BIOACTIVE SUBSTANCES FROM THE *NIGELLA SATIVA* SEEDS

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Abstract. The researches performed in the research grant nr. 34,677/2005-2007 have the aim the establish the effects of different bioactive substances, extracted from seeds of some *Nigella sativa* (Fam. *Ranunculaceae*) genotypes. In experiments performed on labor animals (*Mus musculus L.*), was demonstrated the antistress and immunoprotector effect of different bioactive substances extracted from *Nigella sativa* seeds, minimum doses which induced an cytotoxic effect, as well as induced adulterations at the ultrastructural level (liver and/or spleen). In this study, is analyzed comparative, the radioprotective and immunostimulatory effect of different active biological substances extracted from the *Nigella sativa* seeds, towards of some stress factor, in *Mus musculus*.

Key words: *Nigella sativa; Mus musculus;* bioactive substances; antistress and immunoprotector effect.

[1] Introduction

1.1. The biology of *Nigella sativa* species.

Nigella sativa (Fam. *Ranunculaceae*), is an annual plant, native from Mediterranean area and South-West Asia. The plant reaches to 20 - 30 cm in height, having fin-divided leaf, linear. The flowers with 5 - 6 (10) petals, white or pale-bluish (especially in the main rib region). This species is spontaneously or cultivated. The fruit is a capsule, performed from 3 - 7 follicles, each having numerous seeds of a relative triangular shape, black, strongly odorants. The seeds are used, having a piquant flavor, having numerous bioactive substances.

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1.2. History of the pharmacological uses.

The seeds of *Nigella sativa* were meeting in the Tutankhamen tomb, with the role to help him to "passing in other live". In the Old Testament, in the Isaiah book (28: 25, 27), are specified some particularly features for wheat, black fennel (*Nigella sativa*), barley, spelt and millet. In the Greco-Roman ancient, numerous learned, described some species from *Nigella* genus, their property and useful. *Nigella sativa* was described by Hippocrates, Cato Major, Caius Plinius Secundus (1668) [1], Pedanios Discoridou, (1529) [2], a/o. *Nigella aristata* was described by Pedanios Dioscoridou (1529) [2], Fraas, 1845 [3]; Lenz, 1859 [4], a/o.

In Islamic countries, in Koran is attributed to Mohammed prophet this affirmation "the seeds of black cumin (*Nigella sativa*), ameliorate every disease, except to death" [5]. Avicenna maintains that the black cumin seeds stimulate the body strength, help the human organism after fatigue and melancholy.

In Middle Age, Jacob Theodorus Tabernaemontanus (1588) [6] has described six *Nigella species*. In the **Herbarium** of Péter Melius, edited in Cluj in 1578, at least two of the *Nigella* species (*N. sativa* and *N. arvensis*) are mentioned, one as cultivated species and the other as a weed – both largely used as medicinal plants in about 17 recipes [7], [8].

In the **Unani Tib** medical system, *Nigella sativa* is recommended as remedy for numerous diseases: asthma, bronchitis, rheumatism, inflammatory diseases, stimulating of the digestion and lactation in women, the decrease of the renal calculus forming, treatment of the abscess at the abdomen, eyes or some internal organ level, a./o. The oil was used in dermatology (eczema, burns), treatment of fever, remove the dependence from opionids, a./o.

In present-day, the *Nigella sativa* seeds, present many usefulness in human alimentation (as spice at the bread, chase or other aliment preparation), flavones extraction in the perfumery industry, and for many medical purposes. Thus, the seeds of *Nigella sativa* are used in folk medicine in the Mediterranean area, Middle East and in some countries from Asia, for the promotion of good health and the treatment of many ailments including fever, common cold, headache, asthma and hardness in respiration, rheumatic diseases and various microbial infections, expelling worms from intestines and against constipation, diuretic regulation, the amelioration of the tooth and head smarting pain, ulcerations of the mouth [9]. It is also used against scorpion and spider stings and bites of snake, cat and dog, for lactation increase in young woman, against freckles, warts and leprosy, a.o. [2], [10].

The main active principles of the seeds are represented by fixed oil with unsaturated or saturated fatty acids, volatile oil (nigellone, thymoquinone, a.o.), proteins, alkaloids (nigellicine, nigellidine, nigellamine-N-oxide), coumarins, saponins (triterpenes and steroidal), minerals, fibers [10].

In the last decades, the researches over the *Nigella* sp. seeds constituents and their activity were enhanced. The most important findings were:
- nigellones from the volatile oil, confer protection against the spasms induced by histamines;
- beta-sitesterol, used an antitumour sterol;
- thymoquinone, in the oils from seeds, which obstruct the pancreatic cancer, eliminate the cells affected by the apoptosis process stimulating; recommended as an preventive strategy in chemotherapy; a./o.
The researches performed by our collective, point out the following findings:
- A total extract of alkaloids from seeds, manifest an immunostimulating and antistress effect (against the X-radiations; [9], [11]);
- Saponines, poliholosides and volatile oils present a radioprotective and immunostimulating effect, depending on the genotype, bioactive substance, their concentration, a/o; ([12], [13], [14];
- The bioactive substances activity is influenced by genetic structure (COX-2 gene by example [15];
- Bioactive substances from *Nigella sativa* and other medicinal plants (*Aralia mandsurica*), interaction with TiO_2 nanoparticules, deuterium-depleted water [16], a/o.

