

Ameliorative effects of virgin olive oil, propolis and zinc chloride on lead acetate-induced genetic variation in mice using 16S rRNA sequence

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Abstract

Lead acetate poses numerous health risks and seriously harms living things' genetic makeup. Propolis and virgin olive oil (VOO) are both natural materials that contain anti-genotoxic chemicals as well as a variety of other advantageous properties for 16S rRNA sequencing. The current study was designated to determine the beneficial effects of virgin olive oil and propolis on lead acetate-induced genotoxicity in mice. Twenty-five male albino mice (*Mus musculus*) were divided into five groups of five mice each: the first group served as the control and received no treatment; the second, third, fourth, and fifth groups received 400 mg/kg B.W. of lead acetate orally, either dissolved in distilled water or dissolved in 8 mL/kg of VOO, or dissolved in 150 mg/kg of propolis, or 4 mg/kg B.W. of zinc chloride (ZnCl₂), respectively. The second group exhibited a high genetic distance value, while the fifth group exhibited a low genetic distance value when compared with the control group. Propolis and virgin olive oil were found to be effective in lowering the genetic variation caused by lead acetate, with genetic distances of 0.0018 and 0.0026, respectively, according to the 16S rRNA sequence data. It concluded that propolis and virgin oil reduced lead acetate genotoxicity when compared with zinc chloride.

Keywords: 16S rRNA; Genetic distances; Lead acetate; Propolis; Virgin olive oil, Zinc chloride.

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Introduction

Pollutants in the environment can have a substantial impact on biological processes via the air, water, soil, and food chain, resulting in toxicity in living species. Animal feed, in particular, can be a source of heavy metal pollutants from human agriculture, industry, or careless use, and exposure to these inorganic toxins can result in heavy metal accumulation in multiple organs, producing a variety of catastrophic disorders, including cancer. Lead (Pb) is a well-known highly toxic metal that poses a variety of risks to both environmental and biological systems, disrupting biochemical and physiological functions in animals (Assi et al., 2016) and humans (Briffa et al., 2020).

Due to its pervasiveness and recognized hazardous effects on the environment (Saleh et al., 2003). lead toxicity remains a significant issue in public health (Duzgoren-Aydin, 2007). Lead also contributes to chromosomal abnormalities, which are one of the most serious risks linked with hereditary lead exposure (Fahmy, 1999; García-Lestón et al., 2010), as well as micronucleus production (Celik et al., 2005). Genotoxicity can induce DNA damage, resulting in mutations such as gene mutations, chromosomal abnormalities, and genomic mutations, or it can cause cell death or apoptosis (Maurano et al., 2012). Lead exposure can harm several organs in the body, with severe kidney alterations being documented in experimental animal (Assi et al., 2016)

Numerous attempts to use inexpensive natural anti-inflammatory compounds to modulate the immune system like Virgin Olive oil (VOO) which is high in phenolic compounds that have both antimicrobial and anti-

inflammatory functions via reducing oxidative damage (Cicerale et al., 2010). Furthermore, due to its high quantities of phenolic compounds, tocopherol, and monounsaturated fatty acids especially omega 9, VOO is the most significant processed food product against free radicals and oxidative stress. In addition to these functions, VOO's phenolic compounds are essential in preventing damage to DNA, proteins, and lipids (Ghorbel et al., 2015). These phenolic compounds protect DNA bases from oxidative stress and reduce the risk of cancer (Pessoa et al., 2022).

Propolis is natural complex sticky substance created by bees, with a variety of biological effects, including those related to being an antioxidant, antibacterial, anti-inflammatory, antitumor, and anti-mutagenic based on its phenolic, flavonoid-rich component which includes chrysin, genistein, pinocembrin, vitamins, minerals and other compounds (Bouchelaghem, 2021).

Zinc is particularly renowned as an antioxidant in cases of lead poisoning, as it can increase the antioxidant system either directly or indirectly by stabilizing the chemical components of the antioxidant enzymes (Kataba et al., 2021). Zinc involved in several crucial biological processes such cell division, growth, and differentiation (Soussi et al., 2018). Additionally, Zinc protects cells' DNA from genotoxicity which in turn prevent cytotoxicity (Costa et al., 2022).

