Relationship between body weight and blood biochemical measurements resulted from sniffing lead-containing substances of homeless adolescent in Khartoum City, Sudan

Leila M. A. Hamed[1], Gaafar K. Nogod[2], Hythem S.A. Saeed[3], and *Abdelmonem M. Abdellah[4]

[1]Department of Medical Laboratories, College of Applied Medical Science, Shaqra University, Kingdom of Saudi Arabia,[2]Central Laboratory of Research and Analysis for Drinking Water, Khartoum, Sudan, [3]Dept. of Biochemistry,Faculty of Dental Medicine and Surgery, National University, Sudan, [4]Allahawi for Research Consultation (ARC), Khartoum North, Sudan, respectively.

**Email address of corresponding author: abdelmonemabdallah@hotmail.com*

Abstract:

Sniffing lead-containing substances phenomenon is associated with possible brain damage and severe breathing problems. In this study, a total of 120 sniffers and 40 normal children as control were randomly selected to investigate the effect of sniffing lead-containing substances on the biochemical parameters: Lead (mg/dl), calcium (mg/dl), total protein (mg/dl), albumin (mg/dl), total bilirubin (mg/dl), direct bilirubin (mg/dl), indirect bilirubin (mg/dl), AST (U/L), ALT (U/L), cholesterol and triglyceride. Result indicated that all of the studied biochemical parameters were not significantly affected by age of sniffers, but some parameters such as lead, total bilirubin and indirect bilirubin were slightly increased with the increase of sniffers age. Accordingly, long-term of sniffing lead-containing substances causes negative influence on children's intelligence and behavioral development.

Keywords: *biochemical, sniffers, exposure, albumin, bilirubin, cholesterol, triglyceride.*

Introduction

Previous studies insured that lead in the environment is gradually accumulates in the body and is regarded as one of the most harmful environmental toxins to toddlers (Ferguson *et al*., 2011). Kosnett (2006) reported that lead poisoning may be acute (from intense exposure of short duration) or chronic (from repeated low-level exposure over a prolonged period), but the chronic poisoning is much more common. It has been stated by Chen *et al*. (2005) that most of international long-term follow-up investigation of the effects of lead exposure on neurological dysfunction in children are persistent, especially lead exposure in 2-year-old children, on age that appears to be a critical period for a child's later intelligence quotient and academic achievement. Moreover, Hou *et al.* (2013) reported that exposure to lead and lead chemical compound occur through ingestion, inhalation and dermal contract. Long-term exposure to lead can cause nephropathy and colic-like abdominal pains. It may also cause weakness in finger, wrists or ankles. Lead exposure also may cause small increase in blood pressure and anemia, particularly in middle-aged and older people. The Agency for Toxic Substance and Disease Registry (ATSDR, 2007) stated that only a small amount of ingested or inhaled lead accumulated in bones and the rest excreted by an adult through urine and feces within a few weeks of exposure, however only 32% of lead excreted by children. Brady (1992) stated that the existence of sniffing phenomenon which associated with aboriginal people is a wider context of adolescent mental health problem; adolescent risk-taking and a wide spread volatile substance abuse amongst some sections of general population. Solvent users had higher rates of emotion symptoms (mostly depressive) and abundance of adverse life enters family dysfunction and high rates of relative who have attempted to suicide (Brady, 1992). The amount of lead in the blood and tissues, as well as the time course of exposure, determines toxicity (Hodge, 1992). In Sudan, the background of those substances abuse was young adolescents who have social problems due to lack of family support. Therefore, the objective of this study is to investigate the relationship between age of homeless adolescent and their blood biochemical measurements that caused by these sniffing lead-containing substances. Previous study conducted by Abdalla *et al.* (2017) at different towns in Sudanese cities found that lead concentration level in exposed children was significantly higher at each of Medani Town (0.0035 mg/dl), Khartoum City (0.00313 mg/dl), Atbara Town registered (0.00039 mg/dl), Eldeweam (0.00267 mg/dl) and Elobeid Towns (0.00250 mg/dl) as compared to control. Similarly, Saeed *et al.* (2017) reported that mean blood lead concentration in occupationally exposed workers in main Sudanese cities was found to be 0.0322 mg /dl, whereas in control was 0.0124 mg/dl. A recent studies conducted by Hamed *et al.* (2021a, 2021b) reported that addiction of sniffing lead containing substances increases some of the investigated blood biochemical measurements. Therefore, the objective of this study is to investigate the relationship between body weight of homeless adolescent and their blood biochemical measurements that caused by sniffing lead-containing substances.