In this paper is analyzed the effect of a total polyphenols extract from the *Nigella sativa* seeds, as well as the COX-2 gene role in the regulation of the bioactive substances effect.

Material and methods

Biological material. The experiments were performed on the young female of *Mus musculus*, of about 24 g each, and was investigated the liver ultra structural features.

Bioactive substance. A 0.01% extract of different bioactive substances (alkaloids, polyholoside, saponins, and volatile oils) obtained from different *Nigella sativa* genotypes (populations). The animals were intraperitoneal injected, five injections, one at two days, with 0.5 ml with an 0.01% total polyphenols extract, diluted in DDW (depleted-deuterium water), or in distilled water.

DDW (depleted-deuterium water), is a water with a small amount of deuterium. In this experiment was used a water with 30 ppm deuterium, obtained at National Institute for Cryogenics and Isotopic Technologies from Râmnicu Vâlcea (Vâlcea district, România). Previously experiments effected in *Mus musculus* with different bioactive substances, extracted from different plant species, point out the benefic effect of the bioactive dissolved in a DDW with a content of 30 ppm deuterium [17].

Stress factor. Half of experiment animals were irradiated (entire body), one day after the third injection, with an X-rays source (an RUP 150/300 apparatus (ex

Soviet Union provenance) at the following parameters: 250 kV, 5 mA, 1 mm Al filter, D.F. = 500 mm, dose output 0.528 Gy/min, in a unique dose of 5.28 Gy. The experimental variants are presented in Table 1.

Table 1. Experimental variants in *Mus musculus* experiment with bioactive substances (BAS).

Code	Injection	Bioactive substance	X-rays (Gy)
C (Control)	Distilled water	-	-
C – X	Distilled water	-	5.28
DDW	DDW	-	-
DDW – X	DDW	-	5.28
BAS in distilled water	BAS in distilled	Bioactive substance	-
BAS in distilled water	water	Bioactive substance	5.28
- X	BAS in distilled	Bioactive substance	-
BAS in DDW	water	Bioactive substance	5.28
BAS in DDW – X	DDW		
	DDW		

Electron microscopy investigations. At a day, after the last injection, the experiment animals were sacrificed through section of the jugular vein. For the electron microscopy analysis, the pieces of about 1 mm^3 in volume, from liver, were prefixed in 2.5% glutaraldehyde solution, postfixed in a 1% Milloning fixation and included in EPON 812. The serrated sections of about 90 nm thick were contrasted with acetate de uranyl and lead citrate and analyzed at a TEM Philips CM 120 microscope (in *Ovidius* University from Constanţa), or at a TEM JEM JEOL 1010 electron microscope (in Electron Microscopy Center from *Babeş-Bolyai* University, Cluj-Napoca).

Results and discussions

Experiment with alkaloids total extract. Ultra structural features of the liver.

Control variant. The structural features in the control variant are characteristic for this organ and specie [18]. The hepatic lobule present a centro-lobular vein covered with an epithelium. Around the centro-lobular vein, are disposed the sinusoid capillary. The hepatocytes of polygonal shape are disposed in rows. Every hepatocyte present one (two) nuclei of oval-spherical shape. In cytoplasm are present numerous mitochondria, normal structured, with an electron-dense matrix (Figure 1). Among mitochondria are dispersed a rugous endoplasmic reticule formed from narrow profiles, usually disposed around the mitochondria (Figure 1).

At the vascular pole, the hepatocytes present microvillus evaginated in the Disse space. In sinusoid capillary, there are Kupffer cells with numerous lysosomes and with a normal activity. Smooth endoplasmic reticule, as well as the dictyosomes, is poor represented. The lipids drops are present in a small quantity, being represented through small drops, disposed with predilection toward the vascular pole (in transit; Figure 2).

In Control variant, and in other experimental variants, in hepatocytes were observed some parasite entities (viruses), and some filamentous structures as result of the metabolic activity [19].

Figure 1. Control, mitochondria.

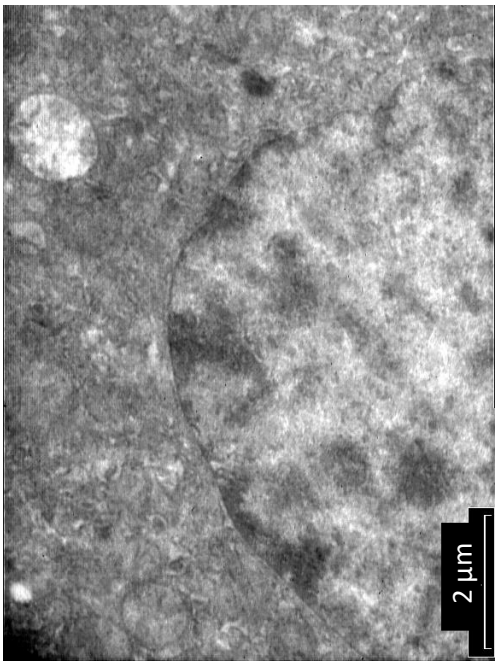

Figure 2. Control, nucleus.

