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Investigating and comparing the effects of lead poisoning in the rate of Flt3 gene expression in Acute Myeloid Leukemia (AML) and the value of its proteins synthesis in adult male rats

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Abstract
Nowadays, cancer is undoubtedly one of the main and most common causes of human mortality. Recent studies have proved the origin and genetic source of many types of cancers. One of the main causes of genetic changes leading to the prevalence of cancer is exposure to various environmental pollutants in the living environment. The present study aims to investigate the possible effects of poisoning with lead compounds on the expression of an important gene of flt3 involved in the development of Leukemia in healthy rats. A total of 48 male rats were used in this study. Animals were generally divided into 6 groups, including control group, 300 mg / kg.bw sodium sulfide poisoning group, 600 mg / kg.bw sodium sulfide poisoning group, 30 mg / kg.bw lead acetate poisoning group, 60 mg / kg.bw lead acetate poisoning group, 600 mg / kg.bw sodium sulfide plus 60 mg / kg.bw lead acetate poisoning group. In this study, gavage was performed on rats for four months and blood samples were taken after this time. Using protein measurement kits, the value of protein was measured and it was found that in the group that received 600 mg / kg.bw sodium sulfide plus 60 mg / kg.bw lead acetate, the value of protein and gene expression increased significantly compared to other groups.

Keywords: AML cancer, lead, Flt3 gene, adult male rat

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INTRODUCTION

Definition of Cancer

Cancer is a disease in which a group of normal cells in the body grows uncontrollably and called as tumor. Tumors can grow and expand in normal tissues and spread into the bloodstream and affect the digestive system and the circulatory and nervous system. Cancer is a group of diseases that occurs as a result of abnormal increase in a number of cells and as a spread in parts of the body (Jadidi et al. 2014) (**Fig. 1**).

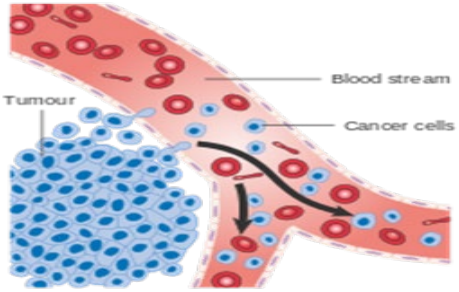

Fig. 1. Cancer Cells (Jadidi et al. 2014)

Acute Myeloid Leukemia (AML) is a malignant hematopoietic disease characterized by the accumulation of abnormal and undifferentiated cells called blasts in bone marrow and disruption in the production of normal blood cells (Kharfan-Dabaja et al. 2006).

The Mechanism of the Effect of Lead in the Body

Gastrointestinal absorption occurs primarily in duodenum. The exact mechanism is unclear and it might involve active transfer or diffusion through the intestinal or intercellular epithelial cells or mineral or organic ionized lead. Blood lead is rapidly taken by red blood cells, where they bind to several proteins in the cell. However, the mechanisms that remove lead from the cell membrane are completely unclear. The results suggest that red blood cell uses two or possibly three pathways for facilitating the transfer of lead across the

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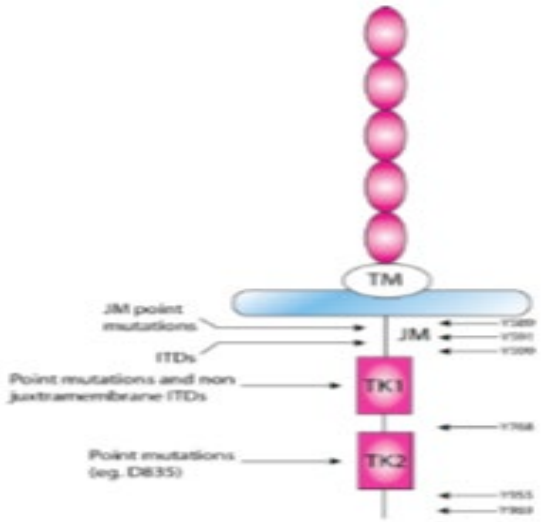

Fig. 2. FLT3 receptor structure (Lemmon 2010)

red cell membrane. The main proposed pathway is an anionic converter that is dependent on Hco3 and is blocked by ion exchange inhibitors.

The second small pathway does not depend on Hco3 and it is not dependent on the anion inhibitor. Lead and calcium may also have a permeability pathway that is likely to be Ca2 +. Lead is transferred by erythrocytes through active pathways including ATpase, Ca2 +, Mg2 (Bannon et al. 2000).

FLT3 (Fms-Like Tyrosin Kinase Receptor)

FLT3 is expressed in the cells of the stem cell and plays the main role in the evolution of the cells. FLT3 mutations can be seen in one third of AML patients (Howlader et al. 2011). FLT3 is located on the chromosome 13q 12 and encodes tyrosine kinase III receptor (Zuo et al. 2009). FLT3 is mainly produced by primary myeloid hematopoietic stem cells and lymphatic precursors and non-adult and adult single cell (Betz and Hess 2010) (**Fig. 2**).