Materials and methods

Area of the study:

The study was carried out in Khartoum State/Sudan during 2013 to investigate the effect of sniffing of petrol and other volatile substances on biochemical and behavioral status of adolescent sniffers. Three rehabilitate centers of homeless were selected namely Tayba (south of Khartoum/for boys, Bashair (Omdurman) for girls Rashad (west of Khartoum) for both young boys and girls).

Sample size:

A total of 120 sniffers were selected from these three rehabilitate centers, 40 participants from each, in addition to 40 non-users as a control subjects. Age of sniffers range between 6-18 years. Blood samples were then collected from the selected sniffers as well as the control to determine lead and calcium concentration, liver function (protein, albumin, bilirubin, AST and ALT) and lipid profile (cholesterol and triglyceride).

Blood sample collection:

A 5 ml of blood from peripheral vein of sniffers (boys and girls) were collected in heparinized container as anticoagulants in order to determine the biochemical parameters. For sample collection, disposable syringes, heparin tubes, cotton, and ethanol were used.

Determination of lead in blood serum and plasma:

The BC-5 Analysis was used for the determination of lead in blood serum and plasma. The blood sample was diluted in deionized water and the analysis was then performed against standards prepared in glycerol to approximate the viscosity characteristics of the diluted samples. Normal serum levels estimated as µg% (Pb 10 – 20) or mg/dl (0.001- 0.002). For determination of serum lead, the sample was diluted1:5 with deionized water. The condition listed in the "Standard Conditions" section was used to determine the concentration of lead. Lead Standards were prepared by diluting the lead stock standard solution described in the Standard Conditions" for lead with 5% (v/v) glycerol solution were also used as a blank solution when determining lead concentration (Butrimovitz and Anal, 1977).

Calcium determination:

Calcium in serum or plasma was stable for 10 days at 2-8ºC. Anticoagulants other than heparin should not be used. The calcium concentration in the sample was calculated using the method described by Cheesbrough (2006).

Protein determination:

Protein in the sample reacts with copper (II) ion in alkaline medium forming a colored complex that can be measured by spectrophotometer. Serum or heparinized plasma was collected by standard procedures. Stable for 8 days at 2 – 8ºC. Anticoagulants other than heparin should not be used. The protein concentration in the sample was calculated using the method described by Cannon *et al.* (1974).

Albumin determination:

The serum albumin concentration was determined using modified bromocresol green colorimetric method as described by Doumas *et al.* (1971). Measurement of albumin is based on its binding to the indicator dye bromocresol green (BCG) in pH 4.1 to form a blue – green colored complex. The intensity of the blue – green colorist directly proportional to the concentration of albumin in the sample. It is determined by monitoring the increase in absorbance at 623 nm.

Albumin + BCG -------- (pH 4.1) ------→ Albumin – BCG complex

Total bilirubin and direct bilirubin determination:

The serum was collected by standard procedures. Bilirubin in serum was stable for 2 days at 2-8 C and protected from light. The bilirubin concentration in the sample was calculated as described by Cheesbrough (2006).

Determination of serum AST

Serum AST activity was determined according to the method described by Reitman and Frankel (1957). The AST catalyzed the transfer of amino group from aspartate to alpha oxoglutarate according to the following reaction:

Alpha-oxoglutarate + L- aspartate --------**AST**----→ L- glutamate + Oxaloacetate

The oxaloacetate formed reacts with 2, 4- dinitrophenyl hydrazine (DNPH) to form hydrazone of keto acid present which subsequently react with sodium hydroxide to form a color. The intensity of this color is proportional to AST concentration. Absorbance of the sample and standard were read against reagent blank at 505 nm. The volume of enzyme activity was obtained from the table provided with kit.

Determination of ALT:

Serum ALT was determined according to the method described by Reitman and Frankel (1957). ALT was measured by monitoring the concentration of pyruvate hydrozone formed with 2, 4-dinitrophenyl hydrazine group from L-alanine to alpha-oxoglutarate according to the following reaction:

Alpha-oxoglutarate + L-alanine ------**ALT**----→ L-glutamate + Pyruvate

Absorbance of the sample and standard were read against reagent blank at 505 nm. The activity of enzyme was determined from the absorbance table U/L.

