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Moringa Extract Protects ALAD Gene Expression in Rats against Pb-Heavy Metal Poisoning

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Abstract

This study investigated the possibility of using *Moringa oleifera* extract to prevent poisoning caused by commercial lead nanoparticles (C.) and induced by pulsed laser ablation (L.) in white Wistar rats. The rats were divided into six groups with seven rats in each group: (1) 160 mg kg^{-1} Moringa seed extract (M.), (2) 5 mg kg^{-1} commercial lead nanoparticles(C), (3) 5.8 mg kg^{-1} nanoscale lead(L), (4) Moringa seed extract + commercial lead nanoparticles (C. M.), (5) Moringa seed extract + prepared lead nanoparticles (L.M.) and (6) control(Con.). After exposure for 45 days, blood was drawn from the animals and the genetic changes of the ALAD gene were studied. Moringa seed extract (160 mg Kg^{-1}) was efficient to protect ALAD gene expression which gave 1.073725, followed by control of 1 and nanoscale lead X Moringa seed extract (5.8 X 160 mg Kg^{-1}) 0.421775. principal component analysis,

spider (radar) and circular analysis were very informative to extract the variance resulted from treatments on ALAD gene expression.

Keywords: ALAD gene, Lead, Rats, Alcoholic extract, heavy metal, Moringa extraction, protection

1. Introduction

Heavy metals are known as elements possessing a particular density of > 5 g m^{-3}. From these metals are lead, cadmium, mercury and arsenic those mainly threaten human health. Therefore, they environmentally ubiquitous elements that cause diverse difficulties in mankind bodies such as loss of bones, kidneys destruction, vascular disorder, abnormalities and carcinogenesis, these metals led to genetic variations in metal transporter1, gluthione related genes and methylenetetrahydrofolate reductase (Joneidi, et al., 2019). agricultural and industrial development has led to an increase in environmental pollution with heavy metals such as lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg) and others, as hundreds of tons of remnants of factories used for these metals as raw materials throw their waste away. Which are later combined with agricultural fields to be transferred to the body after having crop foods. These are the most dangerous health problems currently because of their danger to the formation of the blood, the Hematopoietic system, the central nervous system, the liver, and the kidneys. These minerals disrupt the work of Dysfunction Endocrine hormones energy (Romaniuk et al., 2018) . Production pathways and immunity, even if they are in very low concentrations because they are not used by the body, which leads to their accumulation and poisoning when their concentration in the body exceeds its defensive ability to get rid of them as a result of this accumulation. Most lead toxicity symptoms are due to be occurred via its interference with δ-aminolevulinic acid dehydratase (ALAD) which efficacy of this enzyme are suppressed by Pb (Mukisa et al., 2022). ALAD bio-builds the 2nd phase occurred for biosynthesizing the heme and is acted as internal suppressor on two hundred sixty five proteasomes. So, Ge et al. (2017) illustrated that ALAD had regulated progressive breast cancer via transformation of grown factor-β-mediated epithelial mesenchymal translocation. Moringa plant, represents the most important medicinal plant known in the world. The Moringa tree bears hermaphrodite flowers, single symmetrical, carried on clustered inflorescences, and the fruit is cantilever-like mustard. Moringa plant blooms in the summer and all parts of the plant can be used for food, commercial and medicinal purposes. Moringa is native to the tropics and subtropics (Saini et al.,

2016]. The *Moringa oleifera* plant contains many mineral elements represented in (K, Fe, Ca, Cl, S, P, Si Al, Mg and Na), This plant contains many active ingredients such as nitrile glycosides, niazirin, niazirinin, mustard oil glycosides, pterygospermin, spirochin and benzyl isothiocyanate (Reetu et al., 2020). *Moringa oleifera* seed extract is rich in antioxidants and has a high potential for use as a relief of heavy metal toxicity (Kerdsomboon et al., 2021). Heme levels is associated with ALAS1, ALAD and HMBS mRNAs were minimized with increase of weight gain in rats (Moreno-Navarrete et al., 2017).Therefore, this study was conducted to assess the protection of moringa extracts on ALAD gene expression against the toxicity of heavy metal in wistar rats.

2. Materials and Method

2.1. Plant materials and preparation

The ripening dry pods of *M. oleifera* were harvested from the trees grown in center of desert studies (in Anbar province), Iraq. Consequently, they had classified by a taxonomist in the center, University of Anbar. The seeds were shattered from dried pods and their husk was peeled off. Later, they had deposited at shaded ambient temperature of 45°C to take out the internal-moisture. The seed-samples were later pulverized to prepare their powders via a blender and served it in the refrigerator in airtight containers (±3°C) till be analyzed, modified by (Al-Obaidi et al., 2021).

