Heliyon 5 (2019) e01848

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.heliyon.com

Chronic consumption of calabash chalk diet impairs locomotor activities and social behaviour in Swiss white Cd-1 mice

Bright Owhorji [a], Udemeobong Okon [b], Azubuike Nwankwo [a], Eme Osim [b,*]

[a] Department of Physiology, College of Medicine and Health Sciences, Abia State University, Uturu, Nigeria
[b] Department of Physiology, College of Medical Sciences, University of Calabar, Nigeria

ARTICLE INFO

Keywords:
Neuroscience
Physiology

ABSTRACT

There are safety concerns as regards the consumption of Calabash chalk which is common practice in some localities in Africa and Asia. Calabash chalk contains lead (Pb) and arsenic which are believed to be harmful to the brain and responsible for cognitive dysfunction. It is possible that calabash chalk consumption may affect other neuronal activities in the body such as locomotion and social behaviour. Hence, this present research study investigated the effects of consumption of this diet on locomotion and social behaviour in mice. Forty-five Swiss white mice of mixed sex were randomly assigned into 3 groups of 15 mice each. Group 1 served as control, while groups 2 and 3 received low and high doses of calabash chalk diets respectively. Feeding lasted for 30 days and thereafter their locomotor and social behaviors were assessed. Their locomotor behaviour was assessed using the open field maze while their social behaviour was studied with the aid of nesting behaviour test. Results showed that the calabash chalk diet-fed mice had significantly reduced ($p < 0.05$) line crossing frequency compared to control. The nesting score of the calabash chalk diet-fed mice was significantly lower ($p < 0.05$) compared to control. In conclusion, consumption of calabash chalk impairs locomotion and social behaviour in mice.

1. Introduction

Geophagia, the practice of eating the earth, including soil and chalk (Abrahams et al., 2013) is a practice associated with religious beliefs, medication or as part of a regular diet (Dean and Ma, 2007). A review of literature clearly indicates that geophagia is not limited to age group, race, sex, geophagic region or period, although today the practice is most obviously common amongst the world's poorer or more tribally oriented people and is, therefore, particularly extensive in the tropics (Abrahams and Persons, 1996).

Calabash chalk is a geophagic material popularly consumed in Nigeria and other West African countries for pleasure, and by pregnant women as a cure for nausea (Abrahams et al., 2013). This material is identified by different names such as Calabar stone in English, La Craie or Argile by the French, Mabele Lingala in Congo, Nzu by the Igbo tribe of Nigeria, Ndom by the Efik/Ibibio of Nigeria. It is also known as Ebumba, Poto and Ulo in other places (Abrahams et al., 2013).

Dean et al. (2004) reported Aluminium silicate hydroxide-$Al_2Si_2O_5(OH)_4$, as the major component of Calabash chalk. This is a member of the Kaolin clay group. Multi-elemental analysis was able to quantify 22 elements in calabash chalk including lead at a mean concentration of approximately 40 mg/kg (Dean et al., 2004). In addition, a range of persistent organic pollutants were identified including alpha lindane, endrin, endosulphan 11 and p,pI- dichlorodiphenyl dichloroethane (DDD). Other metals identified included Iron, aluminium, potassium, titanium, chromium, manganese, zinc, nickel as well as the metalloid arsenic (Dean et al., 2004; Ekong et al., 2012).

Research studies on calabash chalk revealed hepatic sinusoidal enlargements, gastrointestinal damaging and anaemic effects (Ekong et al., 2014). Lead and even arsenic constituents of calabash chalk have been reported to be associated with nervous and brain damages as well as learning and behavioural dysfunctions in animals and humans. Our recent study showed elevated anxiety and pain perception in mice following chronic calabash chalk diet consumption (Owhorji et al., 2018).

Since calabash chalk consumption which contains lead, arsenic as well as other neurotoxic constituents affects neuronal components of the body, it becomes necessary to find out the possible effects on locomotion and social behaviour using mice as experimental models.

