

Impact of Simultaneous Exposure to Lead and Efavirenz on Some Biochemical Markers in Wistar Rats

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Abstract

Chronic exposure to heavy metals including lead remains a serious problem for humanity. The current study aims to evaluate the impact of co-exposure to lead (Pb) and Efavirenz (EFV) on some biochemical parameters in blood. Twenty eight Wistar rats were divided equally into four groups respectively orally fed with lead acetate at 10 mg/kg (G_{Pb}), EFV at 20 mg/kg (G_{EFV}), both xenobiotics (G_{Pb+EFV}), and distilled water (G_{Ctrl}). On Day 0 and Day 28, the blood of each animal was collected and biochemical assays were conducted. Data were processed with SPSS 16.0. The results showed a significant decrease in total proteinemia, albuminemia, serum calcium and iron as well as a significant increase in blood urea and uric acid in groups exposed to lead. The aforementioned changes were more pronounced in group G_{Pb+EFV} . Besides, significant increases in total cholesterolemia were observed in G_{EFV} and G_{Pb+EFV} . In contrast, changes in blood glucose and triglycerides were not significant. In conclusion, this study highlights a real problem of public health, in the light of thousands of patients receiving antiretroviral therapy and who are unintentionally exposed to heavy metals.

Keywords: Lead; Efavirenz; Biochemical parameters; Heavy metal; Antiretroviral drug; Wistar rats.

Introduction

The humanity is increasingly confronted to health risks linked to pollution of air, water, soil, fauna and flora by toxic xenobiotics [1,2]. In Benin like most West African States, several studies have reported increasing threats by heavy metal poisoning including lead which occupies a dominating place [3-5]. The levels of this inorganic chemical pollutants are indeed often higher than the maximum allowable concentrations particularly in some drinking water [6,7] and foods commonly consumed [8-11].

Lead is an inducer of oxidative stress [12-16] with proven toxic effects in nervous system [13,17], hematopoietic system, cardiovascular system, reproductive system, liver and kidneys functions [14]. Its absorption is stronger in children [17] and people with protein deficiency or mineral deficiency or excess fat [13].

This could be the case in people immunosuppressed by HIV and who are permanently exposed to the risk of adverse reactions linked to antiretroviral drugs (ARVs) [18,19]. Indeed, despite their efficacy in the improvement of survival of patients [20], the ARVs therapy can induce oxidative stress which is often correlated to disturbances in biological nutritional markers [18,21].

It is in this context that we decided to better know the adverse effects of lead poisoning during antiretroviral treatment. Thus, the current study proposes to evaluate the impact that could have the absorption of lead and Efavirenz on some biochemical parameters. Efavirenz drugs has been selected because it is a very privileged ARVs in pregnant women, children over three years, co-infected patients with HIV and TB according to news recommendations of WHO [20].

Material and Methods

Study area

This work was carried out in Benin, particularly at the Research

Laboratory in Applied Biology, located at Polytechnic School of Abomey-Calavi in University of Abomey-Calavi. It extended from 02 June to 30 December 2013.

Animal material

Twenty eight (28) Wistar rats were used. After their acquisition, the animals aged 2 to 4 weeks were acclimated for 8 weeks in order to fully adapt to their new environment and acquire means weight of about 150 g. Cages were placed in a well-ventilated room with alternating light and dark periods of 12 hours each. Drinking water was available ad libitum and the standard rodent diet was renewed every morning.

Chemical material

It is on one hand, a solution of lead acetate at 10 mg/mL and on the other hand, Efavirenz in powder whose 200 mg were diluted daily in distilled water so as to obtain a 5 mg/mL solution.

Distribution of rats and administration of xenobiotics

Rats were weighed and randomly divided into four (04) groups of seven. The groups were identified according to the following exposure regimes:

- G_{Ctrl} = group of control rats that received 0.5 ml of distilled water

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- G_{pb} = group of rats treated with 10 mg/kg of lead acetate. This dose was chosen in accordance to several studies [13,22].
- G_{Efv} = group of rats treated with 20 mg/kg of Efavirenz. It is the maximum daily dose required in a human child infected with HIV [23]. This choice was done in accordance to the dose used by Adjene et al. [21].
- G_{pb+Efv} = group of rats treated with 10 mg/kg of lead acetate and 20 mg/kg of EFV

The administration of xenobiotics was made through the orogastric tube for 28 days every morning between 7:00 am and 8:30 am.

