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THE RADIOPROTECTIVE EFFECT OF DEUTERIUM DEPLETED WATER AND POLYPHENOLS

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Abstract

In the experiments performed in mouse (*Mus musculus)* the radioprotective effect of Deuterium Depleted Water (DDW, with 30 ppm deuterium) and a 0.01% total extract of polyphenols (from *Aralia mandshurica* cortex) *)*, applied individually or together was tested, towards a sublethal dose of X-rays (5.28 Gy). The animals were intraperitoneal injected (five injections with 0.5 mL each, one at two days), with single DDW, or with a 0.01% polyphenols diluted in DDW. The stress factor was applied one day after the third injection. As radiobiological point the ultrastructural features of the liver was used (following the animal sacrifice a day after the last injection). The X-irradiation of the animals treated independently or combined with DDW and polyphenols extract, manifest a radioprotective effect, especially in the combined action of the two factors. Also, it was established that the application of a single exogenous factors, enhanced the lipid drops from hepatocyte.

Key words: Aralia mandshurica, DDW, liver ultrastructure, polyphenols, radioprotection

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1. Introduction

1.1. Polyphenols

Polyphenols are a group of substances found in plants, which contains more than one of phenol units, or building block per molecule. They are divided generally in: tannins, lignins and flavonoids. The flavonoids are best studied, and include several thousand compounds as flavonols, flavones, catechins, flavonones, anthocyanidins and isoflavonoids. The most abundant polypohenols are condensed tannins, found in almost families of plants (Gironi and Piemonte, 2010).

In vitro, polyphenols have antioxidants properties. Researches performed by Frei and Higdon (2003) established that tea polyphenols manifest antioxidant activity *in vivo*. The anthocyanin pigments and associated flavonoids posses the ability to protect against a myriad of human diseases. In spite of that, they have been notoriously difficult to study with regard to human health. Analyzing six folk medicinal ferns used in the traditional Chinese system of medicine, Chang et al. (2007) established their content in polyphenols and the antioxidant activities. Rahman (2008) underlined that the dietary polyphenols (bioflavonoids) possess antioxidant and anti-inflammatory properties.

Experiments performed in mouse (*Mus musculus*) with a diet supplementation for 5 weeks with polyphenols-rich cereals, point out that they improve several functions and the redox state of mouse leucocytes.

In a synthesis regarding the antioxidant and antimicrobial activity of seeds from different family, is presented also a species of *Aralia*, *A. racemosa*

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(Borchardt et al., 2008). In studies performed on some medicinal plants, Zagnat (2004) established that *Aralia mandshurica* Rupr. et Maxim. posses a very good antistress-adaptogen action.

1.2. Deuterium-depleted water (DDW)

Deuterium-depleted water (DDW) is distilled water, microbiologically pure, with a smaller isotopic deuterium concentration (120 – 20 ppm) than its concentration in natural water (144 ppm D/D+H), (Stefanescu et al., 2004). Somlyai et al. (2004) suggested that some cells can modify the D/H ratio through some molecular mechanisms (probably) implicated in the cell cycle regulation.

The deuterium-depleted water causes the tumor regression in the xenotransplanted mice, while requiring a longer time for *in vitro* multiplication (Somlyai et al., 2004). The decrease of D concentration can appear in the signal transduction pathways thus leading to tumor regression, because the depleted deuterium has an influence on the expression of some genes involved in the tumour regression (Somlyai et al., 2004).

Somlyai (1998) reports the successful treatment with DDW in cancer patients in Hungary The research performed by Somlyai (2001) points out that the reduction of deuterium concentration in water inhibits the ***COX-2*** gene function (genes involved in the prostaglandin synthesis). The experiments performed by Manolescu et al. (2006) in rats and dogs, revealed that a daily treatment with 60 ppm DDW, presents certain detoxification properties, in comparison with the toxic stress generated by cytostatics used in the anti-cancer treatment.

1.3. Reactive Oxygen Species (ROS) and their inactivation by DDW and/or polyphenols

Reactive oxygen species (ROS) are represented through: molecule like hydrogen peroxide, ions like the hypochlorite ions, radicals like the hydroxyl radical, and the superoxide anions.

ROS are formed by different mechanisms: the interaction of ionizing radiation with biological molecules; as an unavoidable byproduct of cellular respiration, or synthesized by dedicated enzymes in phagocyte cells (neutrophils or macrophages). ROS can damage other molecules and the cell structures, through of the free radicals action on the fatty acids side chains of lipids in various membranes of the cell, especially the mitochondrial membranes. The cell defense against the ROS molecules, through some mechanisms: the action of some enzymes (superoxide dismutase, catalase) or through several different antioxidant molecules, as alpha-tocopherol, uric acid, vitamin C, a/o. Similarly, polyphenol antioxidants assist in preventing ROS damage by scavenging free radicals.

In experiments performed in mice, feed with deuterium-depleted water (30 ppm deuterium) time of 15 days, and then irradiated with a dose of 8.5 Gy X-rays, Bild et al. (1999), established a marked intensification of the immune defense and increased proliferation of the peripheral blood cells, under action of the DDW.