2.2.Preparation of extract

The powders of dry seeds (300gms) were subjected to continuous extraction process using Soxhlet via 1000 ml of solvent mixture addition (methanol: Acetone1:1) for 48 hours. The extract was then vaporized in a rotary evaporator. The resulted supernatant was kept at 4°C in a sealed container.

2.2. Stock commercial nanoscale lead

Nanoparticles of lead (Nanoshell) were prepared using methodology according to the method (Sadhasivam et al., 2017). These NP-Pb possessed diameter of <100 nm.

2.3. Nanoparticle preparation

NP-Pb was prepared according to the method (Al-Obaidi et al., 2021). The process was applied using laser

2.4. Experimental animals

Adult white male Swiss Wistar rats (*Rattus norvegicus*), which were collected from the Animal House at the Faculty of Veterinary Medicine at Tikrit University, Iraq, were used in this experiment. Their age ranged from 12 weeks to 14 weeks, and their average weight was 250 g. They were reared under standard laboratory conditions (24 ± 1°C, a normal light cycle, 12 h light and 12 h dark, and fed freely).

2.6. Gene expression Study

2.6.1. Primer

Primers were designed based on the gene sequence present at the NCBI site using Primer 3 plus software available on the site

http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/

http://primer1.soton.ac.uk/primer1.html

The Korean company Bioneer, as shown below processed these prefixes.

ALAD F ACACACCCCAGGAGCCTAGA 20

ALAD R ATGAGGTTGGTGGCACTGA 19

Annealing 60 products 155

Housekeeping gene

GAPDH F CAACTCCCTCAAGATTGTCAGCAA 24

GAPDH R GGCATGGACTGTGGTCATGA 20

Annealing 60 products 118

The gene expression of the ALAD (Aminolevulinic Acid Dehydratase) gene was studied using GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase) gene as a control Normalizing Gene (House Keeping Gene).

2.6.2. RNA extraction

The RNA extraction process was carried out according to the method attached to the AccuZol™ kit supplied by the Korean company Bioneer.

2.6.3. cDNA synthesis

The cDNA synthesis method was used from the extracted RNA samples according to the method attached to the AccuCLNPer® RocketScript RT PreMix kit supplied by the Korean company Bioneer.

2.6.4. Quantitative Real-Time PCR (qPCR Assay)

The qPCR assay of the cDNA samples was performed according to the method supplied with the AccuCLNPer® GreenStar™ qPCR PreMix extraction kit supplied by the Korean company Bioneer.

When the reactive process was accomplished, The data was analyzed.

The results of the Real-Time (PCR) assay were analyzed through the amplification plot based on the Cycler threshold number (CT) value, where the sample is positive when it exceeds the threshold line (Livak et al., 2021). As shown in the equations below.

1…….. ΔCt = Ct of target gene – Ct of a control gene.

2…….. ΔCt = Ct of control gene – Ct of a housekeeping gene.

3…… ΔΔCt = ΔCt (target) –ΔCt (control).

4…….Fold changes = 2-ΔΔCt.

2. Results

2.1. ALAD Expression

by following the effect of the current experiment's parameters on the ALAD gene, it was found that the gene expression Fig. (-1-a,b) table (6) of the samples of the current study was above in the M treatment. While it was for the C. treatment. and Laser and C.M. and L.M. Less gene expression compared to normal. The percentage of gene expression for the M treatment increased by 0.711 from the normal, while the C. treatment recorded a decrease by 4.199 from the normal, and the Laser treatment showed a decrease by 3.6680, while the decrease for the gene expression was for the two treatments C.M. and

Laser M. than normal with a value of 1.299 and 1.527, respectively, which was the highest expression compared to the two treatments of lead nanoparticles only C. And laser,