Blood collection and biochemical analysis

Blood samples were collected on Day 0 and Day 28 from eye vein in a collection tube without anticoagulant (Vacutainer System; Becton Dickinson) such as described in Hassan and Jassim [24]. They were properly labeled and placed directly on a rack into a cool-box containing icepacks. Serums were separated from the blood cells after centrifugations at 2500 rpm. Total protein, albumin, urea, glucose, uric acid, total cholesterol, triglycerides, iron and calcium were measured by using Elitech Clinical Chemistry reagents on analyzer Mindray BS-200.

Statistical analysis

Means and standard deviations for each parameter were calculated using SPSS 16.0 software. Values were checked for homogeneity of variances thanks to the Levene's test. Then, a one-way analysis of variance (ANOVA) followed by the post hoc Bonferroni multiple comparisons test were carried out for comparing mean levels and detect specific significances differences between groups when $p < 0.05$. The rates of changes in all parameters from Day 0 to Day 28 were calculated and the significativity of decrease or increase were evaluated using the Student's t test with a 95 % confidence level. The graphs were designed using Graph Pad Prism software 5.03.

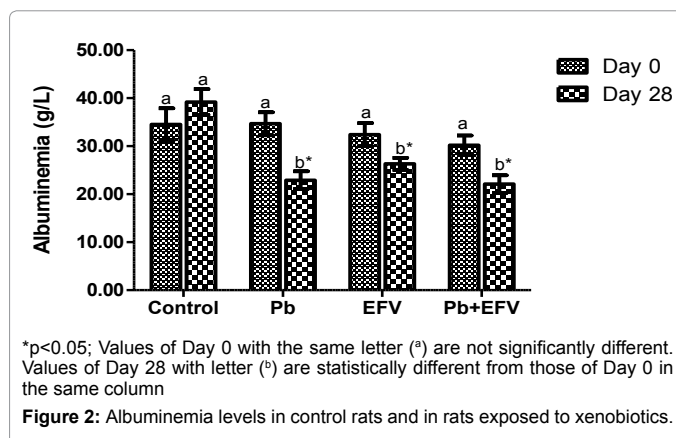
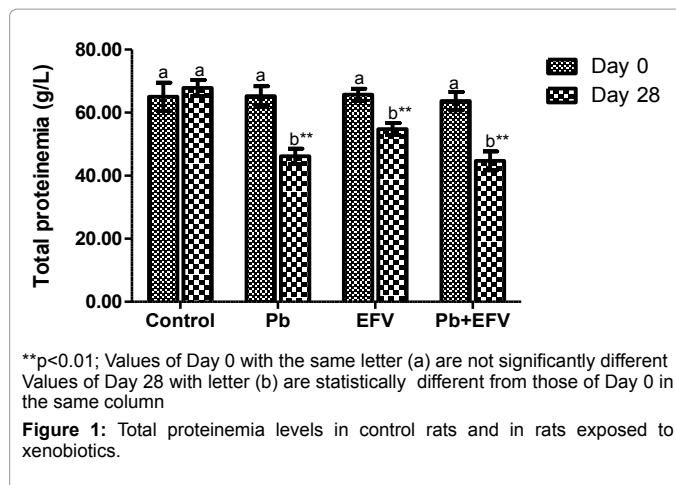
Results and Discussion

Protidic parameters concentrations

Total blood protein: 28 days after exposure, the mean of total proteinemia (Figure 1) was significantly decreased by 29.3% (65.24 to 46.14 g/L) in G_{pb} ($p=0.00662$), by 16.6% (65.67 to 54.79 g/L) in G_{Efv} ($p=0.00205$) and by 29.8% (63.67 to 44.70 g/L) in G_{pb+Efv} ($p=0.00756$). The difference of the rate of decrease in this parameter between the groups G_{pb} and G_{Efv} was significant ($p=0.01871$), but that observed between G_{pb} and G_{pb+Efv} is not.