Control-X. (the X-irradiated effect). Comparatively with the Control variant, under action of the X-rays, were induced some adulterations. The hepatocytes nuclei present an unregulated shape outline (Figure 3). In some of them, the nuclei are hypertrophied, especially the vacuole and pars amorphous components of the nucleolus. The smooth endoplasmic reticule is proliferated, as reaction at the destructive action of the X-rays. Rugous endoplasmic reticule presented dilated cisterns, and little ribosome's associated, because of the diminished of the metabolic activity and of the protein synthesis (Figure 3). Also, was emphasised a depletion of the glycogen. In some hepatocytes, in which are present small focuses of cytoplasm lyses, the nuclei are pycnotic and hyperchrome, as well as a

lipid accumulations under shape of drops of different size. As result of the advanced of the adulteration in the hepatocytes, the plasmalemma was destroyed the cellular compounds being free in sinusoid capillary. In some hepatocytes, the perinuclear space is dilated and the chromatin is uniformly (a degradation process). The Kupffer cell is inactive (Figure 4) and in the Disse space is present accumulation of glycogen.

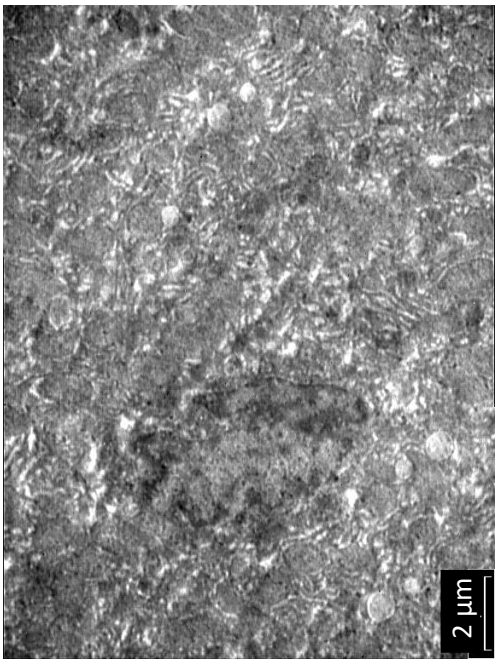

Figure 3. Control-X. Cell with dilated REG, and nucleus with undulated outline.

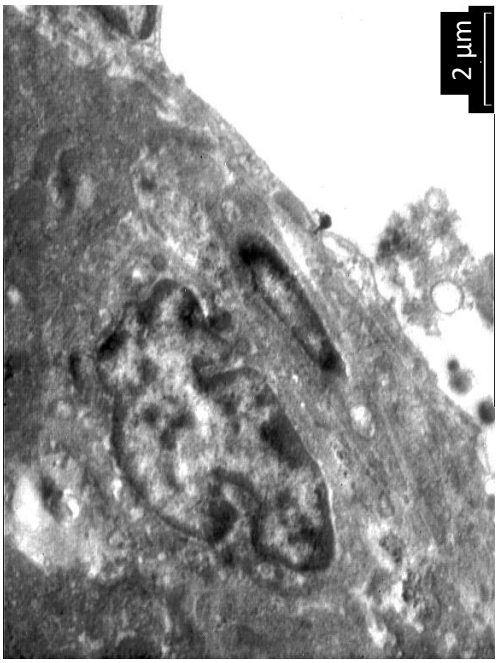

Figure 4. Control-X. Kupffer cell in activity.

DDW effect. In comparison with Control variant, the hepatocytes present some slight adulterations which not affect significant the cell metabolism. The nuclei present a structure almost normally with a spherical-oval shape and with heterochromatin disposed in the electron dense blocks at their periphery. In cytoplasm are present numerous mitochondria which present their matrix slightly electron-dense in comparison with the Control variant. The rough endoplasmic reticule is in an intense activity, in comparison with the Control variant, being disposed in parallel profiles, apt for the protein synthesis, but present some slightly dilatations (Figure 5). Also, the smooth endoplasmic reticulum is hypertrophied in comparison with the Control variant, being implicate in the detoxification process (Figure 5). At the vascular pole, the hepatocytes present numerous microvilli, which denote an intense activity of absorption of the

metabolites. In some hepatocytes, is observed a slightly lipids retention. Not are present the collagen accumulation in the Disse spaces.

The Kupffer cells, present a normal activity, having many lysosomes (Figure 6). In the macrophages are present the primary lysosomes and the cellular residues.