Cholesterol determination:

Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the sample were precipitated with phosphotungeslate and magnesium ions. The supernatant contains high density lipoproteins (HDL). The HDL cholesterol was then spectrophotometrically measured according to Cheesbrough (2006).

Triglycerides determination:
Triglycerides in the sample originates by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometer.The triglycerides concentration in the sample was calculated using the method described by Cheesbrough (2006).

Result

Effect of body weight (kg) of exposures on electrolytes:

1. Lead level:
Lead concentration of exposures was not significantly affected by their body weight, but it the lowest lead level was 0.0018 mg/dl was found to be in the range of (51 - 60 kg of body weight) and highest lead level of 0.0022 mg/dl was found in exposures of body weight ranged between 31 and 40 and 41 and 50 kg. (Table 1 and figure 1).

2. Calcium level:
This parameter was not significantly affected by weight of exposures, but exposures of 41 - 50 kg body weight obtained the highest value (9.38 mg/dl) of calcium level, while the range of both20-30 and 51 - 60 kg of body weight reported the lowest values (9.29 and 9.28) of calcium concentration, respectively (Table 1 and figure 2).

Effect of body weight of exposures on liver function:

1. Protein levels:
The highest value of protein was 6.86 mg/dl which found to be in the range of 41 - 50 kg of body weight, whereas the lowest value (6.73) was found to be in the range of 20 - 30 kg (Table 1 and figure 2).

2. Albumin level:
Table 1 and figure 2 show that albumin level of exposed sniffers was not significantly affected by their body weight, but the highest value (4.11mg/dl) was reported by 51 – 60 body weight, whereas the lowest values (4.07 mg/dl) was obtained by 31– 40 kg body weight.

3. Total bilirubin level:
No significant differences have been shown between ranges of exposure body weight for total bilirubin level, but the result indicated that this parameter was slightly increased with increasing in exposures body weight (Table 1 and figure 3).

Table (1): Effect of body weight (kg) of sniffers on level of some blood biochemical characters.

body weight	Electrolytes		Liver functions							Lipids	
	Lead	Calcium	T. protein	Albumin	T. bilirubin	D. bilirubin	In d. bilirubin	AST	ALT	Cholesterol	Triglyceride
20 - 30	0.0021[a]	9.29[a]	6.73[a]	4.08[a]	0.67[a]	0.31[a]	6.42[a]	19.33[a]	14.29[a]	161.49[a]	40.92[a]
31 - 40	0.0022[a]	9.33[a]	6.80[a]	4.07[a]	0.68[a]	0.33[a]	6.46[a]	20.97[a]	14.13[a]	161.28[a]	39.62[a]
41 - 50	0.0022[a]	9.38[a]	6.86[a]	4.10[a]	0.73[a]	0.33[a]	6.41[a]	21.55[a]	14.32[a]	166.41[a]	41.82[a]
51 - 60	0.0018[a]	9.28[a]	6.80[a]	4.11[a]	0.71[a]	0.35[a]	6.46[a]	21.70[a]	14.30[a]	164.00[a]	41.90[a]
d.f.	115	115	115	115	115	115	115	115	115	115	115
SE±	0.001	0.12	0.13	0.10	0.05	0.04	0.14	1.90	0.26	6.06	1.58
C.V.(%)	150.59	3.03	4.53	5.48	17.75	29.65	4.79	21.19	4.18	8.52	14.28

Means with the same letter in the same Columns are not significantly different at 0.05 level of probability according to DNMR

Fig.(13): Effect of body weight on lead level of sniffers.