Serum albumin: The mean of albuminemia (Figure 2) was significantly decreased by 33.9% (34.68 to 22.90 g/L) in G_{pb} ($p=0.01125$) by 18.8% (32.41 to 26.31 g/L) in G_{Efv} ($p=0.02339$) and by 26.8% (30.19 to 22.10 g/L) in G_{pb+Efv} ($p=0.01601$). The difference between the mean level of serum albumin in G_{pb} and G_{Efv} groups is not significant. It is the same between G_{pb} and G_{pb+Efv} .

Blood urea: From Day 0 to Day 28, the mean of blood urea (Figure 3) was increased by more than 2 times in the groups GPb and $GPb+Efv$, passing respectively from 0.20 to 0.45 g/L ($p=0.00713$) and from 0.21 to 0.48 g/L ($p=0.00065$). Changes in G_{Ctrl} and $GEfv$ groups were not significant. The increase in the rate of the mean level of blood urea in GPb significantly exceeds that observed in $GEfv$ ($p=0.01066$). But there is no significant difference between the increase in GPb compared to $GPb+Efv$.



Blood uric acid concentrations: From Day 0 to Day 28, the mean of uricemia (Figure 4) was significantly increased in all groups except G_{Ctrl} . This increase was by 73.1% (33.02 to 57.16 mg/L) in G_{pb} ($p=0.00223$) and by 90.9% (31.96 to 61.00 mg/L) in G_{pb+Efv} ($p=0.00089$). The difference of the rate of increase in the mean of blood uric acid is significant between G_{pb} and G_{Efv} i.e. 48.5% ($p=0.00131$). But between G_{pb} and G_{pb+Efv} , no significant difference was noted.

Blood glucose concentrations: No significant changes in the mean of blood glucose were noted whatever comparisons made between the four experimental groups (Figure 5).

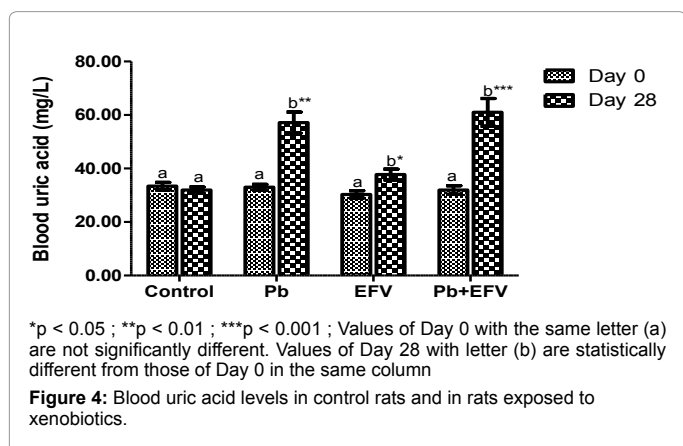
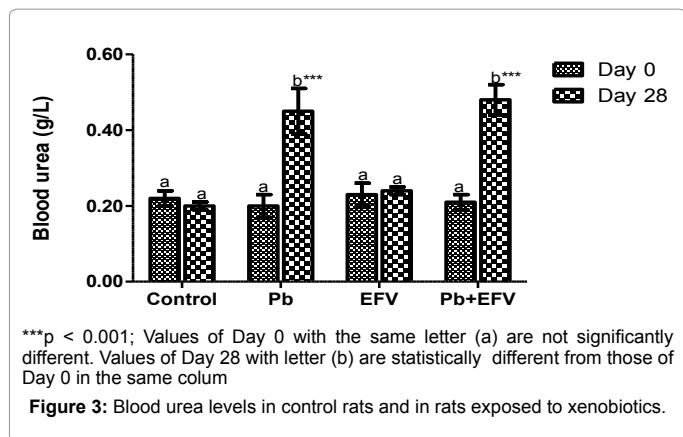
Blood lipids concentrations

Total cholesterolemia: From Day 0 to Day 28, the mean of total cholesterolemia was increased significantly in G_{Efv} ($p=0.00261$) and G_{pb+Efv} ($p=0.00827$) with respective rate of increase of 35.2% (0.80 to 1.08 g/L) and 32.2% (0.86 to 1.13 g/L). The changes in this parameter in the others groups (G_{Ctrl} and G_{pb}) were not significant (Figure 6).