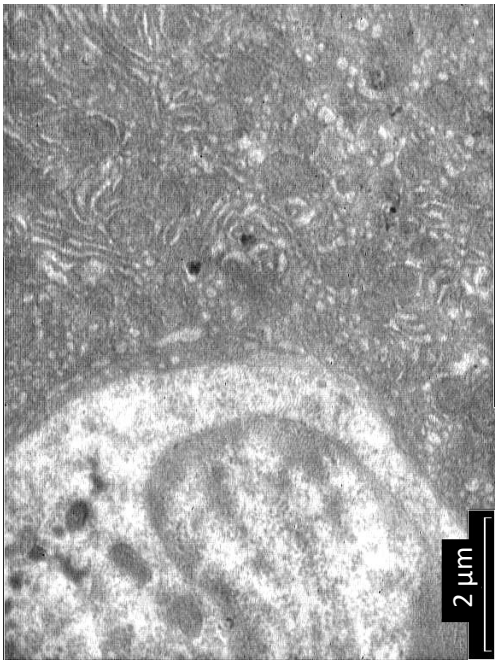

Figure 5. DDW. Hypertrophied SER and RER.

Figure 6. DDW. Kupffer cell with lysosomes.

DDW-X. (The irradiation in the DDW presence effect) On the basis of the slightly adulteration induced by the DDW presence in hepatocytes, the X-irradiation accentuated the adulterations effect of the hepatocyte ultrastructure. This suggests that DDW not present a radioprotective effect. In this variant, the amount of rough endoplasmic reticule is bigger represented in the cell, in comparison with the case of the irradiated animals. Also, in the case of combined action of the DDW and a stress factor (X-rays), the drops lipids is in a smaller number in the cells, in comparison with the Control, unirradiated or irradiated. The nuclei present an irregular contour, and in generally the chromatin is rarefied. The nucleolus is hypertrophied and the vacuole component and pars amorphous are enhanced quantitatively, or with an adulterated structure (Figure 7). The cytoplasm matrix is rarefied, with many lyses area, of small size. Also, was observed a reduction of the number of cytosol ribosomes. Mitochondria are present in a smaller number, being polymorphous as size and shape. As effect of

the X-irradiation, in hepatocytes are maintained a high quantity of rough endoplasmic reticule.

At the vascular pole of the hepatocytes, the plasmalemma of some cells is dense, many compounds migrates in sinusoids (Figure 8).

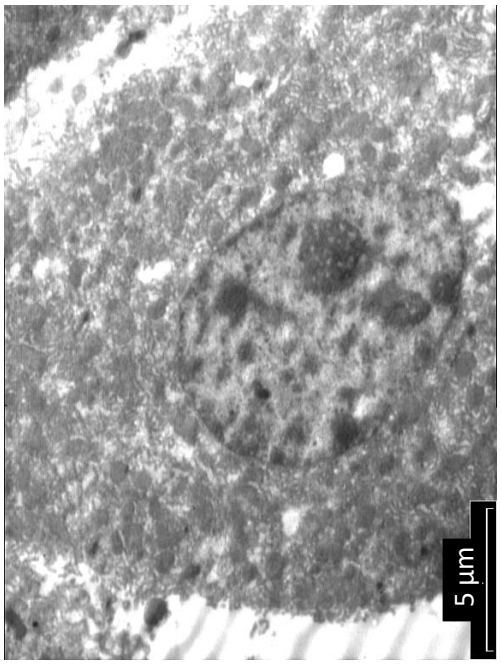

Figure 7. DDW-X-rays. Hypertrophied nucleolus.

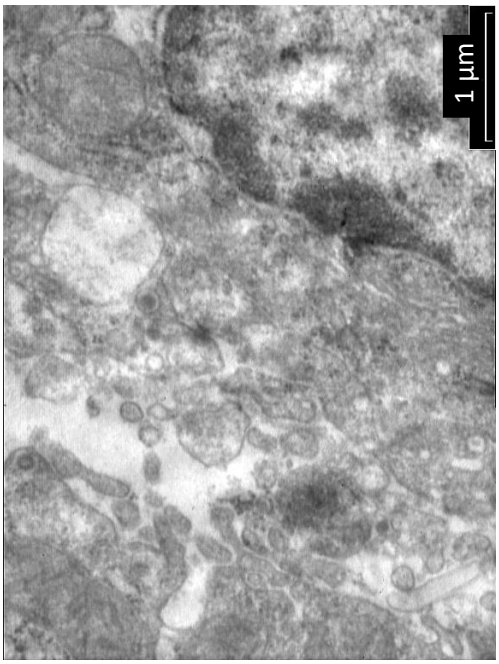

Figure 8. The vascular pole of hepatocyte.