The 16S rRNA gene, specifically the small subunit, is the gene of choice for this study due to its large number of conserved and variable design options for PCR primers, as well as a limited percentage of 16S rRNA gene nucleotide substitution (Hassler et al., 2022). From this point of

view, this study will compare natural remedies as virgin olive oil and propolis to the pharmaceutical compound zinc chloride in treating the effects of lead poisoning on the 16S rRNA gene.

Materials and Methods

Chemicals

Lead acetate 3-hydrate, purity 98%, and Zinc chloride, purity 98%, were obtained from Gene Tech. Virgin Olive Oil purchased from the National Projects Company (SAFY) in Qena, while the Propolis was obtained from Asal Al-Shifa Company Ltd, also located in Qena.

Animals

Twenty-five male albino mice, approximately 6-8 weeks old and weighing 23±2g, were obtained from the Physiology Department at the Faculty of Veterinary Medicine, South Valley University, located in Qena, Egypt. The mice were kept in plastic cages and housed at a suitable temperature of 25±2°C and relative humidity of 55±5% for two weeks with ad-libitum access to food and water to acclimate them to the laboratory conditions prior to the experiment.

Experiment design

The mice were randomly divided into five groups (five mice for each group) as the follow:

- 1- The first group (Control) didn't receive any treatment.
- 2- The second group was treated orally with 400 mg/kg of lead acetate which dissolved in distilled water.
- 3- The third group was treated orally with 400 mg/kg of lead acetate dissolved in 8 mL/kg of VOO.
- 4- The fourth group was treated orally with 400 mg/kg of lead acetate dissolved in 150 mg/kg of Propolis.

- 5- The fifth group was treated orally with 400 mg/kg lead acetate with 4 mg/kg ZnCl₂.

All animals' groups were orally treated with a gavage for 15 consecutive days with a garage. After the end of the experiment, all mice were euthanized, and their kidneys were quickly dissected and stored at -20°C until used for DNA extraction. The DNA extraction was performed within 24 hours of the end of the experiment.

DNA Extraction

The preserved kidney tissues were used for DNA extraction using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) following the manufacturer's guidelines.

PCR Amplification and Sequencing

Amplification of genomic DNA Polymerase chain reaction (PCR) was performed using primers according to (Kim and Dekker, 2018). PCR achieved for the amplification of isolated DNA using a total of 40 µL containing; 1 µL genomic DSNA as a template, 17 µL of nuclease free water 1 µL of each forward and reverse primer, and 20 µL of 2X master mix. The PCR amplification comprised an initial denaturation at 95 °C for 3 min. followed by 30 cycles: denaturation at 94 °C, annealing at 48 °C and extension for 1 min. at 72°C respectively. Cycling was terminated with a 10-min extension at 72 °C. The PCR products were separated and visualized by electrophoresis on a 1.5% ethidium bromide-stained agarose gel. The PCR amplification yielded a single band in each group. All sequence was achieved by macrogen (South Korea). The obtained sequences were submitted to GenBank/NCBI to get the accession numbers.

To calculate the sequence divergences, Kimura 2-parameter distances, a method developed by (Kimura, 1980) were used. While Clustal W, a sequence alignment tool developed by (Thompson et al., 1994), was used to align the sequences.

Statistical analysis

This was done in MEGA version 7.018, a software package developed by (Kumar et al., 2016). The alignment process involved 1000 bootstrap iterations,

a statistical resampling method introduced by (Felsenstein, 1985)

Results

The 16S rRNA partial nucleotide sequences were submitted to GenBank and assigned accession numbers OM746930 to OM746934. The average values for accession numbers, sequence lengths, A+T contents, and C+G contents were calculated for each group and presented in Table 1 and Figure 1.