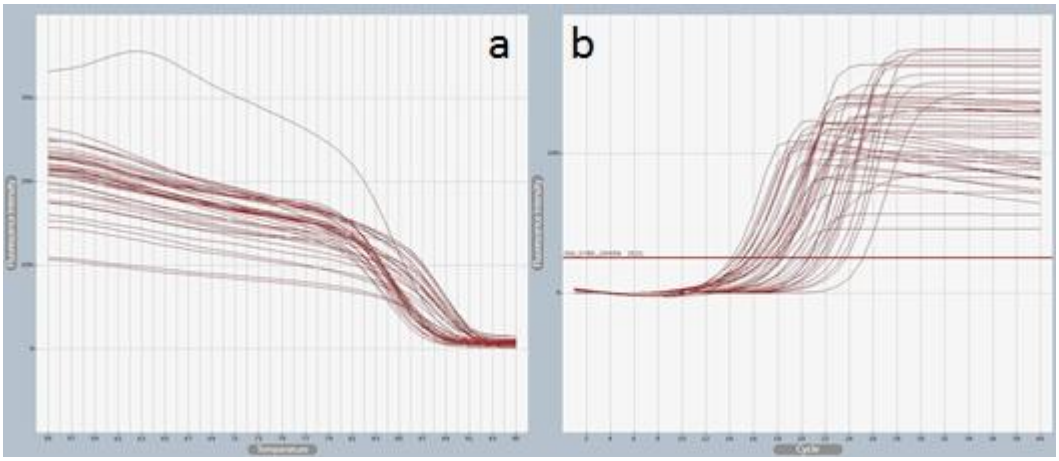

Figure 1(a-b) the comparison and amplification curves for ALAD gene

Table (6) Effect of studied groups on gene expression in the blood of rat

group	levels	qPCR
Control	NONE	1
commercial lead-NP	5 mg Kg^{-1}	0.140475
nanoscale lead	5.8 mg Kg^{-1}	0.32825
commercial lead-NP X Moringa seed extract	5 X 160 mg Kg^{-1}	0.3767
nanoscale lead X Moringa seed extract	5.8 X 160mg Kg^{-1}	0.421775
Moringa seed extract	160 mg Kg^{-1}	1.073725

Figure 2. illustrated the radar distribution of treatments effect on ALAD gene expression. So, this figure extracted that rats treated with 160 mg from seed extract per kilogram was the highest effect on ALAD gene expression, followed by 5.8 nanoscale lead X 160 seed extract. This results are confirmed by figure 3 which derived that the two mentioned treatments possessed 45% of total effect on ALAD gene expression

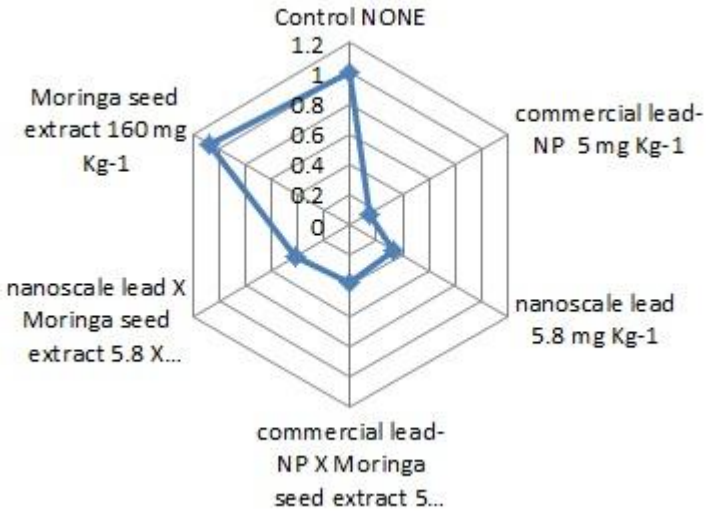

Figure 2 Spider distribution of q PCR of ALAD expression in six groups

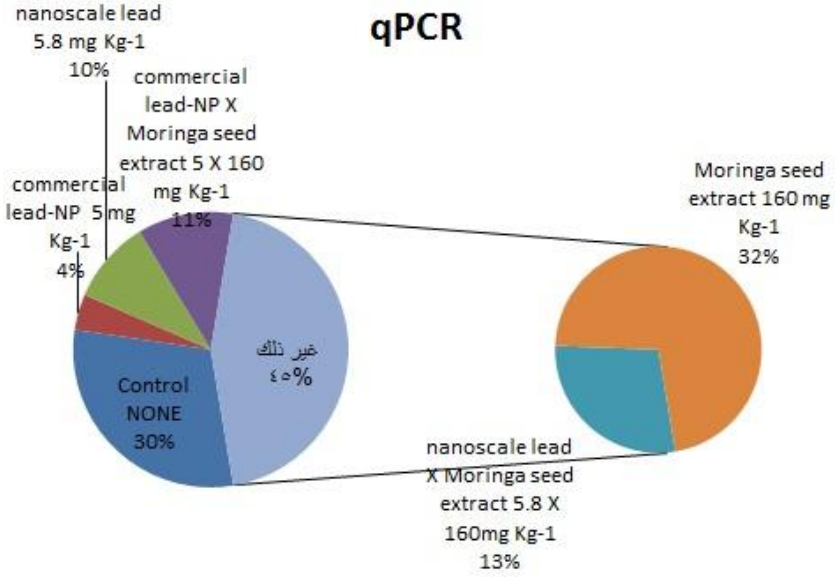

Figure 4 circular distribution of q PCR percentage for ALAD gene expression in six groups

Conclusions

2.2. Principal component analysis

Figure 5 demonstrated that the two principal components extracted 99.998% of total variance resulted from treatments ALAD gene expression in the studied parameters categorized the treatments into polygon with head of control, MS, CLNP, LLNP and CLNPM**.** Where, the control and MS were highly superior in all parameters. Most of parameters are located near to control and MS.

