Arsenic, Mercury, Lead and Iron Induced Lipid Peroxidation in Phospholipids Liposomes and Protective Effect of Fumaric Acid

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Heavy metals like arsenic, mercury, and lead etc, can have toxic effects on living organisms. There is considerable data which confirmed that chronic (or some time acute) exposure to heavy metals can lead to cognitive impairments, developmental delays, and behavioral problems. They are also categorized as human carcinogens, and prolonged exposure has been linked to a number of cancers, including prostate and lung cancer [1]. The health of humans and animals can seriously be threatened by the accumulation of large concentrations of metals in various food and fodder crops cultivated on soil contaminated with metals [2].

Heavy metals can also cause oxidative stress in living organisms. This is caused by an imbalance in the body to detoxify the reactive oxygen species (ROS) or repair the harm they produce [3]. Some of the highly reactive ROS are superoxide radicals, hydrogen peroxide, and hydroxyl radicals. They are produced as natural byproducts of various metabolic processes in the body, including cellular respiration. The oxidative stress (induced by heavy metals) can pay to a wide range of health problems, including neurodegenerative diseases, cardiovascular diseases, kidney damage, and various other chronic conditions [4, 5].

The antioxidants can help to mitigate the harmful effects (of metal induced oxidative stress) by scavenging free radicals and prevent the lipid peroxidation [6]. In the current study, we tried to explore the antioxidant profile of Fumaric acid ($HO_2CCH=CHCO_2H$, with molar mass of 116.074). In 2014, a division of DG Health "the European Commission Scientific Committee on Animal Nutrition" determined the fumaric acid to be partially non-toxic. However, prolonged usage of large doses is likely to create nephrotoxicity [7, 8].

The use of rats or mice for studying the effects of heavy metals like arsenic, mercury, or lead is a complex ethical issue. The ethical implications of using animal models are important they raise critical questions about animal welfare and the moral responsibility to minimize suffering. As public awareness of animal rights grows, researchers face increased scrutiny, which can affect the legitimacy of their work. It depends on research goals, ethical guidelines, and regulatory frameworks in place. The Replacement, Reduction, and Refinement (3Rs) principle is widely accepted in animal research ethics. It inspires the researcher to seek alternatives model for animal testing and improve protocols to reduce the suffering of the animals. Egg yolk contains a significant amount of phospholipids, such as phosphatidylcholine and phosphatidylethanolamine [5]. Other major constituents are Phosphocholine (73.0 %), Hemolysophosphorylchline (5.8%), Phosphoethanolamine (15.0%), Hemolyticphosphoethanolamine (2.1%), and Phosphoacylserine

(0.9%) to name a few. These phospholipids are the primary components of cell membranes and are particularly susceptible to lipid peroxidation. It also contains unsaturated fatty acids, such as linoleic acid and arachidonic acid [9], oleic acid, palmitic acid, and stearic acid which are highly prone to oxidation by free radicals, making them suitable targets of lipid peroxidation [10]. This is exactly we observed in our recent study, where arsenic, mercury, lead, iron and nitric oxide caused significant lipid peroxidation in phospholipids homogenate [5].

This further motivated us to explore the antioxidant potential of fumaric acid against metal induced lipid peroxidation in phospholipids. In order to explore the novelty of the project, on September 6, 2023 we performed bibliometric analysis using Scopus database. Only six documents were noted which contained “oxidative stress" or "lipid peroxidation" or "TBARS" or "antioxidant*" and "fumaric acid" in the titles of the manuscript. While, no results were found which contained the words "fumaric acid" and "phospholipid*" OR "egg" OR "egg yolk" in the titles [11].

Lipid peroxidation was determined by measure TBARS as previously described. We also explored the deoxyribose degradation inhibition potential. The principal idea was to inhibit Fe^{2+}/H_2O_2 -induced decomposition of deoxyribose. We used the method of Halliwell et al. 1989 [12].

Antioxidant activity of fumaric acid was evaluated by monitoring the ability to quench the stable free radical DPPH [13]. Iron chelating ability of fumaric acid was determined by the modified method of Puntel et al. in 2005. While, the hydrogen peroxide scavenging activity fumaric acid was determined by the modified method Chen Y at al. in 1999 [14].

