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Effect of Long-term Exposure to Low or Moderate Lead Concentrations on Growth, Lipid Profile and Liver Function in Albino Rats

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Abstract: Effect of long-term exposure to low or moderate lead acetate (PbAc) on growth, serum lipid profile and some biochemical parameters was investigated. Male albino rats were divided into five groups and given 0, 0.025, 0.05, 0.1 and 0.3% PbAc in drinking water for 11 months. There was a significant decrease in body weight in rats given 0.1 % PbAc at the third month of lead treatment ($p<0.01$) compared to the control group, while the body weight gain was significantly increased at the end of experiment in animals exposed to 0.05 % PbAc ($p<0.05$). Serum concentrations of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglyceride remain unchanged in all lead exposed groups compared to the control group. Similarly, no significant alteration was observed in serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase activities and glycemia in all lead treated groups. In conclusion, the long-term exposure to low lead concentration may conduct to high weight gain and obesity risk. However, no evident for disturbance of serum lipid profile or biochemical liver parameters was observed after long-term lead exposure. Therefore, long term-exposure to low or moderate lead concentrations should not be incriminated to risk of atherosclerosis or impairment of liver function.

Key words: Rat · Lead acetate · Growth · Liver · Serum lipid · Biochemical parameters

INTRODUCTION

Lead is found in our food, water, air and soil. Lead emitted by power plants, smelters and boilers that burn used motor oil is frequently deposited in the soil, where it is taken up by crops [1]. Environmental sources of lead include inhalation of automobile exhaust from gasoline containing alkyl lead additives from ingestion of dust contaminated with lead and from drinking water that had passed through lead piping [2].

Researchers simultaneously made three major discoveries about lead toxicity: lead poising has subtle effects, the effects are permanent and they occur at low levels of exposure. The amount of lead recognized to cause harm is only 10 micrograms per 100 milliliters of blood. Some research shows that lead concentrations below this amount may adversely affect children's physical and mental development [3]

Although characteristic features of lead toxicity were noted by Hippocrates and Nikander in ancient times [4], it remains one of the most important occupational and environmental health problems.

Several studies have incriminated lead exposure to lipid abnormalities and risk of atherosclerosis [5,6].

Dyslipidemia is defined as elevated total cholesterol, low-density lipoprotein cholesterol, or triglycerides; a low high-density lipoprotein cholesterol, or combination of these abnormalities [7]. Indeed, Hypercholesterolemia is a major key modifiable risk factor for coronary artery disease, the leading cause of death in the United States [8].

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The liver performs numerous biochemical functions and it is the site of metabolism of nutrient. Carbohydrates, fats and proteins are all broken down and synthesized there. Many compounds presents in the environments, some which are toxins, are also first metabolized in the liver. Metabolism in the liver usually leads to detoxification of environmental compounds, hence the concept that the liver purifies or cleanses the blood [9]. However, many researchers showed the effect of high lead exposure on liver function alteration but the relation between lead exposure and lipid metabolism remains controversial.

No studies until now reported the effect of lead exposure during a period longer than 11 months on growth, lipid profile and biochemical parameters indicative of liver functions in adult male rats. Therefore, we attempted to investigate in this study whether body weight, body length, serum lipid and liver function parameters are affected in adult male rats after a long-term exposure to low or moderate lead concentrations.

MATERIALS AND METHODS

Chemicals: Lead acetate (ACS reagents, grade > 99 % pure) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid, hydrochloric acid and all chemicals used were of analytic reagent grade.

Experimental Design: Healthy male albino wistar rats were purchased from Pasteur Institute (Algiers, Algeria) were used in this investigation. The animals were maintained under standard conditions of temperature (23 ± 2°C) and natural light-dark cycle.

All animals have free access to drinking water and rat pellet diet (National office for aliment cattle, Bejaia, Algeria). They were allowed 5 days for acclimation prior to experimental treatment.