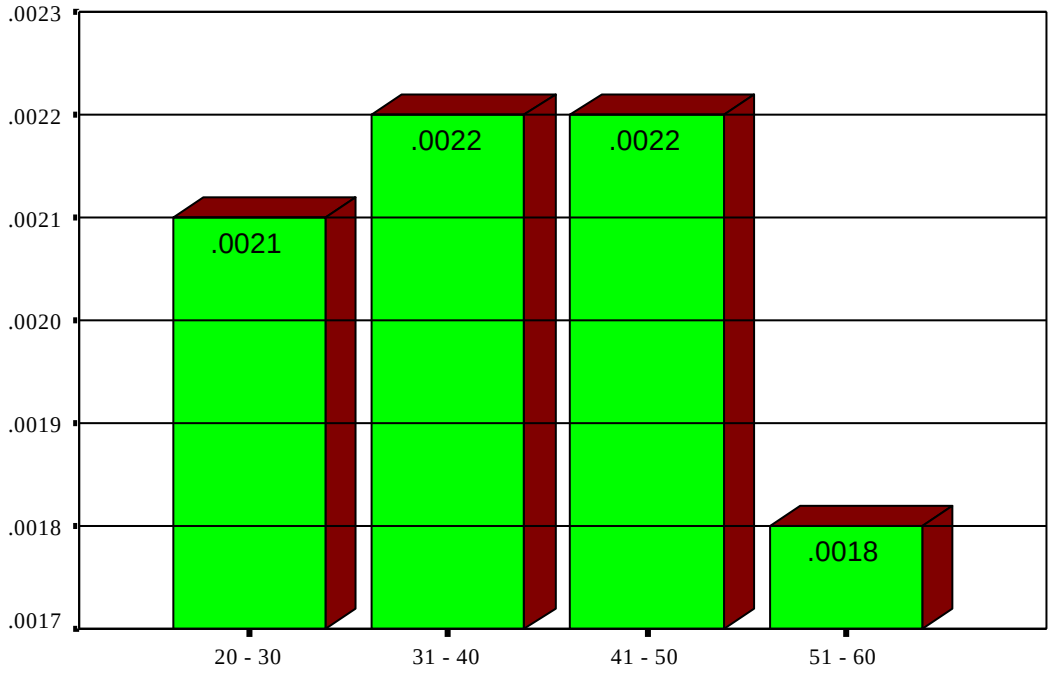

Fig. (1): Effect of body weight on lead level of sniffers.

Fig.(14):Effect of body weight on level of Ca++ and liver function parameters of sniffers.

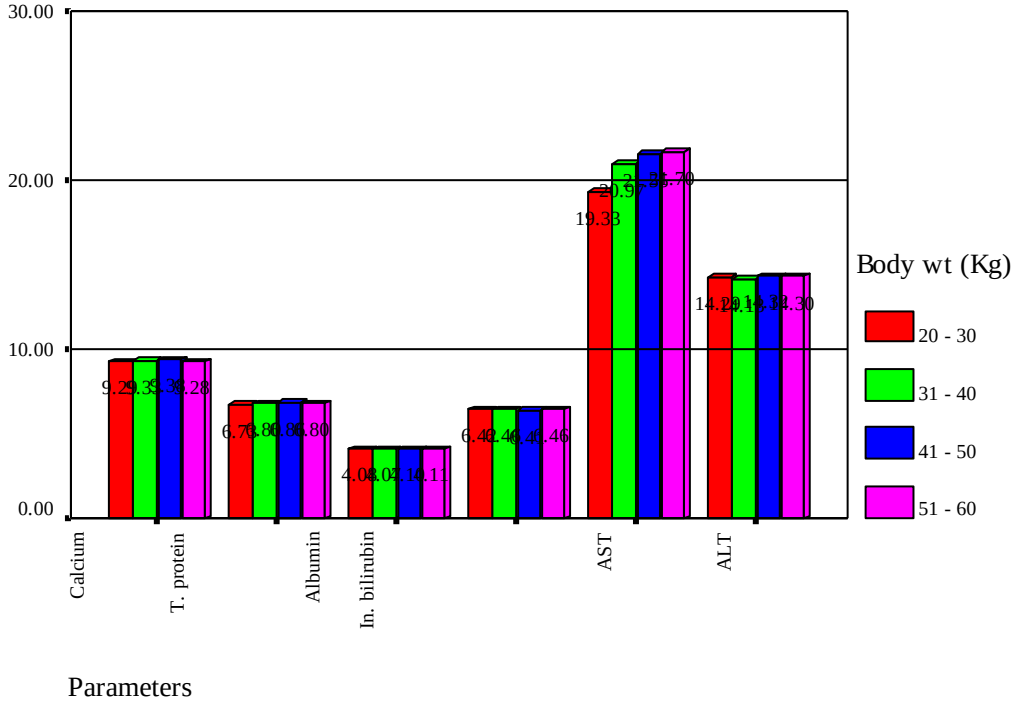

Fig. (2): Effect of body weight on calcium level of sniffers.