* Corresponding author.
E-mail address: emeosim@yahoo.com (E. Osim).

https://doi.org/10.1016/j.heliyon.2019.e01848
Received 10 July 2018; Received in revised form 16 November 2018; Accepted 24 May 2019

2. Materials and methods

2.1. Preparation, storage and analysis of experimental diet

Calabash chalk was procured as a large block from a local market in Calabar and pulverized with manual grinder to obtain a fine powder. The powder was kept in dry and air-tight rubber container from which calabash chalk diets were prepared. Ten percent (10%) calabash chalk diet was prepared by mixing 1 g of calabash chalk with 9 g of rodent chow. This served as low dose. Twenty percent (20%) calabash chalk diet was prepared by mixing 2g of calabash chalk with 8 g of rodent chow. This was high dose. These diets are similar to the percentages ingested by several individuals in their local diets. The calabash chalk was then analyzed for some major and trace elements, using the flame atomic absorption spectrometer (Unicam-939).

2.2. Experimental animals

Forty-five (45) Swiss white CD-1 mice weighing between 22 – 35 g were used for this study. They were kept in well ventilated room at room temperature (28 ± 2 °C) and humidity (85 ± 5%). The animals were kept in a normal 12/12 light/dark cycle and allowed to acclimatize for two (2) weeks before commencement of experiments. They were granted access to feed and clean drinking water *ad libitum.*

2.3. Experimental design

The fort-five mice were randomly assigned into 3 groups of 15 mice per group. Group 1 served as the control and was fed rodent chow only. While group 2 was fed low dose calabash chalk diet, group 3 received high dose calabash chalk diet. The animals were placed on their respective diets for 30 days. This may be considered long term especially for rodents, such as mouse with a life span of only six months. Their food and water intake were measured daily, whereas, their body weight were assessed after every three day interval. Thereafter, their social behavior and locomotor capabilities were assessed. Approval for the use of the animals was obtained from the College Ethical Committee of the Faculty of Basic Medical Sciences, Abia State University Uturu, in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

2.4. Test for locomotion

The Open field maze (OFM) which provides simultaneous measures of locomotion, exploration and anxiety was employed in this study. The apparatus was constructed from white plywood with 72 × 72 cm floor and 36 cm walls. One of the walls is made up of clear Plexiglas floor. The lines divide the floor into sixteen 18 × 18 cm squares from which a central square is marked at the middle of the open field (Brown et al., 1999). Each mouse was scooped up in a small plastic container from its home cage and placed at the center square of the OFM, then allowed to explore the apparatus for about 5 minutes while observing the behaviors (frequency of line crossing, frequency of walling, frequency of rearing, frequency of center square entry and grooming frequency) after which it is returned to its home cage. The OFM was cleaned with 75% ethyl alcohol and permitted to dry between trials to eliminate scent clues left by the previous subject mouse.

2.5. Assessment of social behaviour

Nesting behavior test used by Bender et al. (1996) and Deacon (2006) as an assay for social behavior was used in this present study. The test was conducted in individual home cages. An hour before giving the mice nestling materials, all enrichment objects were removed from the home cages of the mice. About 3.0 g of nestling material was supplied to each mouse in its home cage and allowed for 24 h after which the nests were assessed using the rating scale supplied by Deacon (2006). This assessment was based on what was seen in the home cages of the mice. Extreme care was taken while observation was ongoing, as causing panic on the mouse could result in destroying the nest that was built. The nestling material used was hay.

2.6. Statistical analysis

All data obtained were analyzed by one way analysis of variance followed by post hoc student's t-test using the SPSS Computer program. Results are presented as mean ± SEM and p value less than 0.05 was considered statistically significant.

3. Results

The presence and quantities of the different metallic elements in the calabash chalk is presented in Table 1.

The mean water intake of the low dose group was significantly lower ($p < 0.05$) compared to the control. The value for the high dose group was also significantly lower compared to both the control and low dose groups ($p < 0.05$) (Fig. 1).

The mean daily food intake of both the low and high dose groups were significantly lower ($p < 0.05$) compared to the control (Fig. 2).

The mean body weight change of the low dose group was significantly lower ($p < 0.001$) compared to the control. The value for the high dose group was significantly lower ($p < 0.05$) compared to both control and low dose groups (Fig. 3).

The frequency of line crossing was significantly reduced ($p < 0.05$) in both the low and high dose groups compared to control. However, the line crossing frequency in the low dose group was significantly higher compared to that of the high dose group ($p < 0.05$) (Fig. 4).

While the frequency of rearing of the high dose group was significantly lower compared to control ($p < 0.05$), that of the low dose group was significantly higher compared to high dose group ($p < 0.05$) (Fig. 5).