Alkaloids in DDW (the alkaloids extract diluted in DDW effect). The application of the alkaloid extract in DDW, not affect the normal structure of the hepatocyte. The nucleus presents a polymorphism regarding their shape and the stage of cellular cycle. In some cells, the chromosomes are well structured, the cells being in an intense metabolic activity (Figure 9). In the cells are present an accumulation of lipid drops. The rough endoplasmic reticule is disposed in parallel profiles, having numerous ribosome's, propitious for the protein synthesis (Figure 10). The mitochondria present an electron dense matrix and cristas poor represented. The vascular pole of the hepatocytes, present a different structure, depending on the hepatocytes position. At the hepatocytes situated toward the periphery of the hepatic lobule, the microvillus are evaginated in the Disse space, while at the hepatocytes situated towards the centro-lobular vein, the microvillus are absent.

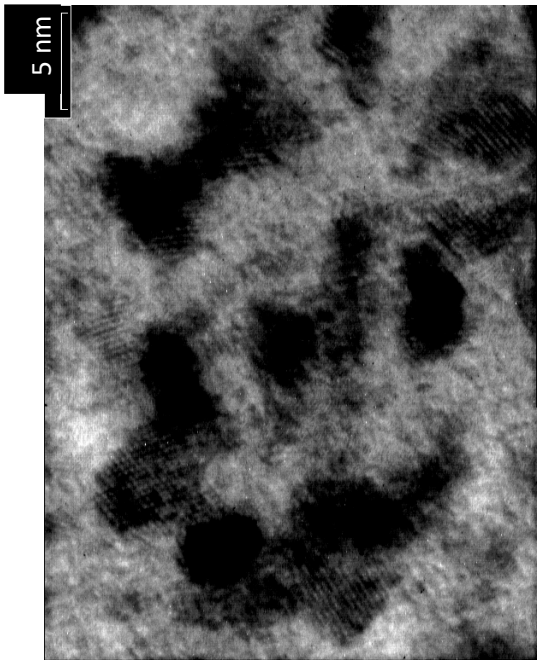

Figure 9. Alkaloids in DDW. Nucleus with chromosomes.

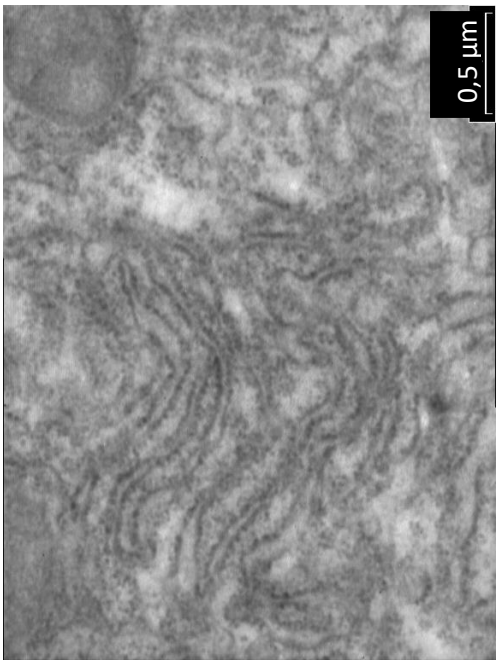

Figure 10. Alkaloids in DDW. RER and mitochondria.

Alkaloids in DDW – X (X-irradiation in the alkaloid extracts presence diluted in DDW). The ultrastructural modification induced by the X-rays, in the presence of the DDW, were major in comparison with the adulteration recorded under the action of the X-rays alone. In some cells, the nucleus is of normal shape, with heterochromatin disposed in blocks in its inner as well as on the inner part of the nuclear envelope. The smooth endoplasmic reticule was in a bigger quantity, and rough endoplasmic reticule presented the slightly the dilated profiles. In other cells, the cytoplasm presents lyses areas, rough endoplasmic reticule cistern dilated and nucleus with rarefied chromatin. The mitochondria presented a slight polymorphism, in comparison with the alkaloid extract application alone. Their matrix is electron-dense and cristas are poor represented. At the vascular pole, the microvilli are rarefied. The Kupffer cells are inactive, without lysosomes. The collagen fibbers are well represented, as well as lysosomes (Figs. 11, 12). Participation of the lysosomal apparatus of hepatocytes in collagen resorption during regression of cirrhosis of the liver was studied by Ryvnyak (1984).

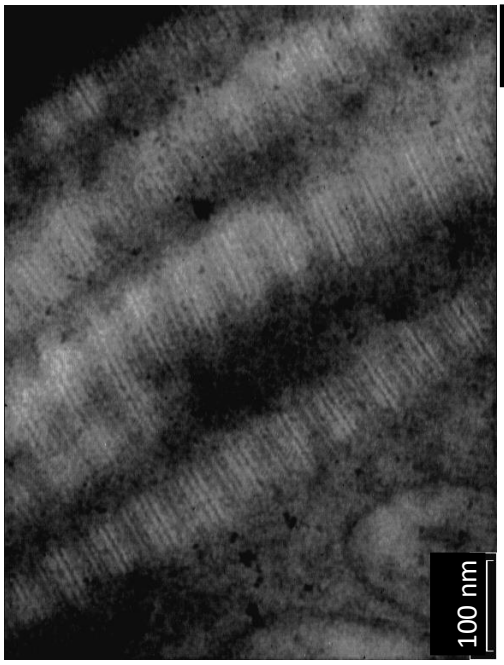

Figure 11. Alkaloids in DDW – X-rays. Collagen fibers.

