

International Research Journal of Biological Sciences _ Vol. 5(1), 14-21, January (2016)

Attenuation effect of *Moringa oleifera* leaves powder on Blood Biochemical disturbance induced in lead-exposed rats

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Received 23rd November 2015, revised 24th December 2015, accepted 4th January 2016

Abstract

The nutritional and phytopharmaceutical composition of Moringa oleifera leaves suggest that this vegetable can prevent toxic effects induced by xenobiotics .The current study aims to assess the prophylaxic capacity of Moringa oleifera leaves powder (MoLP) on biochemical disturbances in lead-exposed rats. Twenty-eight (28) Wistar rats were equally divided into four groups receiving respectively distilled water (Control), MoLP, lead acetate and both lead acetate + MoLP. The treatment was administrated orally for 28 days. Blood samples were collected from animals at day zero (D0) and D28 for biochemical assays. Results showed that lead treatment decreased significantly the levels of total proteinemia (p<0.01), albuminemia (p<0.05), serum iron (p<0.01) and calcemia (p<0.01) compared to control group. MoLP addition to lead treatment produced opposite effect on these parameter levels and reversed the impact of lead treatment. The levels of blood urea (p<0.001), creatinine (p<0.01) and uric acid (p<0.01) increase significantly in lead-treated animals compared to controls. MoLP treatment induced no significant difference in the levels of blood urea, creatinine and uric acid. However, it suppresses the effect of lead treatment. On the whole, these results showed that MoLP administration to rats reverses lead deleterious effects on many circulating biochemical parameters. The results highlight MoLP importance in the attenuation of some biochemical disturbances associated with chronic lead absorption and strengthen arguments in favour of the use of MoLP as food supplement.

Keywords: Lead toxicity, Moringa oleifera leaves powder, Biochemical disturbances, Blood, Rat.

Introduction

Moringa oleifera is a vegetable species to Moringaceae family widespread in tropical and subtropical regions, particularly in sub-Saharan Africa¹. It is increasingly promoted worldwide for its impressive range of medicinal use² and high nutritional value³. Several authors report the use of its leaves, roots, seeds, barks, fruits, flowers and immature pods in the prevention or treatment of many diseases such as diabetes, hypertension, inflammation, bacterial infections, fungal infestations, immune deficiencies, heart disease, cancer, epilepsy, gastric ulcer, urinary disorders, hyperlipidemia, and liver disease².

M. oleifera leaves hydro-alcoholic extract can be used to prevent adverse toxic effects of some antipyretic⁴ and ant tubercular drugs⁵. A detoxifying activity of the same hydro-alcoholic extract was highlighted in lead-exposed rats. Indeed, the seed powder of this plant was used to reduce organic damages induced by arsenic⁶ and lead⁷. However, there are few studies on the antitoxic potential of M. oleifera leaves powder (MoLP), commonly used.

M. oleifera leaf is the most commonly used part of the plant because it's easier accessibility for people⁸. Therefore, it is important to ensure the accuracy of various indications of MoLP which is increasingly sold as food supplement in West Africa⁹. The current study aims at evaluate the prophylactic capacity of MoLP against biochemical disturbances induced by lead exposure.

Materials and Methods

Chemical and vegetal materials: Lead acetate solution at 10% (Manufactured by HACH Company) was acquired at the National Laboratory for Quality Control of Water and Food in Ministry of Health. It was diluted in distilled water for a final concentration of 10 mg/ml.

Mature and fresh leaves of *M. oleifera* were harvested at Come, 60 km from Cotonou in Benin. The plant identity was authenticated by a botanist from University of Abomey-Calavi, Benin. After detaching, the leaves were washed following the procedure described by Sauveur and Broin¹⁰. Then, they were dried away from direct sunlight and dust for fourteen days before to be milled. The obtained powder was recovered in a clean box and kept away from humidity. It was dissolved in distilled water one day before each use for the rats.

Animal and experimental design: The study was done in University of Abomey-Calavi (Benin). Twenty-eight (28) Wistar rats aged 10-12 weeks and weighing between 145-151 g were equally divided into four groups. They were placed in identified cages inside a room where the light was alternated with the darkness per cycle of 12 hours. All animals had free access to water and standard rodent diet which was renewed every morning.