The center square entries of both the low and high dose groups were significantly lower compared to control ($p < 0.05$). However, the value for the low dose group was significantly higher compared to the high dose group ($p < 0.05$) (Fig. 6).

The nesting score of the low dose group was significantly lower ($p < 0.05$) compared to control. The value for high dose group was also significantly reduced compared to both control and low dose groups ($p < 0.05$) (Fig. 7).

Table 1
Analysis of calabash chalk showing the concentration of different elements.

Elements analyzed	Concentration of elements (mg/kg ± SD)
Magnesium (Mg)	1100 ± 100
Aluminium (Al)	160000 ± 15000
Potassium (K)	5500 ± 500
Calcium (Ca)	160 ± 14
Titanium (Ti)	11000 ± 1000
Vanadium (V)	125 ± 10
Chromine (Cr)	130 ± 10
Manganese (Mn)	40 ± 5
Iron (Fe)	15000 ± 1500
Cobalt (Co)	4.1 ± 0.2
Nickel (Ni)	25.5 ± 1.3
Copper (Cu)	15.5 ± 0.7
Arsenic (As)	11.5 ± 0.8
Silver (Ag)	0.50 ± 0.03
Cadmium (Cd)	0.76 ± 0.04
Antimony (Sb)	0.42 ± 0.02
Barium (Ba)	200 ± 10
Thallium (Tl)	0.33 ± 0.02
Lead (Pb)	57 ± 3
Zinc (Zn)	<100 mg/kg

Results are presented as Mean ± Standard deviation.

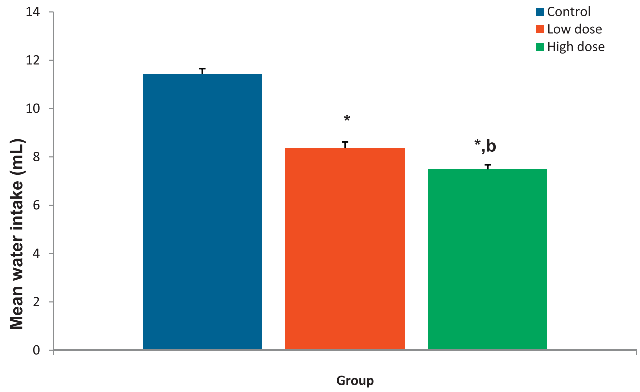

Fig. 1. Mean water intake of the various experimental groups. Values are mean ± SEM, n = 15.*=p < 0.05 vs control; b = p < 0.05 vs low dose.

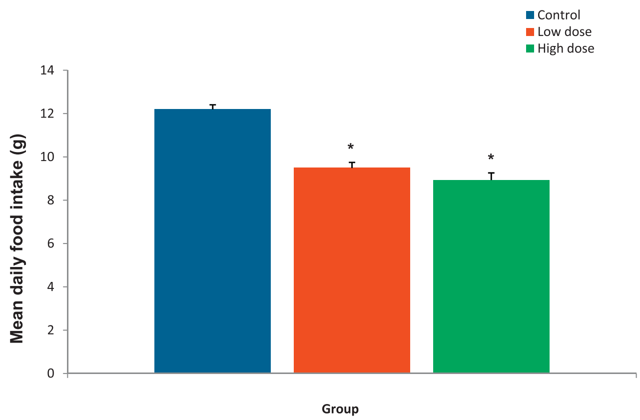

Fig. 2. Mean daily food intake of the various experimental groups. Values are mean ± SEM, n = 15.*=p < 0.05 vs control.

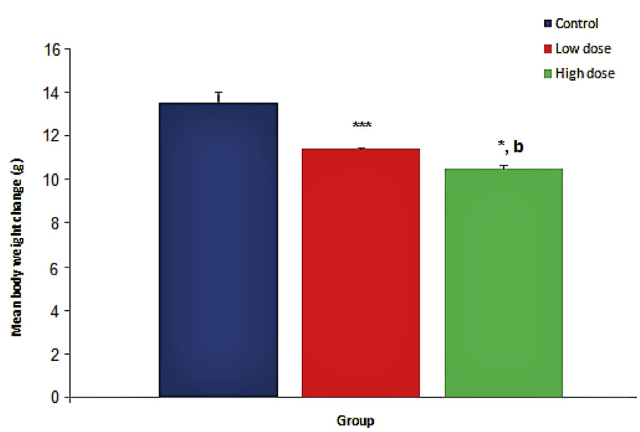

Fig. 3. Mean body weight change of control and calabash chalk fed groups. Values are mean ± SEM, n = 15. *=p < 0.05, ***=p < 0.001 vs control; b = p < 0.05 vs low dose.