As expected, all four metals (Pb, As, Hg and Fe) significantly increased the TBARS formation. Fumaric acid significantly protected against lipid peroxidation at four different concentrations (0.5, 1.0, 2.0 and 3.0 mM). The data is presented is table. 1.

Table 1: Effect of different metals on lipid peroxidation in phospholipids obtained from egg yolk, DPPH radical scavenging and metal chelation potential of fumaric acid. Data are expressed as means ± SEM (n = 3–4). *p<0.05 from respective control by Tukey multiple comparisons test.

S#	TBARS	Metal	Fumaric Acid (Concentrations)			
			0.5 mM	1.0 mM	2.0 mM	3.0 mM
		Iron /0.452 ± 0.007 #	0.352±0.010 a	0.267±0.008 b	0.239±0.005 c	0.232±0.006 c

1		Lead /0.272±0.006 #	0.214±0.007 a	0.181±.005 b	0.156±0.006 c	0.141±0.004 d
		Arsenic/0.538±0.005 #	0.471±0.006 a	0.435±.008 b	0.427±0.008 b	0.361±0.005 c
		Mercury /0.530±0.023 #	0.517±0.006 #	0.462±0.006 a	0.361±0.009 b	0.326±.006 c
2	**DPPH**	**Control**	**10 uM**	**50 uM**	**100 uM**	**200 uM**
		0.2249±0.005 #	0.19475±0.004 a	0.18025±0.004 b	0.1675±0.012 c	0.1215±0.002 d
3	**Fe Chelation**	**Control**	**1 mM**	**5 mM**	**10 mM**	**20 mM**
		0.256±0.023 #	0.20975±.006 a	0.1685±0.003 b	0.15175±0.007 b	0.146±0.004 b

In order to explore its mechanism of action, we performed deoxyribose degradation assay [15]. The Fe+H_2O_2 significantly degraded the deoxyribose. Fumaric acid exerted a modest (non-significant) protection. However, at 10, 50, 100 and 200 uM, fumaric acid significantly scavenged the DPPH radical. In fact, the highest potential (almost 50%) was recorded at 200 uM (Supplementary file 1).

Our results are in pipeline to earlier reports, where Fumaric acid and/or Fumaric acid esters exhibited free radical scavenging properties, immunomodulatory, anti-inflammatory and chemo-preventive effects [16-19]. However, one of the limitations of the present study is the in-vitro or vivo experiments in animal model. Extrapolating the results of lipid peroxidation observed in phospholipids from egg yolk to rat tissues can be challenging. As the lipid peroxidation processes can vary between species due to differences in antioxidant defenses, lipid composition, and metabolic pathways. Therefore, extrapolating results from one tissue type to another, even within the same species can be challenging. In the same vein, there are also some similarities in the basic mechanisms and factors that contribute to lipid peroxidation. For example, both egg yolk phospholipids and the lipids present in rat tissues contain fatty acids, which are liable to peroxidation. The initiation of lipid peroxidation is caused by reactive oxygen species (ROS) including hydrogen peroxide, superoxide radicals, and hydroxyl radicals. These ROS can oxidize lipids and trigger peroxidation reactions.

We can conclude with an interesting report of Kaur et al in 2020, where they reported the protective effect of fumaric acid against cadmium-induced hepatotoxicity in rats [20]. The authors reported

that the rats' livers had increased thiobarbituric acid reactive substances (TBARS) and a decrease of antioxidant enzymes like GSH, SOD, GPx, and CAT activity. Treatment with fumaric acid reversed the damaging effects of cadmium. Heavy metals include lead, mercury, arsenic, and cadmium can cause oxidative stress, which is one of their common adverse impacts. if FA can reduce cadmium-induced hepatotoxicity then we can hypothesize that FA may provide protection against arsenic, mercury, or lead toxicity. Since the chemical features, absorption, distribution, and elimination of heavy metals are different. it is important to approach these extrapolations cautiously because the interactions and harmful mechanisms among various heavy metals might change widely. Further research, including trials on animals, would be required to find out whether fumaric acid has any protective effects against arsenic, mercury, or lead toxicity in order to explore this idea extensively.

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All co-authors equally contributed in project designing, data collections, statistical analysis and manuscript writing.

Conflict of Interest:

None

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