4. Direct bilirubin level:
This parameter was also slightly increased with increasing in exposures body weight, with higher value (0.35) reported in 51 - 60 kg of body weight and lowest value (0.31) obtained by 20 - 30 kg of body weight with an increasing between them estimated by 12.9%. The difference between the ranges of body weight for direct bilirubin level statistically was not significantly different (Table 7 and figure 3).

5. Indirect bilirubin level:
Exposures body weight did not significantly affect their indirect bilirubin level, but both 31-40 and 31-40 and 51-60 kg of body weight reported the lowest value (6.42 and 6.41, respectively) (Table 1and figure 2).

6. AST level:
No significant differences were shown among the range of exposures body weight for the level of AST, but the range 51-60 kg of body weight reported the highest value of this parameter (21.70 U/L), followed by 41-50 (21.55 U/L), 31-40 (20.97 U/L) and finally 20-30 kg (19.33 U/L), (Table 1 and figure 2).

7. ALT level:
This parameter was not significantly affected by exposures body weight, with higher value (14.32 U/L) reported in 41-50 kg of body weight and lower value (14.13 U/L) obtained by 31-40 kg of body weight (Table 1 and figure 2).

Effect of body weight (kg) of exposures on lipid profile:
1. Cholesterol level:
The highest value of cholesterol (166.4 U/L) was obtained by exposures of 41-40 kg of body weight, while the lower value (161.28 U/L) was recorded by 31 – 40 kg but the difference between them was not significant (table 1 and figure 4).

2. Triglyceride level:
Triglyceride level of exposures was not significantly affected by their body weight, but it was slightly increased with increasing in weight of exposures (Table 1 and figure 4).

Relationship between exposures body weight and biochemical measurements:
Table 2 indicates that there was positive and significant relationship between exposure body weight and level of total bilirubin, AST and cholesterol, while the relationship with total protein was significant, but it was negative. On the other hand, the relationship between body weight of exposures and lead, Ca^{++}, albumin, direct bilirubin, indirect bilirubin, ALT and triglyceride was positive and insignificant.

Fig. (15):Effect of body weight on sniffers total and direct bilirubin levels.

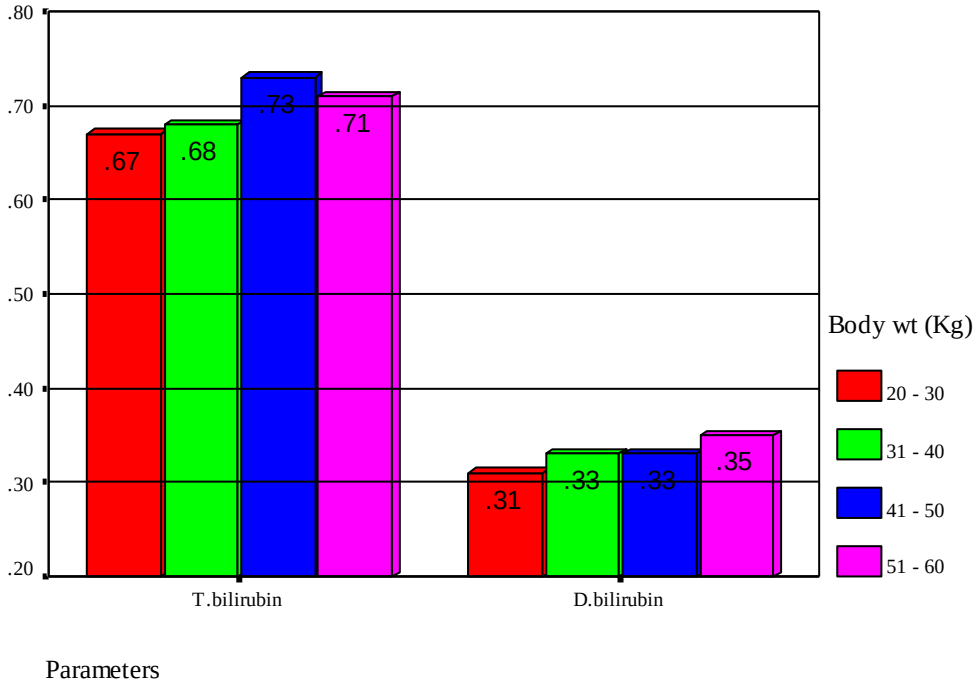

Figure (3): Effect of body weight on sniffer total and direct bilirubin levels

Fig. (16):Effect of body wt on cholesterol and triglyceride levels.

