



Effects of Plant Drugs on Lipid Peroxidation in Irradiated Cells Onion Seeds

SEVIL A. MAMEDLI

*Dr. of Biological Sciences
Institute of Radiation Problems of NAS of Azerbaijan,
370025, Azerbaijan, Baku, Nobel st., 5, app.80
mamedli.sevil@gmail.com*

*It is shown that seed treatment with herbal preparations before irradiation reduced the level of lipid peroxidation in the cells of *Allium cepa*.*

Key words: Gamma irradiation; LP¹; MDA²; *Allium cepa*.



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¹Lipidperoxidation

²Malondialdehyde



Background. Today more than 500 ageing theories and hypothesis based on the actual evidence about the development of this multifactorial and multifocal biological process, manifold of which itself is indicative of a lack of actual knowledge in this field are known. The majority of the existent ageing hypothesis and theories are systematized in the monographs and reviews of the different authors [1]. Although, currently neither of them give comprehensive definition for ageing phenomenon, nevertheless characteristics of this phenomenon comes down to the fact that ageing is a process of life activity weakening, reduction of its adaptive possibilities as well as increase in sensitivity to adverse effect which inevitably evolving in time and naturally proceeded. Representing linking between DNA molecules, between protein molecules as well as between DNA and protein molecules, according to authors' hypothesis [4] which are forming in the natural conditions, involving different metabolites, permanently present in the cells, and are the trigger which predetermines cell ageing, mediated by the inevitable accumulation of DNA damages with ageing.

Thus, ageing theory in conjunction with intensity of free radicals occurrence suggesting that appropriate coverage of antioxidation protective system components in the cells can maintain functional condition of the DNA molecule and stability of transferring of the established in phylogenesis hereditary information by regulation of the rate and direction of redox processes. Thereby, it is the determinant factor of physiological ageing inhibition. It should be noted that these conclusions are embodied in the adaptive and regulatory theory which fundamental position involves relationship between mechanisms of metabolic control and function.

Objects and methods. Irradiation of the onion seeds

Allium cepawas performed on "Issledovatel" apparatus (^{60}Co) at dose rate 0.02 Gy/s and "Rhund" apparatus (^{60}Co) at dose rate 0.5-1 Gy/min. Control and irradiated seeds were couched on the wet filter paper in the Petri dishes at 24°C. Analysis of the lipid peroxidation (LP) rate by the procedure of registration of the malondialdehyde (MDA) quantitative content in plant leaves through colour reaction with thiobarbituric acid by change of the optical density of the stained complex on CФ-26 at 532 nm. Intensity of the lipid peroxidation in plant leaves was evaluated by accumulation of MDA oxidation products in tissues which was measured by colour reaction with thiobarbituric acid [2]. For that purpose, 0.3 ml of the buffer (TrisNaClHCl) was added to the test tube with 0.3 g weight of the plant material, homogenized, and then 2 mL of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) was added, and incubation was performed during 30 min. on boiling water bath with subsequent filtration. Then filtrate was transferred to the cuvette, and optical density was measured on SF-26 spectrophotometer at 532 nm wave length. Environment with reagents was used for control.

Results and discussion.

On the assumption that radioprotective and antimutagenic effects of plant extracts is due to the presence of agents with antioxidant characteristics in their composition, we studied effects of the different natural drugs on intermediate product of lipid peroxidation - malondialdehyde. MDA resulting from chain reaction of peroxidation by free radicals of polyunsaturated fatty acids is not only the indicator of free radicals rate (FR) and peroxide processes, but it also easily reacts with macromolecules. Covalent linking in different variations between DNA and protein molecules resulting from this, embarrass the possibility of reparation and are the trigger which accelerates ageing process causing inevitable accumulation of DNA damages with ageing.

Lipid peroxidation process is induced by free radical oxygen forms which form unstable lipid hydroperoxides able to spontaneous decomposition through attacking of polyunsaturated fatty acid chains. They form complex mixture of mono- and dialdehydes among which MDA was differentiated. Consequently, increase of MDA concentration bears an evidence of intensification of FR processes in the cells. In particular, based on the registration of MDA level increase not only after ionizing or non-ionizing radiation, but as well as under the influence of different ecological factors: tetrachloride carbon, iron ions, cadmium, activated bliomicine, promutagen - acetylaminofluorene, as well as in case of spontaneous faults of cell protective systems due to the body ageing.

Specifically, it was found that a fundament of MDA mutagenic action is related to mechanism of fluorescent products formation, Appeared due to generation of inter-strand cross-linking in DNA molecule, as well as DNA-protein linking which embarrasses the possibility of damages reparation. Participation of

AOF¹ in the formation of ecological and genetic instability of the plants can be determined by their deleterious effect on cell genome (genotoxicity), effects on cellular membranes with change of their permeability (membrane mechanism) and functioning of AOF as the intracellular mediators of signal transduction.