The first group (Control) received 0.5 ml of distilled water. The second group (MoLP) received 500 mg/kg body weight (bw) of MoLP. The third group (Lead) received 10 mg/kg bw of lead acetate. The last group (Lead + MoLP) received both lead acetate (10 mg/kg bw) and MoLP (500 mg/kg bw). The products were daily administered by force-feeding for 28 days. Different probe was used for each kind of solution in order to avoid interferences.

Blood sampling and biochemical analysis: At the beginning (D0) and end of the exposition period (D28), animals were anesthetized by exposure to chloroform vapour. Blood samples were collected by venipuncture in the retro-orbital plexus with a heparinised capillary pipette following the method described by Tehoua et al.¹¹. All specimens in collection tubes without anticoagulant (Vacutainer system; Becton Dickinson) was labelled and centrifuged within 2 hours at 2500 rpm/min for 10 minutes. Total protein, albumin, urea, creatinine, iron, calcium, glucose, uric acid, total cholesterol and triglycerides were measured by using Elitech reagents (ELITech Group, Maizy,

France) on a Mindray BS 200 auto-analyzer.

Statistical data processing: Data were entered in Excel 2010 then exported in SPSS 16.0 software. The mean and standard deviation were calculated for each measured parameter. Values were checked for homogeneity of variances using the Levene's test. A one-way analysis of variance followed by the post hoc Bonferroni's multiple comparisons test was carried out for comparing mean levels and detect specific significances differences between groups. Differences were considered as significant when p < 0.05.

Results and Discussion

Results: Total proteinemia: From D0 to D28, total serum protein level decreased significantly (p<0.01) by 29.3 % in the lead-exposed group (figure-1) compared to control. Rats treatment with MoLP or the combination of lead and MoLP showed no significant variation in total protein level compared to controls. MoLP has allowed keeping 32.2 % of the serum protein level that would be lost under the lead action (p<0.01).

Albuminemia: Albuminemia decreased significantly (p<0.01) by 29.3 % in lead-treated group (figure-2). MoLP addition to lead treatment has reduced significantly (p<0.01) by 34.3 % the risk of decreasing in serum albumin level.

Blood Urea: The level of uremia increased significantly (p<0.001) by 127.8% in the lead treated group (figure-3). No significant variation of uremia was noted in the other groups. The supplementation with MoLP has reduced significantly (p<0.01) by 112 % the risk of increasing in blood urea level.



Total proteinemia level in rats exposed to lead acetate and/or Moringa oleifera leaves powder compared to controls



Figure-2

Albuminemia level in rats exposed to lead acetate and/or Moringa oleifera leaves powder compared to controls



Figure-3

Blood urea level in rats exposed to lead acetate and/or Moringa oleifera leaves powder compared to controls

Creatininemia: Lead treatment induced a significant increase (p<0.01) in creatinine level by 56.2 % (figure-4). MoLP addition to lead treatment has allowed preventing significantly (p<0.01), 34.3 % the risk of increasing in creatininemia level.

Uricemia: The level of uric acid increased significantly (p<0.05) by 73.1 % in the lead treated group and by 30 % in the similar group which received MoLP in addition (figure-5). The difference in those increased level indicate that the

supplementation with MoLP has reduced significantly (p<0.01) by 43.1 % the risk of hyperuricemia.

Serum iron: Serum iron level decreased significantly (p<0.01) by 58.3 % in the lead-exposed group (figure-6). Rats treatment with MoLP or the combination of lead and MoLP showed no significant variation in iron level compared to with the controls. MoLP has allowed preserving 52.7 % of the serum iron level that would be lost under the lead action (p<0.01).



Creatininemia level in rats exposed to lead acetate and/or Moringa oleifera leaves powder compared to controls



Figure-5

Blood uric acid proteinemia level in rats exposed to lead acetate and/or *Moringa oleifera* leaves powder compared to controls

Serum calcium: Serum calcium level decreased significantly (p<0.01) by 21.8 % in lead-treated group (figure-7). MoLP addition to lead treatment has reduced significantly (p<0.01) by 16.8 % the risk of calcemia decreasing.

Blood glucose level: Blood glucose level showed no significant variation in rat group receiving lead alone, MoLP alone or association of both lead and MoLP when compared to control (figure-8).

Cholesterolemia: No significant variation in the serum cholesterol level was noted in the experimental groups compared to the control (figure-9).

Triglyceridemia: The level of triglycerides did not vary significantly in any of the treated groups compared to the control (figure-10).