Triglyceridemia: From Day 0 to Day 28, the mean of blood triglyceride (Figure 7) was increased insignificantly in the four different experimental groups.

Mineral nutrients concentrations

Serum iron: From Day 0 to Day 28, the mean of serum iron (Figure 8) was significantly decreased by 58.3% (1.85 to 0.77 mg/L) in G_{pb} ($p=0.00170$) and by 66.0% (1.74 to 0.59 mg/L) in G_{pb+Efv} ($p=0.00087$).



Changes in G_{Ctrl} and G_{EFV} were not significant (Figure 8). The difference between the rate of decrease in this parameter in G_{Pb} compared to G_{EFV} or to G_{Pb+EFV} was not significant.

Blood calcium: The mean of blood calcium was decreased significantly by 21.8% (80.4 to 62.9 mg/L) in G_{Pb} ($p=0.00429$) and by 24.1% (78.9 to 59.9 mg/L) in G_{Pb+EFV} ($p=0.00142$). In the others groups (G_{Ctrl} and G_{EFV}), the decrease in this parameter was not significant (Figure 9). The decrease in the mean level of serum calcium in the G_{Pb} group exceeds significantly by 15.1% ($p=0.02326$) that observed in G_{EFV} . But between G_{Pb} and G_{Pb+EFV} , no significant difference was observed.

Discussion

Impact of lead acetate on biochemical parameters

The signs of chronic lead poisoning are usually non-specific, discreet and insidious [13]. In this study, the daily dose of 10 mg/kg of lead acetate we used has been considered by some authors as a low dose in rats [25,26]. Saka et al. [13], during their experience wherein increasing doses of lead acetate (25, 50 and 100 mg/kg) have been administered in rats for one week, had also found a highly significant decrease in proteinemia and an increase in blood urea and uric acid concentration. Missoun et al. [27] showed that rats exposure to 1000 ppm of lead acetate in drinking water for 8 weeks causes hypercalcemia.

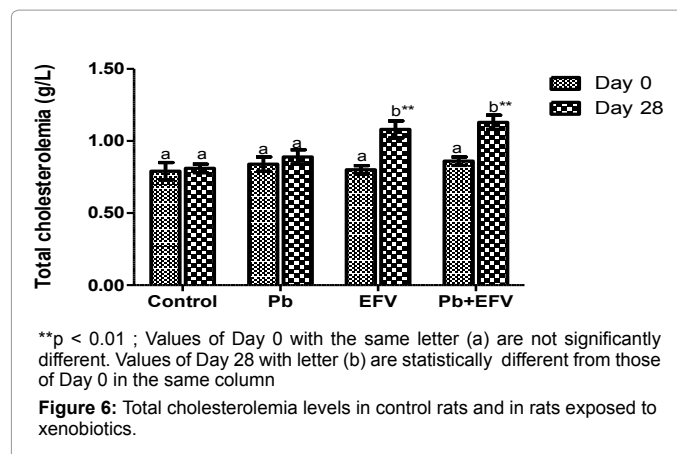
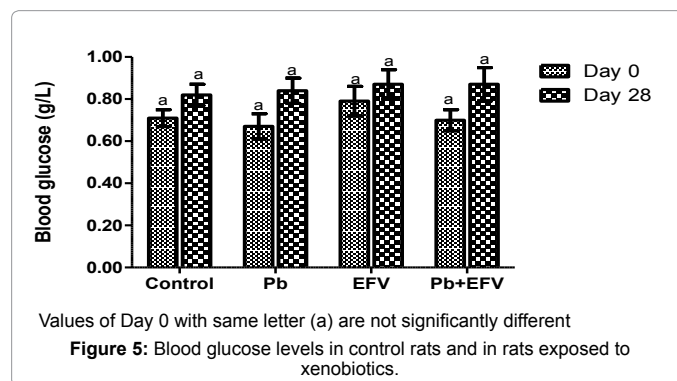
Unlike our results wherein the variability in blood glucose was not affected, Saka et al. [13] report that the lead (from 50 mg/kg) causes a significant increase in glycemia. Missoun et al. [27] on the other hand