Increased production of AOF leads to the formation of signals in the irradiated cells which are transferred to that part of the cell which was not irradiated. These cells response on signals from directly irradiated cells by AOF formation and formation of DNA damaged due to their action.

¹ Active oxygen forms



So-called “bystander effect” occurs; it makes a great contribution to the sizes of cellular contingents affected by genome instability [3,4]. Non-radiation stresses also cause significant, frequently tenfold, increase of AOF quantity. At that, AOFs occurring in the cytoplasmic structure, migrate to different distances and can lead to deleterious effect on membrane structures, organelles, including nucleus, affecting genetic material, membrane degradation, chromosomal aberrations and other disorders.

Nitric oxide system has a significant impact on redox reactions, products of which are presented with AOF. Nitric oxide (NO) is a highly-reactive radical molecule. In water environment superoxide radical O_2^- , reacting with NO, forms peroxynitrite (NOO⁻) – highly-toxic compound able to initiate LP processes, oxidize NH- and SH-groups of proteins, lead to irreversible inhibition of mitochondrial complexes [5]. Experiments proved that compounds of different classes which have radioprotective action (aminothiols, isotiuronium derivatives, thiazolines, indolealkylamines, etc.) inhibit NO formation in an organism.

In this regard we had a task to study the relations between the level of radiation-induced LP in plants in terms of accumulation of its marker – malondialdehyde (MDA) and treating with plant extracts. However in this dose range, in the mode without processing, it increased 2-fold; and with processing - only to 1.5 times as an average. Another unique feature of rutin and extracts of nut and coral bean treatment impact was increase of their action at higher doses. Thus, at 1 Gy, decrease of MDA ranged from 88% to 95%, and at 15 Gy it was much lower: 60% to 72% (Table 1).

Thus, it was shown that treatment of seeds with rutin, walnut and coral bean extracts before irradiation led to reduced MDA concentration in plant tissues obtained from irradiated seeds.

Radioprotective action of these compounds under the studied subject seems to be of significant importance, because MDA forming during unsaturated fatty acids peroxidation, contained in many food products moreover has the ability to form mutagenic nitrosamines in the presence of nitrites, nitrates, and carbonyl compounds.

Table 1. Effects of gamma irradiation, extract of walnut, extract of coral bean and rutin on the level of malondialdehyde accumulation in *Allium cepa* onion

Modes of the experiment	Irradiation dose, Gy					
	0	1	2.5	5	10	15
Control	0.50±0.01 (0.42-0.56)	0.56±0.01 (0.53-0.58)	0.74±0.01 (0.65-0.79)	0.87±0.01 (0.78-0.91)	0.96±0.02 (0.87-1.04)	1.17±0.01 (1.12-1.21)
Nutextract	0.50±0.01 (0.42-0.56)	0.49±0.01 (0.47-0.52)	0.60±0.01 (0.54-0.62)	0.72±0.02 (0.62-0.81)	0.80±0.00 (0.78-0.81)	0.70±0.01 (0.65-0.76)
P	<0.001	<0.001	<0.001	<0.001	<0.001	–
Coral Bean extract	0.50±0.01 (0.42-0.56)	0.51±0.01 (0.45-0.55)	0.65±0.00 (0.64-0.66)	0.67±0.00 (0.65-0.69)	0.78±0.01 (0.74-0.83)	0.84±0.01 (0.81-0.87)
P	<0.05	<0.001	<0.001	<0.001	<0.001	–
Rutin	0.50±0.01 (0.42-0.56)	0.53±0.00 (0.51-0.55)	0.61±0.02 (0.51-0.67)	0.71±0.01 (0.65-0.76)	0.71±0.02 (0.64-0.80)	0.78±0.01 (0.74-0.84)
P	<0.05	<0.001	<0.001	<0.001	<0.001	–

In this regard it is pertinent to note that one of the consequences of membrane lipids over oxidation which directly correlates with free radicals content in cells is change of the biomembrane barrier function and consequently increase of free fraction of organelle-specific enzymes. It is also known that one among many biological manifestations of such cell changeover to new biological condition is expressed in escalation of mutations occurrence, accumulation of which accelerates natural ageing. All of this gives us a conclusion that presence of anti-radical and anti-oxidative properties in natural and synthetic compounds is one of the most important factors for exhibiting of their non-specific anti-carcinogenic action. Therefore, reduction of MDA content resulting from plant extracts effect, suggesting their anti-oxidative properties. Taking designated relation between radiation-induced genome instability induction and oxidative processes into account, these compounds should be considered as promising for inhibition of the instable genome development.



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Author Biography



SEVIL A. MAMEDLI *Dr. of Biological Sciences Institute of Radiation Problems of NAS of Azerbaijan, 370025, Azerbaijan, Baku, Nobel st., 5, app.80*