Figure-6

Serum iron level in rats exposed to lead acetate and/or Moringa oleifera leaves powder compared to controls



Figure-7

Serum calcium level in rats exposed to lead acetate and/or Moringa oleifera leaves powder compared to controls

Discussion: This study showed that the administration of 10 mg/kg bw of lead acetate to Wistar rats induce a decrease in serum protein and albuminemia levels. These findings are in agreement with some published results¹². Who reported hypoproteinemia in rats exposed to lead acetate during a week? Hypoproteinemia in lead-exposed rats is followed by a blood urea increase. This can be explained by proteins catabolism increasing¹² from which amino acids are converted into ammonia leading to urea. Lead affects the excretion function of

nephrons, the structural and functional unit of the kidneys¹³. This justifies the observed hypercreatinemia which is a specific markers showing the decrease in kidneys capacity to purify blood and excrete urine. The increase of blood urea and creatinine in lead intoxicated rats is due to a nephropathy characterized by glomerular and/or tubular damages¹⁴. These biochemical disturbances were correlated with the hyperuricemia observed in the lead-exposed rats. It was reported that lead can induce oxidant/antioxidant system imbalance¹⁵.

International Research Journal of Biological Sciences . Vol. 5(1), 14-21, January (2016)

Lead induced tubular reabsorption of uric acid¹⁴ which is an oxidative stress biomarker¹⁶. The observed decreasing serum iron and calcium level may be correlated to this oxidative condition. The lead competes with these two metal ions, by expelling them from the biding sites of proteins and other transport macromolecules. Iron and calcium deficiency may facilitate the rapid absorption of lead that may in return accentuate their deficiency¹⁷. The released iron because of the lead, contributes to amplify the oxidative stress¹⁸ by catalysing the synthesis of free radicals and other pro-oxidative molecules

that can alter the metabolism¹⁶. The serum iron is rarely free¹⁶. It appears in blood only under pathological conditions after its releasing from transport proteins because of the reactive oxygen species.

In this study, glycemia has not been affected by lead intoxication. Similar results have been reported¹⁹, but some authors have found hypoglycaemia in lead-exposed rats²⁰ while others¹² reported hyperglycaemia in rats treated with the same dose of lead.



Figure-8

Blood glucose level in rats exposed to lead acetate and/or Moringa oleifera leaves powder compared to controls



Figure-9

Total cholesterolemia level in rats exposed to lead acetate and/or Moringa oleifera leaves powder compared to controls



Triglyceridemia level in rats exposed to lead acetate and/or Moringa oleifera leaves powder compared to controls

Cholesterol and triglyceride are the two major blood lipids. Lead intoxication didn't affect the cholesterol level. This result is in accordance with that of some authors²¹, but it contrasted with those of others²² who reported increased circulating free cholesterol in rats exposed to lead acetate during one month. Blood triglyceride levels did not vary significantly. These results also contrasted with those of other authors²¹ who showed that administration of 10 mg/kg bw decrease triglyceride levels in rats.

The administration of Moringa oleifera leaves powder in leadexposed rats has preserved them against protein, iron and calcium deficiency. This observation can be explained by the richness of Moringa oleifera leaves in proteins, iron and calcium²³. The maintaining of normal blood level of each of those nutrients contributes to reduce significantly lead intestinal absorption just as its mobilization and distribution in tissues. It also helps the excretion of lead from the body¹⁷. The antioxidant molecules such as phenolic substances, flavonoids, ascorbic acid, α -tocopherol, and oligo-nutrients brought by Moringa oleifera leaves1,8 improve the activity of the primarily antiradical system by reducing the oxidative stress²⁴. This fact is confirmed by the significant lowering of blood uric acid and the normal blood level of urea and creatinine observed in rats which received both lead acetate and Moringa oleifera leaves powder. Other authors²⁵ observed that Moringa oleifera leaves can decrease blood urea level.

This confirms the diuretic property of *Moringa oleifera*². The preventive effect of *Moringa oleifera* is to reduce level of reactive oxygen species (ROS) by stimulating activities of superoxide dismutase, catalase and glutathione peroxidase just as increasing level of tissues glutathione and metallothionein⁶.

The detoxification property of *Moringa oleifera* is also due to its high concentration in methionine and cysteine, which are amino acids with sulfhydryl group that improve heavy metals chelation²⁶.

Conclusion

The results of study highlight that *Moringa oleifera* leaves powder has antitoxic properties. It has prevented some lead deleterious effects on many circulating biochemical parameters in rats. These arguments are in favour of the use of *Moringa oleifera* leaves powder as food supplement especially in chronic lead-exposed animals.

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