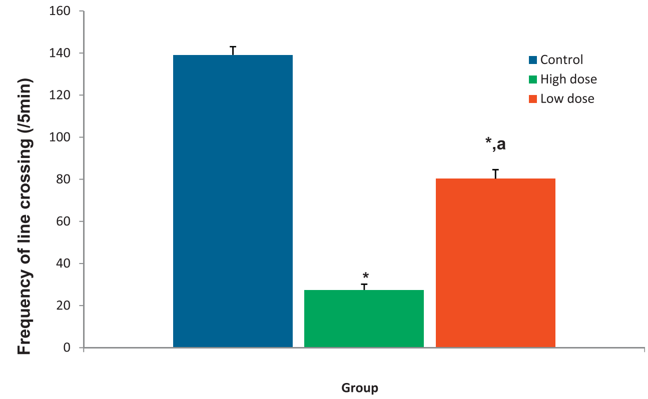

Fig. 4. Effect of consumption of calabash chalk on the frequency of line crossing in the open field maze test of the different experimental groups. Values are mean ± SEM, n = 15. *=p < 0.05 vs control; a = p < 0.05 vs high dose.

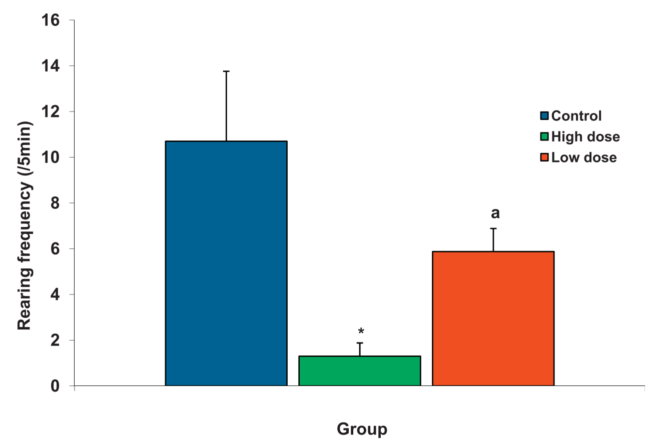

Fig. 5. Comparison of the frequency of rearing during the open field maze test in the control, low and high dose groups. Values are mean ± SEM, n = 15. *=p < 0.05 vs control; a = p < 0.05 vs high dose.

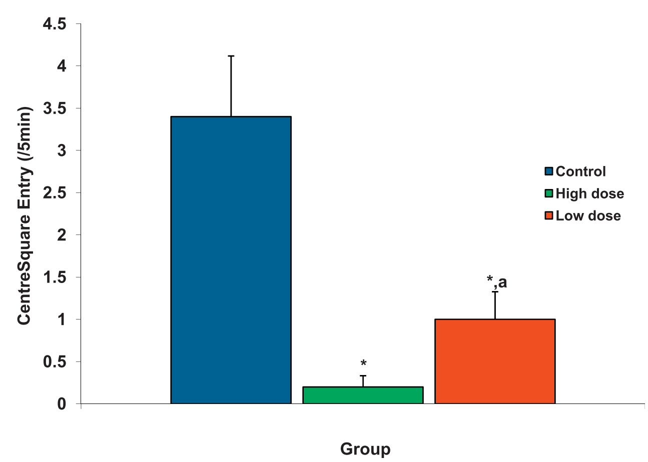

Fig. 6. Effect of consumption of calabash chalk on the centre square entry in the open field maze test of the different experimental groups. Values are mean ± SEM, n = 15. *=p < 0.05 vs control; a = p < 0.05 vs high dose.

4. Discussion

In our recent study, we showed that the chronic consumption of calabash chalk elevated anxiety and pain perception in mice (Owhorji et al., 2018). The ED_{50} used for this present study was derived from the LD_{50} of our previous study (Owhorji et al., 2018).