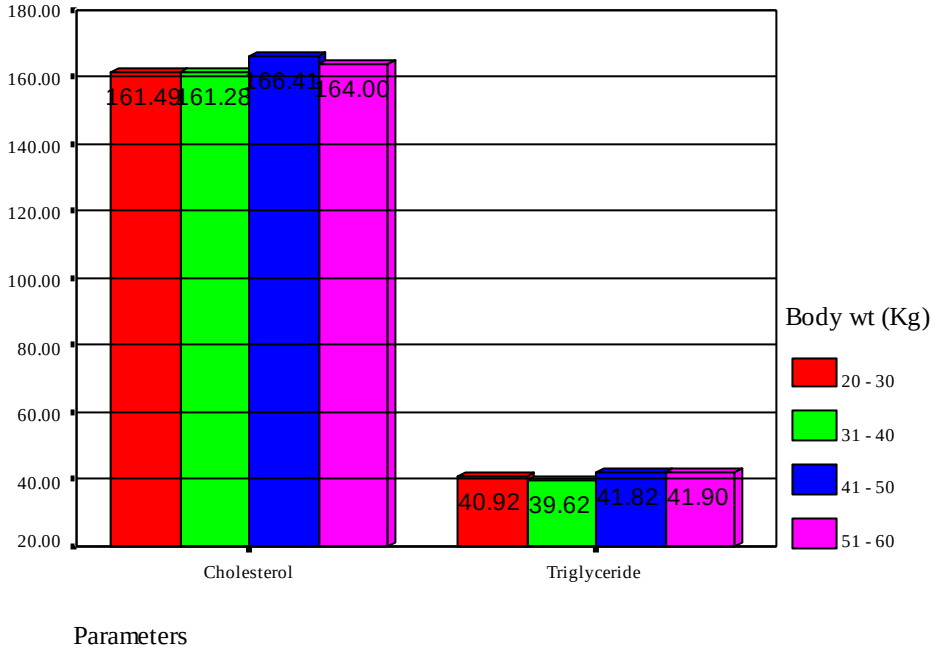

Figure (4): Effect of body weight on cholesterol and triglyceride levels

Table (2): Relationship (regression) between body weight and blood characters of sniffers.

variables	B-value	d.f	SE±	t-value	sig.
Lead	1.200	118	0.0001	1.21	ns
Calcium	- 0.004	118	0.003	1.45	ns
T.protein	0.007	118	0.003	2.37	*
Albumin	0.002	118	0.002	0.68	ns
T.bilirubin	0.003	118	0.001	2.06	*
D.bilirubin	0.002	118	0.001	1.72	ns
In d.bilirubin	0.003	118	0.003	1.04	ns
AST	0.096	118	0.044	2.15	*
ALT	0.004	118	0.006	0.60	ns
Cholesterol	0.307	118	0.140	2.19	*
triglyceride	0.036	118	0.059	0.61	ns

ns: Not significant. *: Significant at 0.05 level of probability.

Discussion The findings of the present study indicated that for lead and calcium levels there is no significant difference between body weight of lead exposure and their biological parameters, although Needleman *et al.* (1990), Bellinger *et al.* (1992) and Rogan *et al.* (2001) mentioned that children biological susceptibility to lead is greater than that of adults, because the developing human brain undergoes rapid growth, development and differentiation, and lead can interfere with these extraordinary complex and delicate process. In the present study, other factors, e.g., age of sniffers, duration of sniffers and the nature of the sniffed substances may be more affected than body weight. In the present study, the results showed that the heaviest weight (51 – 60 kg) obtained slightly higher mean of AST (21.7), cholesterol (164.00 mg/dl) and triglyceride (41.90 mg/dl) as compared to the lowest weight (20 – 30 kg) who obtained 19.33, 161.49 and 40.92, respectively. Similar results were also reported by Brady (1992), Brady and Torzillo (1995), Burns (1996) and Mosey (1997). Polyaniski (1986) stated that symptoms stand to appear in children generally at around 60 mg/dl, whereas in adults can occur at level above 40 mg/dl, but are more likely to occur only above 50 – 60 mg/dl. Meanwhile, Cava and Hor (2011) stated that symptoms may be different in adults and children, and the main symptoms in adults are headache, abdominal pain, memory loss, kidney failure, male reproductive problems and weakness, pain, or

