, development, and differentiation. Thus, these interactions contribute to interindividual differences in susceptibility to adverse effects of metals in men (29).

According to a study by Sharpe *et al.* (30) sertoli cells proliferate during the foetal, neonatal, and pre-pubertal periods and each of these periods is particularly sensitive to the adverse effects of metals. Testicular toxicity in rats has also been shown as a good predictor in human subjects (31). Arsenic and cadmium which are classified as the first and seventh most hazardous substances (32) were significantly increased in the DSE group. Arsenic and its compounds have been reported to disrupt ATP production when exposed to animals by way of water drinking (33). The observation in this study corroborates other studies that reported that cells exposed to arsenic showed a considerable depletion in ATP and glycogen levels in the liver and other tissues. Cadmium has been reported to cause severe damage to spermatogenic epithelium in an animal model (34). Other reports have shown that at concentrations greater than (0.003 mg/L), cadmium had direct injury on the testes by damaging germ cells and Sertoli cells and subsequently reducing sperm quality (34). The increase in cadmium level in the dumpsite-exposed animals also confirms the fact that cadmium accumulates in the male reproductive organs, especially the testis of both humans and animals (35). Cadmium has been shown to negatively affect accessory sex organ functions (10).

The increase in testicular and epididymal weights (36) has been reported in cadmium exposure which corroborates the findings of this study. The increase in the organ weights generally indicates an excessive accumulation of interstitial fluid and maybe a sensitive indicator of decreased sperm production (36). Some studies have earlier reported that cadmium mediated histological changes in the testes, epididymis and accessory sex organs (37). Moreover, exposure to cadmium, lead and arsenic may contribute to prostate cancer development and some reports have associated their exposure with increased serum prostate specific antigen (PSA) (10). Metals can affect the male reproductive system directly when they target specific reproductive organs. When there is an accumulation of metals in the testis, epididymis, prostate, seminal vesicle, they impair progressive sperm motility (38). Metals affect the secretory function of the seminal vesicles leading to the loss of fertility, libido or impotence (39).

The seminal plasma is a mixture of secretions derived principally from the major accessory sex glands such as the seminal vesicle, prostate, Cowper's glands with minor contributions from the epididymis, ampulla gland etc. Since neither the testis nor epididymal sperm contains fructose, the immotile spermatozoa encounter fructose only after it has been intermixed with seminal plasma during ejaculation. This occurs at a crucial moment when the sperm requires a high degree of motility requiring a source of quick available energy. This energy is provided by the metabolic process of fructolysis during which lactic acid is formed. Since growth and secretory function of the accessory sex glands are under the control of the testis, the level of fructose in seminal plasma appears to be a precise indicator of the function of the Leydig cell system of the testis. The decreased fructose level as well as an increased accumulation of heavy metals in the testis observed in this study is consistent with the earlier report. Scientific evidence suggests that hypofunction of these accessory sex organs affects sperm motility and sperm chromatin stability which may produce infertility (41). The seminal fructose concentration is an assessment of the seminal vesicle and could also give useful indications of the male reproductive function (42).

The level of cellular adenosine triphosphate (ATP) during anaerobic conditions had been widely assessed using LDH activity because it is a stable enzyme. LDH is an oxidoreductase enzyme that catalysis the interconversion of pyruvate and lactate. Cells release LDH into the bloodstream after tissue injury. Animals exposed to the dumpsite had a significant depletion of LDH activity in the serum. Inhibition of LDH which is a key enzyme of glycolytic pathway caused by the dumpsite exposure in the absence of oxygen which is needed for ATP production would slow down the metabolic pathway in charge of energy production. This outcome supports (43,44) who had previously reported that patients with abnormal spermatogenesis had low levels of ATP. Additionally, ATP is necessary for the synthesis of cholesterol, ketone bodies and fatty acids. In aerobic conditions, pyruvate is converted to acetyl CoA catalyzed by pyruvate dehydrogenase which may be oxidized in the tricarboxylic acid (TCA) cycle to produce ATP. The findings in this study thus suggest that heavy metals in the dumpsite could disrupt ATP production by inhibiting pyruvate dehydrogenase when competing with the phosphate group thus uncoupling oxidative phosphorylation. This inhibits energy-linked decrease of NAD+, mitochondrial respiration, and ATP synthesis in the serum. It is interesting to note that a different interplay of events occurred in the testis of the DSE animals, as there was an increase in the level of LDH activity in the testes. Lactate dehydrogenase is the functional marker of ATP and it is required in its production. LDH is also connected with existence and development of germ cells. The increase in testicular LDH activity thus observed in this study may indicate an adaptive mechanism by the testes to assuage germ cell death and azoospermia in DSE rats.

The significant correlation between the testicular LDH, mercury and copper, as well as fructose and nickel and lead in the DSE animals further buttress the fact that the heavy metal bioaccumulation in the testis affected the energy supply to the testis thus the reason for the adaptive mechanism. Polychlorinated biphenyls (PCBs) are one of the groups of persistent organic pollutants and they have been linked to reproductive difficulties (45). In this study PCBs were absent in the testes of the DSE rats. According to a study (46), the PCB levels in the AWSD topsoil were below detection limit which would have been the reason it was not detected in the testis of the DSE rats. This however does not rule out the possibility of the presence of organic pollutants at the dumpsite as there was an accumulation of polyaromatic hydrocarbons (PAHs) according to their study.

Conclusions

The direct exposure to a municipal dumpsite elicits detrimental effects on the energy supplies needed for male reproductive activity. The exposure compromised testicular LDH and decreased the LDH activity in the serum which are markers of cellular ATP. The inhibition of LDH activity and a decrease in fructose levels observed could be possible mechanisms by which direct dumpsite exposure elicited energy depletion and toxicity in the male rat. PCBs were absent thus the biotoxic effects and energy depletion could be linked to heavy metal interactions which was additive, synergistic, antagonistic and individual in nature. Indeed, an alteration in LDH and fructose levels would be expected to compromise the energy supplies to germ cells. The human male has relatively low fertility potential compared with other mammals and is much more susceptible to metal toxicity, there is therefore the need for a critical assessment and discouragement of siting residential areas near dumpsites.

Conflicts of interest

The authors declare they do not have any conflicts of interest.

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