The mice that consumed high dose of the calabash chalk diet recorded the least water intake, followed by the low dose group. Similar results were observed in their food intake. The calabash chalk diet may have been unpalatable to the mice and so reduced their craving for food and water. It is also possible that calabash chalk has inhibitory effects on hunger and thirst centers (located in the hypothalamus) to reduce their food and water intake respectively.

The change in body weights of the mice fed the different calabash

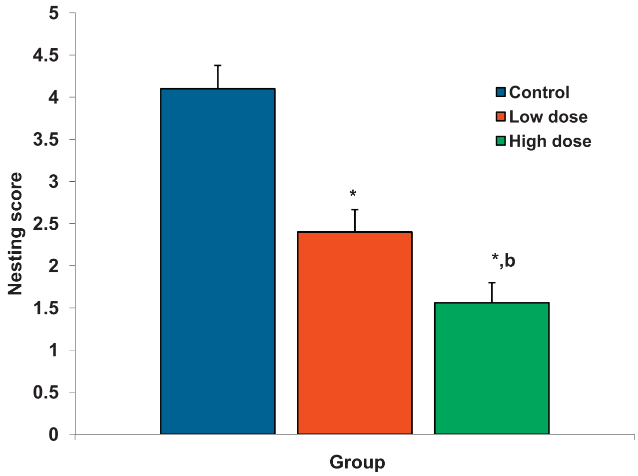

Fig. 7. Effect of consumption of calabash chalk on the nesting score in the social beheaviour test of the different experimental groups. Values are mean ± SEM, n = 15. *=p < 0.05 vs control; b = p < 0.05 vs low dose.

chalk diets were significantly reduced when compared with control. The reason for this reduction in their change in body weight is uncertain. It may however, be attributed to the reduction in food intake of mice fed calabash chalk diet as shown by our results.

Following the consumption of calabash chalk diets, the line crossing frequency was reduced in the low and high dose groups compared to control. This parameter is a measure of the horizontal locomotor activity. The lower the line crossing frequency of a mouse, the lower its locomotor activity. Hence, the locomotor activity of the mice fed calabash chalk diets was reduced compared with control.

Rearing frequency in the open field maze is usually used as a measure of the vertical locomotor activity. It also serves as measure of exploration and anxiety. It is a standard method that has been used as a measure of anxiety (Lever et al., 2006). From our study, it was observed that mice placed on calabash chalk diet had reduced rearing frequencies. A low frequency of rearing indicates decreased locomotion and exploration and/or a higher level of anxiety. Consequently, increased levels of anxiety lead to decreased exploratory behavior and vice versa. This is because increased anxiety will result in less locomotion and a desire to stay close to the walls of the field (Ennaceur, 2014). This explains the low locomotor activities observed in the mice fed calabash chalk diets.

The center square entries of the low and high dose groups were significantly lower than control. This also confirms the low locomotor activity of the mice fed calabash chalk diets, because the lower the center square entry, the lower the locomotor or exploratory behavior which is the tendency to investigate a novel environment.

Animal locomotion arises from complex interactions among sensory systems, processing of sensory information into patterns of motor output and the musculo-skeletal dynamics that follow motor stimulation (Biewener and Daniel, 2010). Since No observable motor impairments were seen in the mice prior to the tests, the reduced locomotor activity observed in the mice placed on calabash chalk diets may be due to the presence of lead in such diet. This neurotoxicant which is present in large quantities in calabash chalk might have influenced locomotion by altering the cellular genetics of the central pattern generators (neuronal networks in the spinal cord that are able to produce rhythmic movements) by oxidative stress (Sanders et al., 2009). It is also likely that other constituents not studied may be responsible.

Nesting behaviour sheds some light on important disorders of human behaviour like autism. Abnormal behaviour shown by mice could imply a deficit associated with autism spectrum disorder. Mice placed on the calabash chalk diets exhibited poor social behaviour by having significantly lower nesting scores than the control as shown by their inability to fluff up nesting materials to build up their nest. The high dose group exhibited the poorest social behaviour. This could be due to the depression of their central nervous system by the neurotoxicants: lead and arsenic present in calabash chalk (Tyler and Allan, 2014).