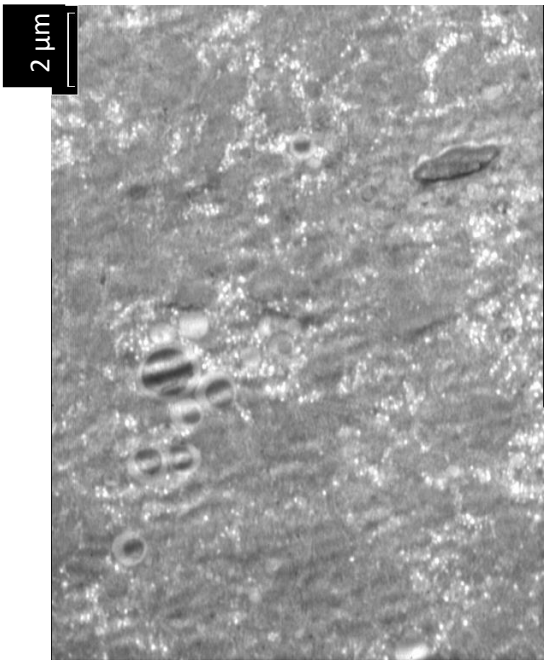

Figure 12. Alkaloids in DDW – X-rays. Collagen fibers and lysosomes.

Alkaloid diluted in distilled water (the effect of alkaloid extract diluted in distilled water). The cell ultrastructure is not affected in the administration case of the alkaloids dissolved in distilled water. The nucleus ultrastructure is normal, with fine blocks of heterochromatin dispersed in its inner or near nuclear envelope (Figure 13). The mitochondria present also a normal structure with a matrix compact and long and numerous cristas in inner. The endoplasmic rugged reticule and the smooth endoplasmic reticule are well developed (Figure 14). In some cells, the endoplasmic canalicules are dilated having a cistern structure. The vascular pole of the hepatocytes, present numerous vilosity. Near the sinuous capillary are present Kupffer cells in metabolic activity.

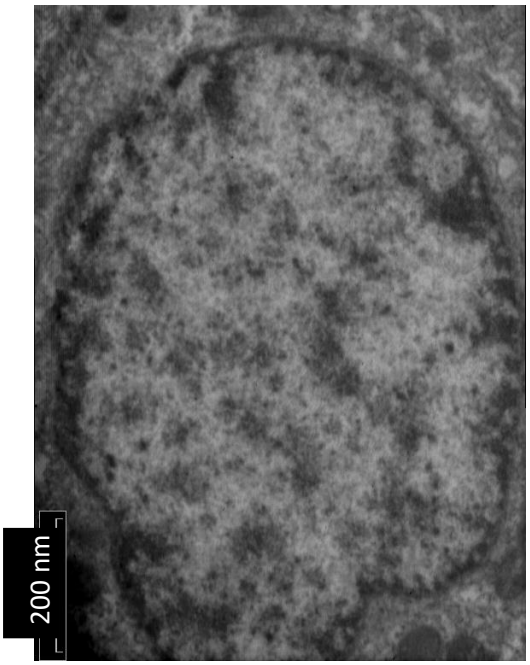

Figure 13. Alkaloids in distilled water. Nucleus with normal structure

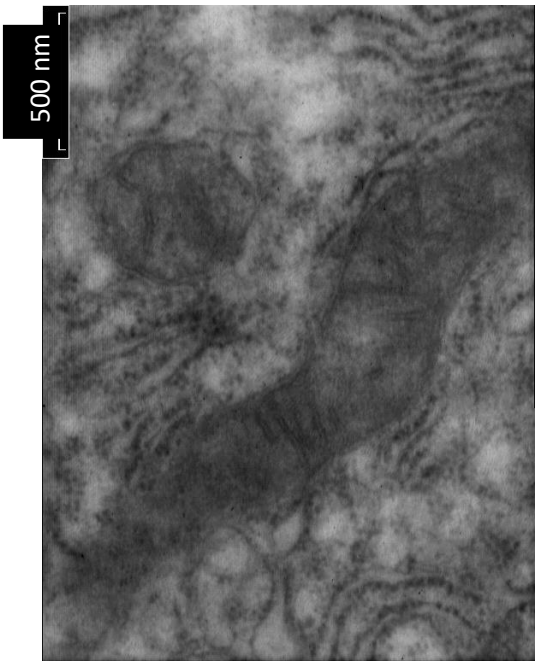

Figure 14. Alkaloids in distilled water. Mitochondria and RER.