Animals of 6-7 weeks old, with a mean body weight of 151 ± 18 g were divided into five groups (9-10 rats per group) including the control group. Four groups were given 0.025; 0.05; 0.1 and 0.3% PbAc in distilled water. The control group was given acetic acid solution containing an equivalent amount of acetate to the highest lead. One milliliter of 5 N HCl was added to each litter of solution to preclude precipitation of insoluble lead salts and even in solution of control group [10,11]. The concentrations of PbAc in our study were chosen on the basis that they regroup low to moderate concentrations [12]. The experiment was conducted during 11 months (43 weeks).

At the end of experiment, presence of brick red stain that can occur in the region of the head or back of some animals was registered and graded from 0 to 2 according to intensity of colour (absence: 0, light brick red spot: 1 or dense brick red spot: 2) and frequency is then calculated for each class.; the colour stain was recorded by the same person in blind manner. However, all animals remained active during lead treatment period.

Body weights, water and food consumptions were measured once per month for 11 months of lead exposure, except for the 7th month. The quantities of water and food intakes were measured during one week per month and then they were divided by the overall body weights in the group. The average daily consumption of water and food throughout all experimental period were calculated and they were reported to 100 g of body weight.

At the end of experience, all animals were fasted overnight, light anaesthetized with diethyl ether, weighed and the body length from nose to anus was measured to calculate a body mass index (BMI= weight /length^2). Blood was collected via the retro-orbital sinus. Then, animals were sacrificed by cervical dislocation; liver was excised, cleared of adhering fat and weighed.

Determination of Biochemical Parameters: Blood was collected into two tubes. The first tube contained fluoride oxalate for plasma glucose measurement and the second tube contained blood sample allowed to clot at room temperature. The serum was separated by centrifugation and immediately analyzed for biochemical parameters. For lipid determination, the serum was aliquoted and frozen at -20°C until analysis. The serum concentration of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) were determined by enzymatic colorimetric method using the following Roche kits for Cobas Integra 400 plus autoanalyzer: TG, TC second generation , HDL-C and LDL-C plus second generation.

While, serum albumin, total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL) and plasma glucose were determined automatically using Cobas Kits for Roche/Hitachi Cobas c 501 systems autoanalyzer.

Statistical Analysis: All data were presented as mean ± SE. Levene's test was used to control the homogeneity of variances. One-way ANOVA test was performed to check

significances of differences among the groups followed by Dunnett's post hoc test to compare treated groups with the control group.

If variances were not equal, the Kruskal–Wallis test was conducted followed by the Mann–Whitney U test. P<0.05 was taken as significant.

All statistical analyses were performed using the SPSS package program, version 17.0.

RESULTS

Clinical Signs: The apparition of brick red stain in hair was not affected by lead exposure in all rat groups (Table1).

Body Weight Evolution During Lead Exposure Period: There was a significant decrease in body weight in rats given 0.1 % PbAc at the third month of lead treatment (p<0.01), when compared to the control group and it was normalized later (Fig.1). However, an increase in the body weight of rats exposed to 0.05 % PbAc was observed after the third month of lead exposure (Fig. 1), which trends to significance differences at the 5th and 9th month of experimental period as compared to the control group (p<0.08; p<0.06) respectively.

Total Water and Food Consumption, Body Weight Gain, Length and Body Mass Index at the End of Experimental Period: No significant differences were observed in the average daily of overall water and food intakes throughout lead treatment period in all treated animals compared to the control group (Table 2). Interestingly, the body weight gain was significantly elevated at the end of experimental period in animals exposed to 0.05 % PbAc compared to the control group (p<0.05). Moreover, the BMI of these animals was elevated as comparing to the control group but the difference was not statistically significant.

The BMI and the body length remain unchanged in other treated groups (Table 2).

Absolute and Relative Liver Weights: There were no significant differences in the absolute liver weights in all lead treated rats. On the other hand, the relative liver weights were significantly increased in animals exposed to the highest PbAc concentration (p<0.05) comparatively to the control group (Fig.2).

Serum Lipid Profile: As shown in table 3, TC and TG exhibited higher values in the group exposed to 0.05 % PbAc, while LDL-C concentrations tend to be lower in all treated groups. These perturbations are not statistically significant compared to the control group.
The level of HDL-C and atherogenic index remain similar in all rat groups.