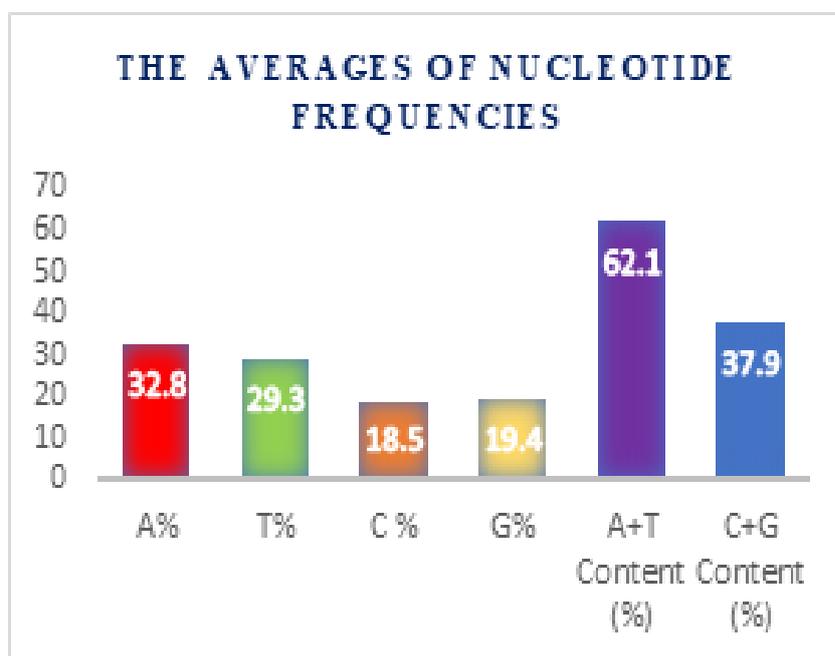


Table1. Accession numbers, nucleotide frequencies and their averages of (16S rRNA) gene in the five groups

Group	Accession number	Base pair Length	Nucleotide Number %				A+T Content (%)	C+G Content (%)
			A%	T%	C %	G%		
Control	OM746930.1	555	32.8	29.4	18.5	19.3	62.2	37.8
Lead acetate	OM746931.1	555	32.8	29.2	18.5	19.5	62	38
Lead acetate+VOO	OM746932.1	548	32.9	29.2	18.4	19.5	62.1	37.9
Lead acetate+propolis	OM746933.1	554	33.0	29.3	18.6	19.1	62.3	37.7
Lead acetate+ZnCl ₂	OM746934.1	548	32.7	29.2	18.6	19.5	61.9	38.1
Average %	-	552	32.8	29.3	18.5	19.4	62.1	37.9

The P-distances, which indicate the genetic distance between the control group and treated groups, ranged from 0.0000 to 0.0036%. The overall distance value among all groups was 0.00%. The largest P-distance of 0.0036 was observed between the control group and the group treated with lead acetate, suggesting that lead acetate had a significant genetic impact. Details of the P-distances for all groups can be found in Table 2.

Based on the results of the 16S rRNA sequencing, the P-distances between the control group and the group treated with both lead acetate and virgin olive oil were

0.0026, which is an improvement compared to the distance value observed in the group treated with lead acetate alone.

Similarly, the P-distances between the control group and the group treated with both lead acetate and propolis were both 0.0018, which is lower than the distance value observed in the group treated with lead acetate alone. These findings suggest that propolis has an ameliorative effect that reduces the genotoxic effects of lead acetate. Finally, the lowest distance value of 0.000 was observed between the control group and the group treated with both lead acetate and ZnCl₂.

Table 2. Pairwise distances based on (16S rRNA) gene among the five groups

Group	Control	Lead acetate	Lead acetate+ VOO	Lead acetate+ propolis	Lead acetate+ ZnCl ₂
OM746930.1 Control		0.0036	0.0026	0.0018	0.0000
OM746931.1 Lead acetate	0.0073		0.0036	0.0041	0.0025
OM746932.1 Lead acetate+ VOO	0.0037	0.0074		0.0026	0.0026
OM746933.1 Lead acetate+ propolis	0.0018	0.0091	0.0037		0.0000
OM746934.1 Lead acetate+ZnCl ₂	0.0000	0.0037	0.0037	0.0000	

Discussion

In this investigation, the choice of primers was crucial for short-amplicon 16S rRNA gene sequencing from the large ribosomal rRNA (16S rRNA) gene in each of the five groups, as reported by (Abellan-Schneyder et al., 2021). The final alignment of 555 bp was obtained, with 548 of them having variable and conserved sites, respectively, these findings are corroborated by those of (van der Kuyl et al., 1995) who reported a high degree of conservation of mitochondrial genes in different animal species. The A+T content was higher than the C+G content, with an average of 62.1, due to the rarity of GC-rich regions. The rate of GC-poor or GC-rich fragments is lower than average, and in many cases, nil, especially in simpler

organisms, as reported by (Benjamini and Speed, 2012).