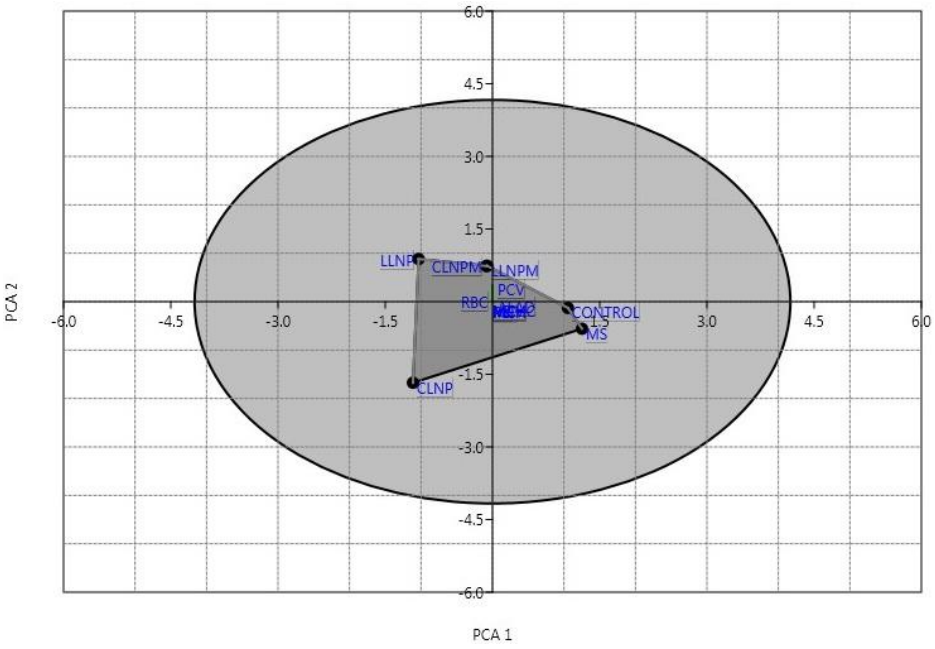

Figure 5 principal component analysis of treatments and blood parameters

Discussion

The ALAD gene is responsible for the expression of ALAD in all tissues of the body, but to a greater extent in red blood cells (Puspitaningum et al., 2018). The enzyme δ-aminolevulinic acid dehydratase (ALAD also known as Porphobilinogen synthase) catalyzes the formation of Porphobilinogen, the initial step in the synthesis of Hemoglobin and that inhibiting this enzyme leads to a lack of hemoglobin, which causes anemia (Cangelosi et al., 2017). Lead interferes in the biosynthesis of heme by interfering with the enzymes necessary for its synthesis, including the enzyme ALAD (aminolevulinic acid dehydrase) and then hemoglobin decreases, as the active site of ALAD contains a Zn (II) ion, Pb (II) is replaced by an ion(Kshirsagar et al., 2016). Zn(II) leads to disturbances in the structure of the active site (Aoki et al., 2017) and which may be attributed to the basic elements in Moringa seeds that reduce lead absorption (Aborisade et al., 2019).

The reason for recording the highest expression in treatment M may be due to Moringa containing polyphenols that help protect DNA from damage caused by free radicals (Khalil et al., 2020) and this was observed in the groups given Moringa seed extract.

The decrease in gene expression in the rest of the treatments may be because lead increases oxidation, which negatively affects the vital processes in the body of the organism. Oxidative stress is a

key concept of the mechanism of lead poisoning. One of the main effects of lead toxicity involves disruption of the gene expression of the genes encoding δ-ALAD, ferrochelatase, and ALAS (Charkiewicz and Backstrand, 2020) table (6).

Conclusions

From current trial, it could be concluded that Moringa was very efficient to protect ALAD enzyme in blood of rats. This efficiency could be due to the high content of polyphenols compounds. These active compound possess the efficacy of antioxidant agents. Some statistical tools are very informative to confirmed the results such as principal component analysis, spider (radar) and circular analysis.

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