Biochemical Parameters Levels: Serum AST, ALT, ALP, LDH activities and glycemia levels showed an increase in rats exposed to the highest PbAc dose, while the concentrations of serum TP and albumin tended towards lower values in all lead treated animals when compared with the control group, but the differences were not statistically significant. However, glycemia and all serum biochemical parameters were unaffected in other lead exposed groups (Table 4).

Table 1: Frequency of hair colour in long-term lead exposed rats.

Groups	Control	0.025% PbAc	0.05% PbAc	0.1% PbAc	0.3% PbAc	LS
Total brick red stain (%)	11.00 ± 5.68	7.33 ± 7.78	7.00 ± 9.49	14.67 ± 7.78	12.00 ± 7.89	NS
Light brick red spot (%)	7.00 ± 4.83	4.89 ± 5.80	1.00 ± 3.16	4.89 ± 5.80	4.00 ± 5.16	NS
Dense brick red spot (%)	4.00 ± 8.43	2.44 ± 7.33	6.00 ± 9.66	9.78 ± 11.60	8.00 ± 10.33	NS

Data are expressed as mean ± S.D of (9-10) animals per group.
LS: level of significance. NS: not significant

Table 2: Total consumption of water and food, body weight gain, length and body mass index (BMI) in rats after long term lead exposure.

Groups	Control	0.025% PbAc	0.05% PbAc	0.1% PbAc	0.3% PbAc	LS
Total water intake (ml/100g/day)	12.64 ± 6.59	13.77 ± 8.04	14.14 ± 6.83	12.96 ± 6.61	11.21 ± 4.98	NS
Total food intake (g/100g/day)	7.62 ± 2.5	7.70 ± 3.22	7.53 ± 2.72	7.53 ± 2.58	7.59 ± 2.07	NS
Body weight gain (g)	278.16 ±28.70	302.53 ± 8.99	326.55 ±59.29*	304.6 ± 50.56	263.96 ±26.15	
Length (cm)	25.65 ± 0.71	26.5 ± 0.87	26.05 ± 1.17	26.22 ± 0.83	25.55 ± 0.98	NS
Weight /length2 (BMI)	0.63 ± 0.05	0.62 ± 0.04	0.68 ± 0.08	0.65 ± 0.07	0.65 ± 0.06	NS

Data are expressed as mean ± S.D of (9-10) animals per group.
LS: level of significance. NS: not significant
* Significantly different from the control value at p <0.05.

Table 3. Serum lipid levels and atherogenic index in rats exposed to various concentrations of lead acetate for 11 months.

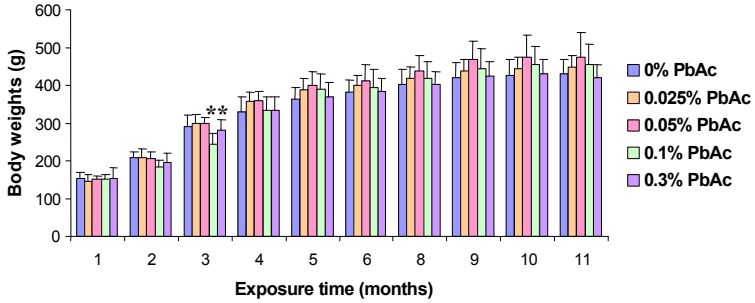
					Atherogenic index (units)		
Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	TC/HDL-C	LDL-C/HDL-C	LS
Control	74.1 ± 16,29	54.50 ± 15.28	61.1 ± 13.15	11.60 ± 3.81	1.21 ± 0.05	0.19 ± 0.04	NS
0.025%	67.33 ± 8.29	54.11 ± 21.03	56.78 ± 7.97	9.11 ± 3.69	1.19 ± 0.06	0.16 ± 0.08	NS
0.05%	80.60 ± 14.83	69.63 ± 39.96	65.40 ± 11.94	10.40 ± 4.55	1.23 ± 0.09	0.16 ± 0.05	NS
0.1%	72.00 ± 19.70	58.44 ± 21.61	61.00 ± 10.93	10.56 ± 3.50	1.17 ± 0.18	0.17 ± 0.04	NS
0.3%	73.10 ± 14.14	58.44 ± 22.32	60.20 ± 10.78	10.78 ± 4.97	1.21 ± 0.09	0.18 ± 0.08	NS