Alkaloid diluted in distilled water – X. X-irradiation in the alkaloid extracts presence (diluted in distilled water). The presence of the alkaloid diluted in distilled water, has a radioprotective effect, in comparison with the ultrastructure features recorded at the alkaloid presence diluted in DDW.

The nucleus present a normal structure with heterochromatin fine disposed in its inner or near inner nuclear envelope. Also, the nucleolus presents their structural components, being in an intense metabolic activity (Figure 15). Some of the mitochondria present a normal structure, while other presents an adulterated structure. In this case, their matrix present some rarefied regions, and the cristas are a few and of a short length.

The endoplasmic reticule is well represented, sometime their canalicles being dilated, of a cistern aspect (Figure 16). The vascular pole of the hepatocytes is in generally, well represented. The sinusoid capillary present sometimes in its inner, hepatocytes and cell detritus. The cells Kupffer are pre-eminent in the sinusoid capillary.

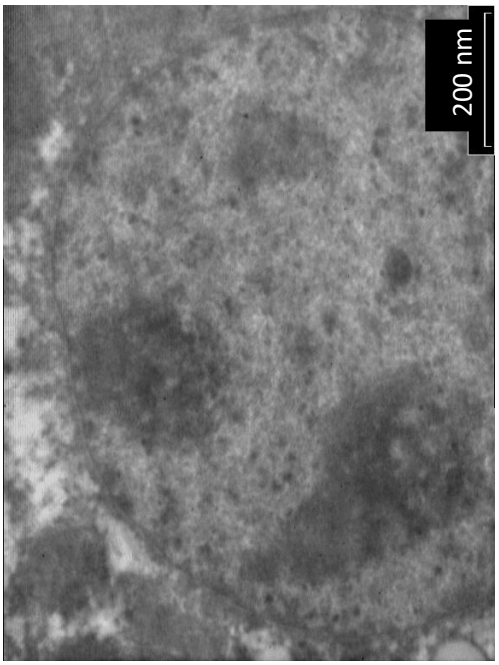

Figure 15. Alkaloids in distilled water – X-rays. Nucleus with normal ultrastructure.

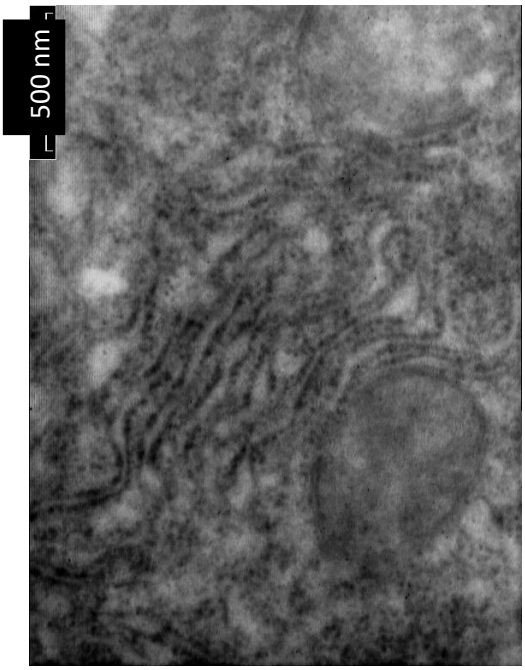

Figure 16. Alkaloids in distilled water – X-rays. Mitochondria and RER.

2. The interaction between a bioactive substance (total alkaloids extract) and COX-2 gene. Under action of the two exogenous factors (DDW and X-rays), the hepatocyte is reversible adulterated. In a hepatocyte with a normal mitotic activity, the effect of the alkaloids extract was dependent on the cells (hepatocytes) position, toward the cento-lobular vein. In the lymphocytes from the Malpighi lymphatic corpuscle, with a high mitotic activity, the total extract of alkaloids diluted in DDW, present a radioprotection effect.

The diminished of the deuterium content from water, probably inhibited the COX-2 gene function. This gene is implied in the prostaglandin synthesis. The alkaloids extract, amplified this action in the cells with quick mitotic activity, normal or pathological.

3. Experiments with polyholosides and saponins from *Nigella sativa*.

Saponins from *Nigella sativa*, unirradiated animals. Are induced slightly, reversible modifications as: a dilatation of the Centro lobular vein and erythrocytes disposed on the endothelium. Around the centro lobular vein, the hepatocytes are ordaining disposed. Also, is present a stasis process at the centro lobular vein level. In nucleus from the pericentrolobular hepatocytes, the heterochromatin is pulverized.

Saponins from *Nigella sativa*, irradiated animals. In the centro lobular vein is present a stasis phenomenon, the erythrocytes being adherent on the epithelium of the centro lobular vein. In sinusoid capillary, the erythrocytes are disposed under a roll shape. There is present a reduced amount of nuclear chromatin, which is homogenous, disposed in fine rows.

Polyholosides from *Nigella sativa*, unirradiated animals. Present a stasis process in the centro lobular vein, and a slightly stasis process in the sinusoid capillary. In the nuclei, agglomerations of heterochromatin are present at the periphery. Nucleoli are hypertrophied.