Several studies have confirmed the adverse effects of lead acetate exposure on DNA and chromosome integrity. For instance, Fahmy (1999) revealed that mice given lead acetate at doses of 50,100,200 and 400 mg/kg b.w. had significantly enhanced the proportion of chromosomal aberration in bone-marrow. Acharya et al. (2003) declared that a rise in the number of chromosomal abnormalities caused by lead acetate demonstrates a reduction in DNA synthesis fidelity, failure of DNA repair mechanisms, and may lower fertility. Rats given high dose (400 mg/kg) of lead acetate without treatment exerts worst toxic consequences. Furthermore, Zhang et al. (2014) demonstrated how pb

occupies four sites in the DNA of mice through minor grooves to cause damage to the DNA structure and these finding agreed with (Taha et al., 2019) who discovered that high lead doses caused a vast scope of toxicity and had a negative impact on growth performance, liver and kidney functions; these negative effects were accompanied by DNA damage.

The improved results of the 16S rRNA sequences in the lead acetate+ VOO-treated group compared to the lead acetate-treated group can be attributed to the presence of olive oil, which contains a significant number of antioxidants, particularly phenolic compounds. These compounds have been shown to have a protective effect against the genotoxic effects of lead acetate, preventing damage to DNA, proteins, and lipids, as reported by (Fki et al., 2007).

The phenolic constituents of olive oil possess potent free-radical scavenger and metal chelator properties, which contribute to alleviating oxidative stress, as reported by (Visioli et al., 2002). Therefore, the phenolic components of virgin olive oil may play a crucial role in the diet-based prevention of free radical-induced DNA damage and oxidative stress-related diseases, such as cancer (Erol et al., 2012).

Virgin olive oil has been found to have potent therapeutic benefits that can help prevent the development of amyloid plaques in chronic conditions such as cancer, neurological disorders, and cardiovascular diseases. Furthermore, it has been shown to be effective in preventing osteoporosis, as reported by (García-Gavilán et al., 2018). Additionally, the hepatoprotective and anti-inflammatory benefits have been observed in intoxicated mice which is attributed to the antioxidant activity of olive oil (Habibi et al., 2021).

The lower P-distances observed between the control group and the lead acetate+ propolis-treated group compared to the group treated with lead acetate alone can be attributed to the presence of strong antioxidants, known as flavonoids, found in propolis. These flavonoids have been shown to scavenge free radicals and protect cell membranes from lipid peroxidation. Propolis also has anti-inflammatory, anti-cancer, hepatoprotective, and immune modulating activities. Additionally, propolis possesses anti-oxidative properties that reduce the levels of malondialdehyde (MDA) and peroxidative damage to rat mitochondria (Barary et al., 2022).

The lowest P-distances observed between the control group and the lead acetate+ZnCl₂-treated group in the 16S rRNA sequences suggest a beneficial effect of ZnCl₂ in reducing the genotoxic effects of lead acetate. This is consistent with findings reported by Badkoobeh et al. (2013) and Zhang et al. (2021), who reported that zinc plays a crucial role in several important biological processes, including DNA repair and protection of DNA strands from damage.

Conclusion

The use of 16S rRNA sequencing to determine genetic variation in mice caused by lead acetate exposure is a useful tool. The data from the present study suggests that propolis and virgin olive oil, as natural products, have beneficial effects in mitigating the genetic variation induced by lead acetate in mice. These findings support the use of propolis and virgin olive oil as food adjuncts to inhibit the harmful genetic effects of several chemicals, particularly lead.

Ethics approval and consent to participate.

All procedures of the present study were carried out according to the ethical committee of the animal use in Faculty of Science South Valley University Qena, Egypt with approval number (008/11/22)

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

NSM and EN designed the study design, carried out the experimental procedures and statistical analysis, wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Abbreviations

VOO: virgin olive oil; ZnCl₂: Zinc chloride

Data availability

All data generated or analyzed during this study are included in this published article.

Consent for publication

Not applicable

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