Data are expressed as mean ± S.D of (8-10) animals per group.
LS: level of significance. NS: not significant

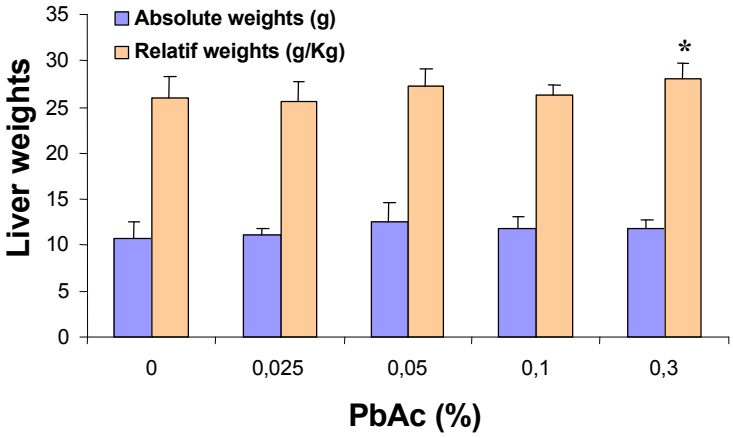
Table 4: Glycemia and serum biochemical parameters levels in all lead treated rats.

Groups	Control	0.025% PbAc	0.05% PbAc	0.1% PbAc	0.3% PbAc	LS
Glucose (g/l)	0.95 ± 0.18	1.01 ± 0.14	1.05 ± 0.16	0.99 ± 0.21	1.12 ± 0.20	NS
Albumin (g/l)	42.36 ± 3.01	39.80 ± 2.41	41.87 ± 2.03	41.08± 2.84	41.18 ± 2.79	NS
TP (g/l)	75.73 ± 4.72	74.10 ± 4.31	72.63 ± 2.28	71.23 ± 4.17	72.44 ± 4.47	NS
AST (UI/l)	122.3 ± 24.16	110.56± 14.21	126.00 ± 34.37	121.25 ± 37.19	131.22 ± 48.33	NS
ALT (UI/l)	45.6 ± 7.99	37.33 ± 4.87	45.3 ± 8.94	43,88 ± 10.75	48.5 ± 12.70	NS
ALP (UI/l)	70.4 ± 13.75	66.89 ± 12.96	73.3 ± 14.05	83.56 ± 18.16	79.44 ± 20.31	NS
TBIL (mg/l)	1.1 ± 0.32	0.89 ± 0.33	0.89 ± 0.33	0.89 ± 0.33	1.00 ± 0.00	NS
DBIL (mg/l)	0.50 ± 0.53	0.22 ± 0.44	0.22 ± 0.44	0.56 ± 0.53	0.40 ± 0.52	NS
LDH (UI/l)	323.33 ± 117.96	394.43 ± 155.05	365.00 ± 151.49	314.56 ± 124.20	402.33 ± 134.50	NS

Data are expressed as mean ± S.D of (7-10) animals per group.
LS: level of significance. NS: not significant



Values are mean ± SD of (9-10) animals per group.
** p < 0.01 against the control group.
Fig. 1: Body weight evolution of rats during the period of lead exposure.



Values are mean ± SD of (9-10) animals per group.
* p <0.05 against the control group.
Fig. 2: Absolute and relative liver weights in lead-treated and control groups.

DISCUSSION

In this study, the effect of long-term lead exposure of rats on growth, lipid profile and biochemical parameters were examined. The brick red stain observed in some animals in our study was not due to lead exposure so we can consider that the appearance of this hair colour is evident in elderly rats after this long experimental period. Nevertheless, Grant *et al.* [13] observed a distinctly higher proportion of animals lead exposed 9 months to 0.025 % PbAc. They had extremely poor hair coats consisting of yellow fur and patches of marked hair loss, while Khan *et al.* [14] observed a ruffled hair coat from 2nd week of lead administration in mice given 100 mg/Kg body weight during 42 days.