tingling in the extremities. Polyaniski (1986) also mentioned that signs that occur in children at blood lead levels exceed 100 mg/dl include encephalopathy, such as bizarre behavior, discoordination and apathy. It is more common for lead poisoning to build up slowly over time. This occurs from repeated exposure to small amount of lead. In this case, there may not be any obvious symptoms. Overtime, even low lead levels exposure can harm a child's mental development and the death problems get worse as the level of lead in the blood gets higher (Polyaniski, 1986).

Davis and suendsgaard (1987) and Mushak *et al*., (1989) concluded that lower levels of lead in children (10 mg/dl) cause adverse effects on the central nervous system, kidney, hematopoietic system, decrease intelligence and impaired neurobehavioral development, whereas severe lead exposure (blood lead levels > or = to 380mg/dl) can cause coma, convulsions and even death. Low lead levels also has many other effects such as decreasing stature or growth (Bornschein *et al*., 1986 and Shulka *et al*., 1989), decreasing hearing acuity (Schwartz and Otto, 1987) and decreasing ability to maintain a steady posture (Bhttacharya *et al*., 1988). Mohammed Ali (2007) observed that lead levels in Khartoum State exposure was significantly increased with age of exposures (20 – 46 years), while Ali (2007) showed slight reduction in Pb levels with age (11 – 16 years) of 60 sniffers in the same stated. Similarly, it has been recently reported by Hamed *et al*. (2021b) that all of the biochemical parameters that studied were found to be not significantly affected by age of sniffers, but some parameters such as lead, total bilirubin and indirect bilirubin were slightly increased with increasing sniffers age.

Conclusion and recommendations

The study concluded that there is no significant difference between body weight of lead exposure and their biological parameters changes, in spite of the fact that children biological susceptibility to lead is greater than that of adults. Generally, slight increase in biological parameters under the study may be correlated to the increase of body weight of lead exposure. Creating lead-safe community occurs by elimination of products with dangerous lead levels and timely mechanisms to share information about lead sources, including toxic properties, across government agencies. Meanwhile, the author of this work believe that more studies should be carried out in the field of sniffing phenomenon and its impact on socio-economic and health status associated with sniffers and their surrounding environment.

Acknowledgement

The authors would like to thank everyone who contributed to complete this work.

References

Abdalla FAB, Saeed HAS, Abbas AA and Abdellah AM (2017). Lead poisoning from cars exhausts among primary school children in Sudan. Asian Journal of Science and Technology, 08(10): 5970-5973.

Ali ZH (2007). The effect sniffing lead on blood of adolescent's homeless ainls gasoline sniffers in Bashair camp Khartoum. Sudan. Master of Science in biochemistry. Omdurman Islamic University.

ATSDR (Agency for Toxic Substances and Disease Registry). (2007). Toxicological profile for Lead. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Bellinger DC, Stiles KM.and Needleman HL (1992). Low-level lead exposure, intelligence and academic achievement: A long-term follow-up study. Pediatrics 90:855-861.

Bhattacharya A, Shukla R, Bornschein R, *et al.* (1988). Postural disequilibrium quantification in children with chronic lead exposure: A pilot study. Neurotoxicology 9:327-340.

Bornschein RL, Succop PA and Krafft KM (1986). Exterior surface dust lead, interior house dust lead and childhood lead exposure in an urban environment. In: Hemphil DD, ed. Trace substances in environmental health. Vol. 20. Columbia, MO: University of Missouri 322-332.

Brady M (1992). Heavy Metal. Canberra:Aboriginal studies press, AIATSIS.

Brady M and Torzillo P (1995). Petrol sniffing down the track. *Medical Journal of Australia, 160*(21), 176–177.

Burns C (1996). An End of Petrol Sniffing. Ph D thesis, Sydney University.

Butrimovitz 1GP and Pudy WC (1997). (1977). The determination of lead in by Atomic Absorption Spectrometry. Ann. Chim. Blood plasma Acta 94.63 Pb (30).