METHOD

In this study, 48 adult male Wistar rats with an approximate weight of 200 ± 10 g in the range of 2.5 to 3 months were used. The rats were prepared from the Razi Serum Institute of Karaj and were studied according to the ethical principles of working with laboratory animals approved by the Ministry of Health and the regulations of the Ethics Committee of University.

The animals were generally divided into 6 groups (each contained 8 animals), which included:

1- Control group: received no specific treatment

2- Sulfur poisoning group (300 mg / kg.bw): received 300 mg / kg.bw sodium sulfide daily for 4 months in gavage.

3- Sulfur poisoning group (600 mg / kg.bw): received 300 mg / kg.bw sodium sulfide daily for 4 months in gavage.

4- Lead poisoning group (30 mg / kg.bw): received 30 mg / kg.bw lead acetate daily for 4 months in gavage.

5- Lead poisoning group (60 mg / kg.bw): received 30 mg / kg.bw lead acetate daily for 4 months in gavage.

6- Lead and sulfur poisoning group: received 600 mg / kg.bw sodium sulfide plus 60 mg / kg.bw lead acetate daily for 4 months in gavage.

To examine the value of FLT3 gene expression, Pishgam Company primers, manufactured by South Korea, were used. Primers were designed by Oligo software. YWHAZ, as a gene with very little changes in expression, was used as the reference gene for internal control. The sequence of primers designed for Flt3 and YWHAZ genes is presented in **Table 1**.

In **Table 2**, the protein synthesis kit was purchased from Pars Toos Company of Mashhad and transferred to the genetic laboratory for the synthesis of protein.

Statistical Method

The obtained results were analyzed by SPSS 23 software. ANOVA and Tukey tests were used to examine the relationship and the significance of the data. All analyses were performed according to the significance level of $P < 0.05$.

FINDINGS

Using protein measurement kit, the protein was measured and inter-group comparison was performed in **Table 3**.

Protein absorption was performed and comparison was made between the groups in **Table 4**.

The results of these tests show that it cannot be conclusively claimed what mechanism is involved in these biologic changes, since various factors are involved in the mechanism of the effect of lead in living organisms in **Figs. 3** and **4**. Therefore, other extensive studies are required for mechanism of action and the effect of these living organisms to provide a specific model for the treatment of cancer patients. After consuming sodium sulfite and lead acetate for 4 months

Table 1. FLT3 gene primer design

Primer Name	Primer seq. 5'-3'	Length	Tm	Amplicon size (bp)
Flt3-F	GAGACCGTTACAAACCAAGATCTGC	25	60.5	163
Flt3-R	ACCCCTTCACTCCGATGCTTC	21	60.7	

Table 2. YWHAZ gene primer design

Gene Name	Oligo	Primer seq. 5'-3'	Length	%GC	Amplicon (bp)
YWHAZ	F	TCGAGTATTGAGCAGAAGACGG	22	44	157
	R	GCCAAGTAGCGGTAGTAGTCAC	22	40	

Table 3. The statistical results of the effect of sulfur and lead poisoning at different doses on the protein level. This table presents the mean and standard deviation

Tested groups	mean±SD of level of protein (mg/ml)
Control	0.008879±0.29620
Sulfur poisoning group: 300 mg / kg.bw sodium sulfide	0.011062±0.30000
Sulfur poisoning group: 600 mg / kg.bw sodium sulfide	0.014317±0.30829
Lead poisoning group: 30 mg / kg.bw lead acetate	0.012572±0.30113
Lead poisoning group: 60 mg / kg.bw lead acetate	0.020604±0.29371
Sulfur and lead poisoning group: 600 mg / kg.bw sodium sulfide +60 mg / kg.bw lead acetate	0.009968±0.28114

Table 4. The statistical results of the effect of sulfur and lead poisoning on different doses on protein absorption. The table presents the mean and standard deviation

Tested groups	mean±SD of protein absorption (mg/ml)
Control	0.02577±1.0580
Sulfur poisoning group: 300 mg / kg.bw sodium sulfide	0.03308±1.0688
Sulfur poisoning group: 600 mg / kg.bw sodium sulfide	0.04219±1.0929
Lead poisoning group: 30 mg / kg.bw lead acetate	0.03688±1.0738
Lead poisoning group: 60 mg / kg.bw lead acetate	0.06071±1.0500
Sulfur and lead poisoning group: 600 mg / kg.bw sodium sulfide +60 mg / kg.bw lead acetate	0.01605±1.0602