Polyholosides from *Nigella sativa*, irradiated animals. The liver presents structure characteristics for an intense metabolic activity. In some cells two nuclei are in an adjacent position, suggesting a mitotic division activity. The chromatin is fine disposed, being present also the NAB's structure (nucleolus associated body's), which indicate an intense metabolic activity as result at the animal adaptation at the action of this stress factor.

4. Experiment with polifenols from *Aralia mandshurica*.

Control. Hepatocytes present a normal ultrastructure. They present usually one (two) nuclei, with smooth outline, and numerous mitochondria. The rugous endoplasmic reticulum is well represented, while the smooth endoplasmic reticulum has a discrete presence, being evidently through many vesicles. In hepatocytes are present a small amount of lipids drops. Dyctiosomes, glycogen, a/o. The Ito cell present a small amount of lipids and the Kupffer cell is in a normal activity.

Control – X rays. In hepatocytes are present numerous lipid drops. As result of action of a stress factor, the nucleus outline is unregulated, in nucleoli being present some vacuolarizations as well as in the heterochromatin areas. In mitochondria, the crista numbers is reduced. In the Ito cell are present small lipid drops. The Kupffer cell is active, having lysosomes, pynocitosis vesicles, and the cell rests.

DDW. The cell react at this exogenous substance, being affect the water metabolism. Thus in hepatocyte is present a big amount of lipid drops. Nucleus is normal, but presents an enhanced of the vacuolar component of the nucleoli. Also, take place a proliferation of the smooth endoplasmic reticulum, this organelles having an important role in the detoxification processes. In hepatocyte is present a small amount of glycogen. The Kupffer cell presents lysosomes in metabolic activity.

DDW - X rays. DDW action as a scavering of the free radicals, the amount of lipid drops being reduced and lesions induced by X-rays being limited. Thus nucleus and mitochondria present a normal ultrastructure. Smooth endoplasmic reticulum is well represented, the glycogen is in a small amount, and collagen

fibers are absent. In Ito cell are present some lipid drops, of different size. Kupffer cell present lysosomes and phagocytes products.

Polyphenols in DDW. Hepatocytes present a normal structure, the nucleus having a smooth, normal outline. In cell are present numerous lipid drops, and the glycogen is practically absent. Cellular organelles (mitochondria, endoplasmic reticulum) present a normal structure. In the Ito cell the lipid drops are practical absent. The Kupffer cells contain lyzosomes, hematin and destroyed material. Capillaries without stasis processes.

Polyphenols in DDW – X rays. The presence of the polyphenols in DDW in the time of the stress factor action (a sub lethal dose of X-rays), confer a total protection vs. X-rays, the hepatocytes having a structure similar to Control. In the Kupffer cell are present primary and secondary lyzosomes with residual corps (phagocyte of cellular rests destroyed). Some sinusoid capillaries are slightly congestion and microvillus preeminent in the Disse space.

Conclusions

Under action of a source of X-rays of sublethal value (5.28 Gy), the hepatocyte ultrastructure is adulterated, especially the nucleus, mitochondria and endoplasmic reticule. Also, are affected the quantity of the lipids drops and glycogen amount from the cells, as well as the Kupffer cell ultrastructure.

Under action of the DDW, are established some slightly adulteration of the hepatic cells, which didn't affected significantly the cell metabolism. In principally, DDW adulterate the lipid metabolism, in hepatocyte being present a great number of lipid drops.

The DDW presence in the time of X-irradiation of the animals, reduce very significantly the injuries produced by X-irradiation at the liver level, the ultrastructural features being appropriate to Control animals.

The application of the alkaloid extract in DDW, affected the normal structure of the hepatocytes.

The alkaloid extract administered in distilled water, did not affected the normal structure of the hepatocytes, their structure being similarly with the Control.

The presence of the alkaloid extract diluted in distilled water, in the time of the animal irradiation, manifested a protector effect at the liver level.

The experiments performed with a total extract of alkaloids from *Nigella sativa* seeds, offer informations about the COX-2 gene activity. The COX-2 gene activity (implied in the prostaglandin synthesis), is affected by the cell content in deuterium. The reduction of deuterium content from water has as effect an inhibition in the COX-2 gene function.

The acute X-irradiation of the whole body in the presence of polyholosides extract from Nigella sativa seeds, manifest a radioprotective effect at the liver level in *Mus musculus*.

The saponins extract from *Nigella sativa* seeds, applied alone, induced a slightly adulteration of the liver structure.
The presence of saponins extracted from *Nigella sativa* seeds in the time of X-irradiations of the animals, induce a slightly protection.
A 0.01% total polyphenols extract from *Aralia mandshurica*, diluted in DDW, not induced cytotoxic effects.
The X-irradiation of the animals in the presence of DDW and a total polyphenols extract from *Aralia mandshurica*, manifest a strong stressprotector effect.

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