Cannon DC, Olifzky I and Inkpen JA (1974). Protein. Cli – chem. Principle and technics,zed. Cannon and winkelman editors. New York, PP. 407 – 421.

Cava RJ, Hor YS. Cava RJ (2011). Pressure Stabilized Se-Se Dimer Formation in $PbSe_2$". *Solid State Sciences***13**: 38–41. Bibcode:2011SSSci..13.38B.doi:10.1016/j.solidstatesciences.2010.10.003.

Cheesbrough M (2006). District laboratory practice in tropical countries, part 1, 2rd addition, Cambridge University press, New York – USA, 350, 404

Chen A, Dietrich KN, Ware JH, *et al.* (2005). IQ and blood lead from 2 to 7 years of age: Are the effects in older children the residual of high blood lead concentrations in 2-year-olds? Environ Health Perspect 113(5):597-601.

Davis JM and Svendsgaard DJ (1987). Lead and child development. Nature 329:297-300.

Doumas BT, Waston WA and Bigg hG, (1971). Albumin standard and the measurement of serum albumin with bromocresol green. Cli- chem. U.S. 31, 87 – 96.

Ferguson A, Bursac Z, Kern DF. Arkansas (2011). People Participating in Lead Education (APPLE): results of a Lead-safe training program. J Community Health; 36(3):367–374.

Hamed LMA, Nogod GK, Saeed HSA, Abdalla FAB, Abdellah AM and Abbas AA (2021a). The Effect of Addiction to Sniffing Lead-containing Substances on Blood Biochemical Measurements of Homeless Adolescents in Khartoum City, Sudan, Greener Journal of Environmental Management and Public Safety, 10(1): 1-9.

Hamed LMA, Nogod GK, Saeed HSA, and Abdellah AM. (2021b). Correlation between age and blood biochemical changes of homeless adolescent resulted of sniffing lead-containing substances, International Journal of Green and Herbal Chemistry, 10(1): 015-025.

Hodge AT (1992). Roman Aqueducts & Water Supply. London: Duckworth. ISBN 0-7156-2194-7.

Hou Y, Burkhard B and Müller F (2013). Uncertainties in landscape analysis and ecosystem service assessment. Journal of Environmental Management 127, S117–S131

Kosnett M.J. (2006). "Lead". In Olson, K.R. Poisoning and Drug Overdose (5th ed.). McGraw-Hill Professional. p. ISBN 0-07-144333-9.

Mohammed Ali SA (2007). Determination of lead in Air and Blood Samples from People in Khartoum State-master of science in biochemistry. Omdurman Islamic University.

Mosey A (1997). Report on petrol-sniffing in central Australia alcohol and other drugs program. Alice Springs: Territory Health services, unpublished report.

Mushak P and Carocetti AH (1989). Determination of numbers of lead –exposed American children as function of lead source. Integrated summery of a report to U.S. congress on childhood lead poisoning. Sources of U.S. child lead exposure, 210-229.

Needleman HL, Schell A and Bellinger D (1990). The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report. N Engl J Med 322:83-88.

Polyanskiy NG, Fillipova NA (1986). ed. Аналитическая химия элементов: Свинец [*Analytical Chemistry of the Elements: Lead*] (in Russian). Nauka.

Reitman S and Frankel M (1957). Acolorimetric method for the determination of serum glutamate oxaloacetatic acid and pyruvic acid transaminases *American of Clin-Pathol*., 28: 56 – 63.

Rogan WJ, Dietrich KN, Ware JH (2001). The effect of chelation therapy with succimer on neuropsychological development in children exposed to lead. N Engl J Med 344(19):1421-1426.

Saeed HSA, Abdellah AM, Abdalla FAB and Abbas AA (2017). Effect of air lead pollution on blood bilirubin and lactate dehydrogenase levels among occupationally exposed workers in main Sudanese cities. IOSR Journal of Applied Chemistry (IOSR-JAC) 10(1): 47-52

Schutz A, Skerfving S and Ranstam J (1987). Kinetics of lead in blood after the end of occupational exposure. Scand J Work Environ Health 13:221-231.

Shukla R, Bornschein RL, Dietrich KN (1989). Fetal and infant lead exposure: Effects on growth in stature. Pediatrics 84:604-612.