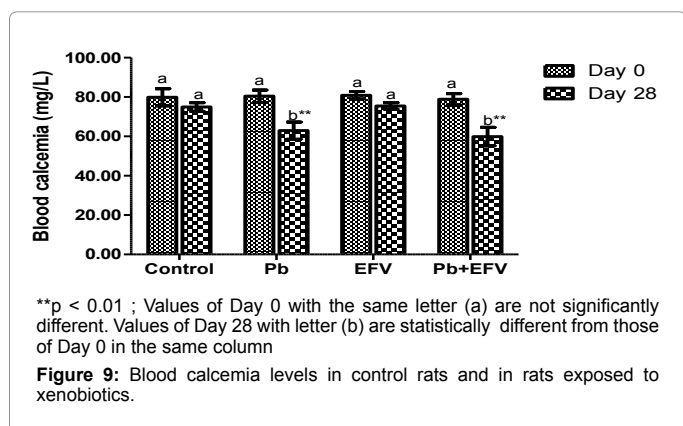
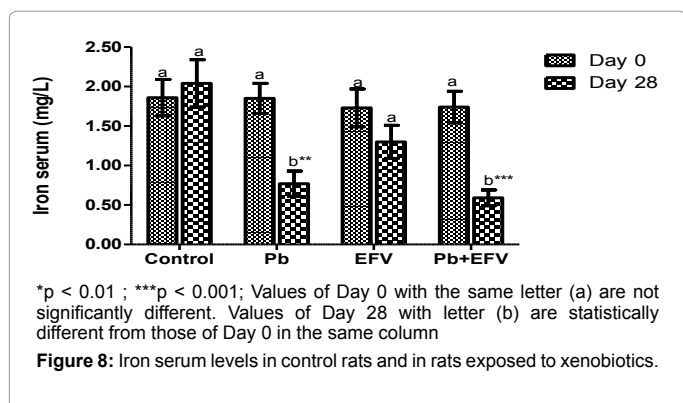
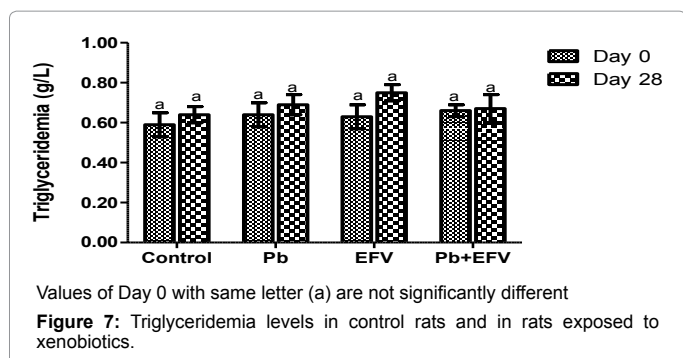
have found that lead acetate induce a decrease in this parameter.

Furthermore, the insignificant changes in total cholesterolemia after lead exposure is in accordance with findings of Hassan and Jassim [22] but is opposed to those of Moussa and Bashandy [28] who have noted an increase in this blood lipid level after rats exposure to lead (via drinking water containing 20000 ppm of lead acetate) for a month. Triglyceride concentrations have remained normal according to our results unlike those of Hassan and Jassim [22] who found that administration of lead acetate at 10 mg/kg induce a decrease in triglyceridemia in rats.