Body mass index (BMI) is a simple index of weight-for-height that is commonly used to classify underweight, overweight and obesity in adults [15]. Low and high body mass index values haves been shown to increase health risks and mortality.

Obesity also adversely affects plasma lipids, especially increasing triglycerides and decreasing the cardio-protective levels of high density lipoprotein cholesterol [16].

Our results indicated that the body weight of rats exposed to 0.1 % PbAc was significantly reduced at the third month of experimental period followed by normalization despite the continuation of lead exposure. Similarly, the body weight loss was regained later in rats treated with an identical lead concentration [12]. However, the normalization of body weight after one month of lead exposure was also observed by other authors in rats treated with higher lead concentration than that used in our experiment [17,18]. Hence, we can suggest that lead can affect the body weight in the first period of exposure and the long duration of exposure allows the body to adapt to this toxic, then it can regain its lost weight.

At the end of experimental animal, the body weight gain was significant increased in animals exposed to 0.05 % PbAc without an increase in total food intakes throughout the experimental period. However, no significant lead effect on feed consumption and the feed conversion ratio was found in broiler chickens exposed to 200 mg/kg added to the diet for 42 days, while the body weight was significantly decreased [19].

Likewise, body weight gain was not affected in our previous experiments conducted in rats treated for 6 months with similar lead concentrations [20]; therefore, we can suggest that the chronic exposure to low lead dose may conduct to high body weight gain and obesity risk.

In our research, the body length and BMI remain unchanged in all treated groups compared to the control group. Thus, the long-term exposure to low or moderate lead concentration does not affect neither the body length nor BMI.

Contrariwise, it was reported that lead exposure reduced in rats, somatic growth, longitudinal bone growth and bone strength during the pubertal period [21].

Indeed, there was no association observed between maternal blood lead and childhood BMI or attained height [22].

In our experiment, the absolute liver weights were normal in all lead treated groups, while the relative liver weights were significantly increased in animals exposed to the highest PbAc concentration.

In accordance with our results, no change was observed in the absolute or relative liver weights in offspring rat exposed 9 months to 0.025 % PbAc, an identical concentration used in our experiment [23].

The liver was slightly enlarged in lead exposed mice [14] and moderate hepatomegaly with normal liver function tests was reported in the case of an adult battery worker [24].

The increase in hepatic weight was associated with liver lipid accumulation [25] and TC and TG were significantly increased in rat liver extracts after 3 weeks of lead acetate treatment [5]. Furthermore, hepatic cholesterogenesis was enhanced in rats exposed to low lead concentrations [26].

Although lipids and biochemical parameters in liver were not determined in our study, Pearson's correlation showed no correlation between serum lipids or biochemical parameters and relative livers weights of animals exposed to the highest PbAc dose. The only independent variable that tended to correlate to these relative liver weights was PAL (0. 643, $p = 0.06$).

Alkaline phosphatase is a liver enzyme that is frequently measured. This enzyme is usually found in the walls of the intra- and extra-biliary ducts. Elevation of alkaline phosphatase may indicate an injury to the biliary cells [27].

It was shown that lead exposure induced liver histopathological alterations in rats [28] and damage in Human Liver Carcinoma (HepG2) Cells in vitro [29].

Few studies have reported the effect of long-term exposure to low or moderate lead concentrations on serum lipid and biochemical parameters of liver in animal or human.However, increased serum concentration of TC and LDL-C and low concentration of HDL-C are major cardiovascular risk factors [30].

Our results revealed no significant changes in serum TC, TG, LDL-C, HDL-C levels and atherogenic index in all rat groups after this long period of lead exposure. Therefore, long-term exposure to low or moderate lead concentration is not responsible of disturbances in serum lipid profile and atherosclerosis risk. Similarly, there was no significant difference in plasma TC and TG levels in rats exposed to low lead concentrations for 12 weeks [26] and serum TC concentrations were not changed in rats given 250 mg lead/l every week for the four weeks of treatment [31] and in rats poisoned intragastrically with small doses of lead for 7 weeks [6]. On the contrary, a significant elevation of plasma TC was found in rats orally exposed for 90 days [32], while serum TC and TG were significantly increased in rats exposed 3 weeks to lower lead acetate concentration than ours.