In conclusion, consumption of calabash chalk diet impairs locomotion and social behavior in mice. The major components of the calabash chalk such as lead and arsenic cross the blood-brain barrier to cause different effects including neurotoxicity in different parts of the brain (Cleveland et al., 2008; Ekong et al., 2014). While not ruling out the effects of other metals and micro organisms contained in the calabash chalk, it is possible that neurobehavioural deficits observed in this present study may be associated with the neurotoxicity of lead and arsenic. We however, did not study the effects of various metals and micro organisms in calabash chalk on locomotion and social behaviour. Our study shows that long term consumption of calabash chalk diet impairs locomotor and social behaviour in mice. If these results are applicable to man, the liberal consumption of calabash chalk should be discouraged.

Declarations

Author contribution statement

Eme Osim: Conceived and designed the experiments; Wrote the paper.

Bright Owhorji: Performed the experiments; Wrote the paper.

Udemeobong Okon: Analyzed and interpreted the data; Wrote the paper.

Azubuike Nwankwo: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Abrahams, P.W., Persons, J.A., 1996. Geophagy in the tropics: a literature review. Geogr. J. 162 (1), 63–72.
Abrahams, P.W., Davies, T.C., Solomon, A.O., Trow, A.I., Wragg, J., 2013. Human geophagia, calabash chalk and Undongo: mineral element and nutritional implications. PLoS One 8 (1), e53304.
Bender, B.S., Cottey, R., Bell, W., Taylor, S., 1996. Body temperature and nesting behaviour following influenza challenge in mice: effects of age. Mech. Ageing Dev. 86 (1), 1–9.
Biewener, A., Daniel, T., 2010. A moving topic: control and dynamics of animal locomotion. Biol. Lett. 6 (3), 387–388.
Brown, R.E., Corey, S.C., Moore, A.K., 1999. Differences in measures of exploration and fear in MHC – congenic C57BL/6J and B6-H-2K mice. Behav. Genet. 29, 263–271.
Cleveland, L.M., Minter, M.L., Cobb, K.A., Scott, A.A., 2008. German. Lead hazards for pregnant women and Children: part 1: immigrants and the poor shoulder most of the burden of lead exposure in this country. Part 1 of a two-part article details how exposure happens, whom it affects, and the harm it can do. Am. J. Nurs. 108 (10), 40–49.
Deacon, R.M., 2006. Assessing nest building in mice. Nat. Protoc. 1 (3), 1117–1119.
Dean, J.R., Ma, R., 2007. Approaches to assess the oral bioaccessibilty of persistent organic pollutants: a critical review. Chemosphere 68 (8), 1399–1407.
Dean, J.R., Deary, M.E., Gbefa, B.K., Scott, W.C., 2004. Characterization and analysis of persistent organic pollutants and major, minor and trace elements in calabash chalk. Chem 57 (1), 21–25.
Ekong, M.B., Ekanem, T.B., Sunday, A.O., Aquaisua, A.N., Akpanabiatu, M.I., 2012. Evaluation of calabash chalk effect on femur bone morphometry and mineralization in young wistar rats: a pilot study. Int. J. Appl. Med. Res. 2 (2), 107–110.

Ekong, M.B., Peter, A.I., Ekanem, T.B., Eluwa, M.A., Mbaduga, C.C., Osim, E.E., 2014. Calabash chalk's geophagy affects gestating rats' behaviour and the histomorphology of the cerebral cortex. Int. J. Br. Sci. 2014, 394847.

Ennaceur, A., 2014. Tests of unconditioned anxiety- Pitfalls and disappointments. Physiol. Behav. 135, 55–71, 2013.

Lever, C., Burton, S., O'Keefe, J., 2006. Rearing on hind legs, environmental novelty, and the hippocampal formation. Rev. Neurosci. 17 (1-2), 111–133.

Owhorji, B.I., Okon, U.E., Osim, E.E., 2018. Calabash chalk chronic diet consumption elevates anxiety and pain perception. World J. Pharmaceut. Res. 7 (12), 82–94.

Sanders, T., Liu, Y., Buchner, V., Tchounwuo, P.B., 2009. Neurotoxic effects and biomarkers of lead exposure: a review. Rev. Environ. Health 24 (1), 15–45.

Tyler, C.R., Allan, A.M., 2014. The effects of Arsenic exposure on neurological and cognitive dysfunction in human and rodent studies: a review. Curr. Environ. Health 1 (2), 132–147.