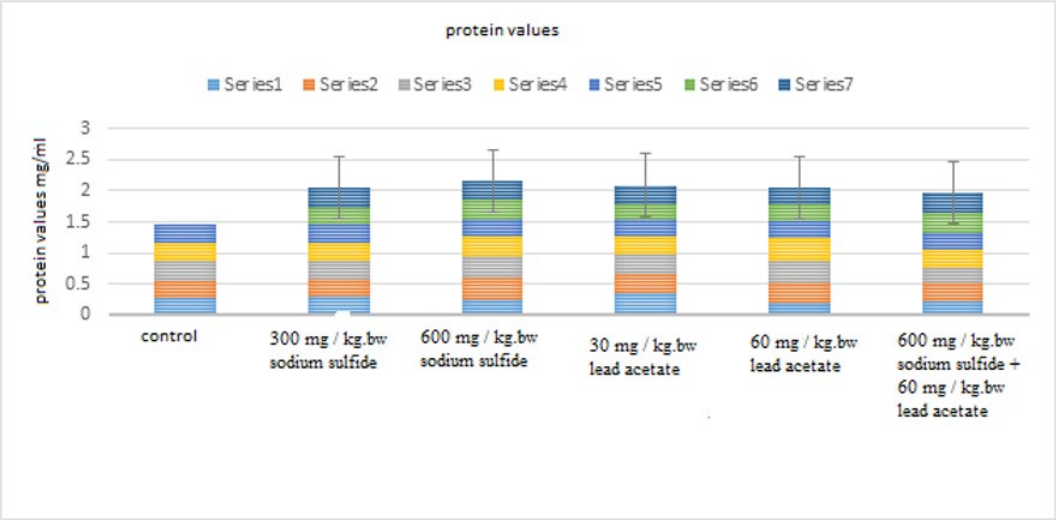

Fig. 3. Comparison of the effect of sulfur and lead poisoning on different doses on the value of protein in the studied groups

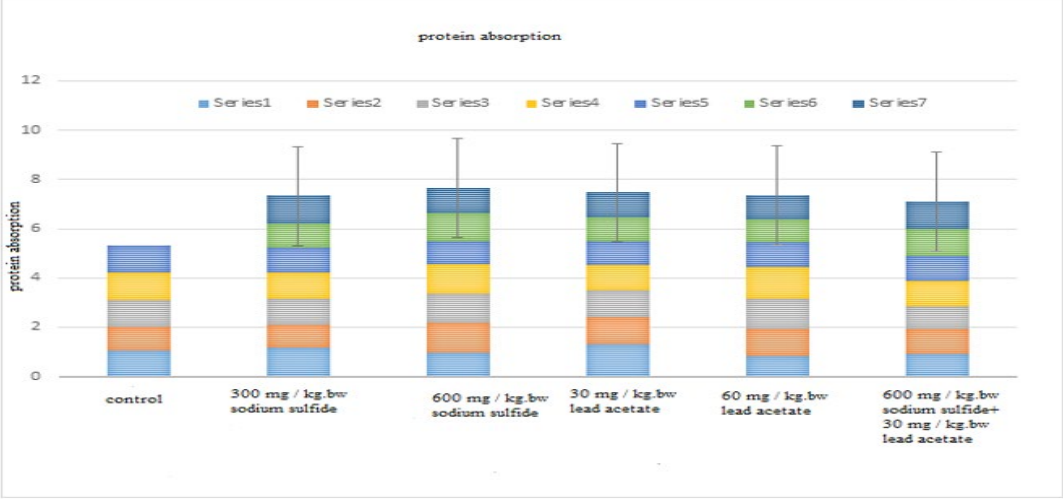

Fig. 4. Comparison of the effect of sulfur and lead poisoning on different doses on protein absorption in the studied groups

and taking blood of rats, the bloods were transferred to Dr. Goodarzi Lab and the protein concentration and absorption were tested by Nanodrop device and the results were obtained as follows:

- In this study, the protein concentration showed a significant increase in the rats received 60 mg / kg.bw lead acetate plus 600 mg / kg.bw sodium sulfide (F6 group) in comparison to the control group.

- In this study, the protein absorption showed a significant increase in the rats received 60 mg / kg.bw lead acetate plus 600 mg / kg.bw sodium sulfide (F6 group) in comparison to the control group.

CONCLUSION

One of the causes of leukemia is genetic factors. Thus, any genetic study of cancer has high importance. Mutation in genetic fields is also dependent on various factors, including environmental factors and one of the environmental factors involved in this regard is the presence of lead acetate (Fuller et al. 2002). Researchers have reported much importance about genes and proteins and their role in the production of natural and cancer cells. One of their important discoveries has been the role of mutated genes in the production of cancer cells. Environmental factors that cause genetic mutations are being identified. Using various molecular methods, we are able to determine the power of expression of defective genes and proteins (Tachdjian et al. 2002).

Being exposed to lead in the workplace significantly increases the DNA breakdown. The cause of cancer is the degradation of DNA. The effects of lead by repairing the damaged DNA with other chemicals have been investigated. Several studies conducted on animals have shown that lead compounds are the cause of cancer in these animals (Fracasso et al. 2002). The immune system can be the target of lead poisoning. Given the harmful effects of lead and the increasing risk of exposure to it, several methods have been considered by researchers to cope with it (Ashry et al. 2010). There are more than 100000 types of chemical compounds in the nature that directly or indirectly impose their effects on cytoplasm and cell nucleus, leading to genetic disorders and mutations (Pakin 2006). In the children and adults with AML, there is a poor prediction for FLT3 mutation (Armstrong SA et al., 2004).The expression of FLT3 occurs in cells such as myeloid and lymphoid that are proliferated (Adolfsson et al. 2005).

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