The association between lead exposure and high serum lipid levels is biologically plausible and could be due to either increased synthesis or to impaired feedback inhibition [5].

Our findings showed a reduction of LDL-C levels in all treated groups with no significant differences compared to the control group, while LDL-C concentration was significantly decreased after 2, 4 and 6 weeks of lead [28]. However, a significant increase in LDL-C was found by Newairy and Abdou [5].

Several studies in human have shown that occupational lead exposure induces alterations [33,34] or no changes [35, 36] in serum lipid profiles.

Aminotransferases are the most frequently used and most specific indicators of hepatic injury and represent marker of hepatocellular necrosis [37].

In the current study, serum AST, ALT, ALP, LDH activities and glycemia were slightly higher in rats exposed to 0.3% PbAc but levels serum TP and albumin tended to be lower in all lead treated groups. These perturbations are not statistically significant compared to the control group.

Nevertheless, no significant changes were observed in serum biochemical parameters or glycemia between other lead treated groups and the control group.

Our research showed that long-term lead exposure did not produce alteration in serum biochemical parameters indicating no presence of hepatocyte injury, hence liver function was not affect.

Our results were similar as that reported by Herman *et al.* [38] who reported no changes in serum TP, albumin, TBIL and DBIL, ALP, AST and ALT activities in well-nourished rats exposed 10 months to 0.05% which was an identical lead concentration used in our experiment animal.

The activities of serum AST and ALT were significantly increased in lead exposed rats [14]. However, experiments conducted in male albino rats receiving a single dose of intramuscular administration of lead acetate (100 µmol/kg body weight) showed a significant increase in serum TP and ALP, AST and ALT activities after 3 and 24 h of lead administration [39].

Activities of ALT, AST and ALP were significantly increased in rats given daily lead acetate in diet as 500 mg/kg after 2, 4 and 6 weeks of treatment [28] and in rats exposed to higher PbAc concentration in drinking water during a shorter period than ours while TP and albumin were significantly decreased in these studies [40,41].

An increase of ALP activity was reported in rats orally exposed to lead for 15, 60 and 90 days [42]. However, experiment conducted in adult cows reared in polluted environment around different industrial units indicated a significant positive correlation between blood lead and serum ALT and AST and a negative correlation with serum TP and albumin [43].

On the other hand, it was reported that oral administration of lead acetate (100 mg/kg body weight) to experimental rats decreased significantly the activities of ALP, AST and ALT after 4 months of treatment [44].

In our research, glycemia and LDH remain unchanged in all lead exposed groups. Similarly, blood glucose was unaltered in neonate rats exposed to high lead dose for 8 weeks [45], while serum glucose level was significantly decreased in rats exposed to oral lead administration for 20, 40 and 60 days [46]. However, an increase in LDH activity was recorded in rats following lead subacute intoxication [47].

Several studies in human have demonstrated a disturbance in serum liver tests following occupational lead exposure [48 -51], whereas TP, LDH and ALP levels were within normal levels in battery and muffler repair workers [52]. However, serum AST, ALT activities and TP were normal in family manufacturing lead acid batteries [53].

It was shown in the case of low blood lead levels that blood lead levels were significantly correlated with AST and ALT levels in non-smoking women [54].

Obviously, the route of lead exposure, lead doses and the longevity exposure explain partially the heterogeneity in the findings of lead effect on lipid metabolism and biochemical parameters of liver in animal or human.

Regarding the previous reports, an alteration of serum lipid or biochemical parameters was observed after a short period of lead exposure, hence we can suggest

that the observed of no pronounced perturbation of these parameters in our study may be due to an adaptation phenomenon that can be occurred after a long-term lead exposure probably by recovery of feedback control.

In conclusion, our findings reveal that the long-term exposure to low lead concentrations through drinking water can contribute to high weight gain and obesity risk while a moderate lead exposure can induce a significant increase in the relative liver weight with no evident disturbances in serum lipid profile or biochemical liver parameters. Thus, we should not incriminate the long-term exposure to low or moderate lead concentrations to risk of atherosclerosis and impairment of liver function. Hence, these findings reassure the young peoples who are exposed weakly or moderately to lead in their lives.

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