The decrease in total proteinemia and in serum albumin particularly might be due to an alteration of their metabolism into the liver. This interpretation is supported by Saka et al. [13] and Fowler and DuVal [29] who claim that in case of aggression by xenobiotics, the hepatic metabolism of proteins is generally altered towards defense systems production and neoglucogenesis. Indeed, amino acids contained in protein compounds are catabolize under actions of transaminases, with ammonia production (highly toxic) leading to urea, the final form of nitrogenous waste excretion [13,30]. That excretion is done at the level of nephrons which is the structural-functional unit of kidney. Therefore, the increases in blood urea often reflect a nephropathy characterized by glomerular and tubular lesions [30]. This dysfunction is confirmed by the increase in blood creatinine which shows a decrease in excretory power of nephrons and even a tendency to renal failure [13,29]. Several authors have proven a close relationship between the intensity of lead poisoning and increased of blood urea, creatinine and uric acid.

The increase in blood uric acid apart from the gout that could result





is a corollary of saturnine nephropathy [13,30]. The hyperuricemia observed in our study is also a marker of oxidative stress linked to a proliferation of pro-oxidative substances such as reactive oxygen species as asserted in [31] and [32]. This assumption of oxidative stress related to lead exposure could justify the decrease in serum iron and blood calcium. Indeed, according to Probius [33], serum iron measured by the spectrophotometric method represents the pool of iron bound to carrier proteins such as transferrin, ferritin, ceruloplasmin and some chelating agents. Under normal physiological conditions, it is this type of iron that is detectable in the body [31]. But when, for any reason it is released, there is a decrease in the proportion measured. This free iron becomes pro-oxidant [34,35] that catalyzes the reactive oxygen species formation. In addition, iron deficiency induced by lead absorption could also be explained by a competitive binding of lead and iron at the level of binding sites of iron [13]. This phenomenon of competition also exists between calcium and lead, hence lower calcemia observed in rats intoxicated by lead. That heavy metal takes the place of calcium

on binding sites and disturbs several cellular or molecular processes mediated by the latter [33,36,37]. According to Hammad et al. [38] and Bruening et al. [39], the gastrointestinal absorption of lead can be significantly reduced by a diet rich in calcium and iron.

The insignificant change in blood glucose indicates that lead has not had adverse effects on the pancreas unlike findings of Ramirez-Cervantes et al. cited by Saka et al [13]. These authors found a significant increase in blood glucose levels in subjects with saturnine and therefore directly attributed to the deleterious effects of lead acetate on the pancreas.

Biochemical disturbances associated with Efavirenz

The administration of Efavirenz at a daily dose of 20 mg/kg led to a significant decrease in total proteinemia and serum albumin as well as a moderate increase in blood uric acid and total cholesterolemia. Indeed, metabolism and mechanism of action of Efavirenz like those of many other antiretroviral drug promote oxidative stress [18,21]. Adjene et al have highlighted lipid peroxidation marked by the significant increase in Malondialdehyde level and decrease in blood superoxide dismutase in rats force-fed with Efavirenz (600 mg/70 kg) for 30 days. The moderate hypercholesterolemia found after Efavirenz absorption in our study is strengthened by the report of Kirchner in 2012 [40] who assert that Efavirenz may be responsible of lipodystrophies characterized by increase in triglycerides and LDL cholesterol and with decreased HDL cholesterolemia.

2-2. Impact of co-administration of lead acetate and Efavirenz

In the conditions of our experiment, the alterations in biochemical parameters were more frequent and severe in rats exposed to lead than in those exposed to Efavirenz. Thus, the decreased rate of total protein and serum calcium as well as the increased rate of uremia and of uric acid were significantly higher under lead administration than Efavirenz. Indeed, the dose of 10 mg/kg/day of lead is high enough [22,25]. To induce a higher toxicity within a short period of time (28 days). Moreover, lower doses than the one used in our study were reported to induce evident toxicity in rats [41]. Taken separately, lead [12,14,22,25,41] and Efavirenz [18,19,21] induced toxicity effect. However, in this study, we did not detect additive effects or inhibition between the two compounds.

Conclusion

This study aimed to evaluate some biochemical impacts associated with lead and Efavirenz intoxication. According to our results, it appears that the damages induced by lead were more important than those caused by Efavirenz. However, we did not find any significant additive or antagonist effect when both xenobiotics were co-administrated. Further studies are needed with the same dose of Efavirenz and a lower dose of lead which will be administered for a longer period of time.

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