In experiments performed in rats intoxicated with chromium (20 ppm/kg), after 30 days of treatment with and without deuterium depleted water (30 ppm/L) administered by gavage, Cărpinişan et al. (2010), established that deuterium-depleted water modified significantly positive both the evolution of the hematocrit and the leukocyte formula. Corneanu et al. (2006) established the radioprotective effect in the combined treatment DDW + bioactive substances extracted from *Nigella sativa* or *Aralia mandshurica*, against the X-rays in mice. This action was established through analysis of the liver and/or spleen ultrastructure. The results obtained by many researcher groups, underlined this research direction, being recorded many patents in this field.

2. Experimental

2.1. Biological material and bioactive substances

This experiment was performed with young females of *Mus musculus*. From the *Aralia mandshurica* cortex, was extracted, through usual methods, the polyphenols, from the acetone fraction. In this experiment was used an extract of 0.01% polyphenols, diluted in DDW or in distilled water. The animals were intraperitoneal injected, one at two days (five injections) with 0.5 ml from the 0.01% polyphenols extract solution. The other bioactive substance was depleted-deuterium water (DDW), with 30 ppm deuterium, produced at the N.R.-D.I. of Cryogenics and Isotopes Separations, Râmnicu Vâlcea, România.

2.2. Stress factor

Half of the experiment animals were irradiated (entire body), one day after the third injection, with an X-rays source (an RUP apparatus, ex Soviet-Union provenance), at the following parameters: 250 kV, 5 mA, D.F. = 500 mm, 1 mm Al filter, dose output 0.528 Gy, in a unique dose of 5.28 Gy. The experimental variants are presented in Table 1.

Table 1. Variants in *Mus musculus* experiment

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

2.3. Electron microscopy investigations

One day after the last injection, the animals were sacrificed through the jugular vein section and the biological material harvested (liver) was used for ultrastructural investigations. Pieces of about 1 mm^3 of liver, were prefixed in a 2.5% glutaradlehyde solution (2 1/2 hours), postfixed in a 1% osmic acid solution (1 ½ hours), and then embebbing and included in EPON 812. The serrated sections of about 90 nm thickness were contrasted with uranyl acetate and lead citrate and analyzing at a JEOL JEM 1010 electron microscope in Electron Microscopy Centre, *Babes-Bolyai* University from Cluj-Napoca.

3. Results and discussions

3.1. Control

The liver from the Control variant, present a normal ultrastructure (Begnescu, 1980, Corneanu et al., 2006). The hepatocytes present one and sometime two nuclei, with a smooth outline. The mitochondria are numerous, usually of spherical shape. Rugous endoplasmic reticulum is well represented, but the smooth endoplasmic reticulum has a discrete presence (Fig. 1). Also in hepatocyte are present dictiosomes, glycogen a/o. The Kupffer cells are in a normal activity. The Ito cells present a few lipid drops, or without lipid drops (Fig. 2).

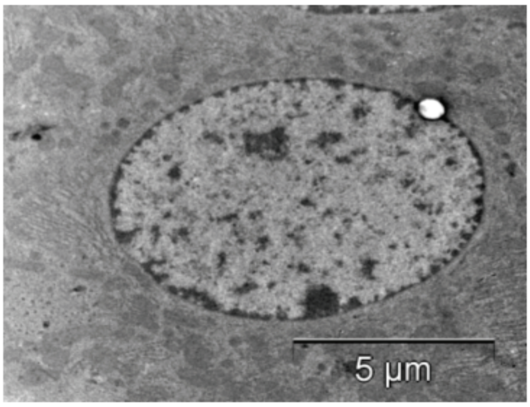

Fig. 1. Hepatocyte with normal ultrastructure

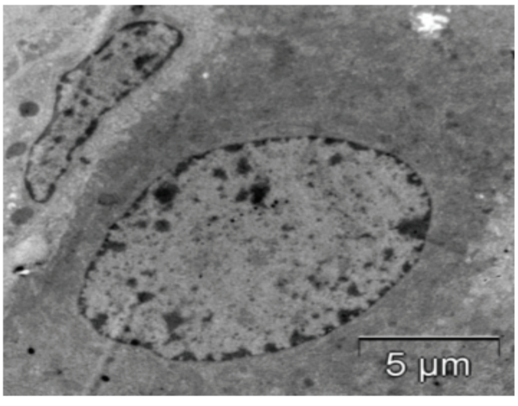

Fig. 2. Hepatocyte and Ito cell

3.2. Irradiated control

In the hepatocyte are present numerous lipid drops. Gokhberg et al. (1978), in experiments performed in mice, established that the administration of a stress factor caused the appearance of many lipid inclusions in the cytoplasm of the hepatocytes, the basis for fatty infiltration of the liver. In present experiment, the presence of numerous lipid drops in hepatocyte cytoplasm, in the irradiated Control, can have a similar cause: the action of a stress factor – X-rays. The mitochondria present a reduced number of crista. Due to stress factor action, the nucleus, with an unregulated contour, presents some vacuolarization in the nucleoli (Fig. 3). Sometime, the heterochromatin is vacuolarized, the nuclei being picnotic. If in the hepatocyte are present numerous lipid drops, in the Ito cells are present a small number of lipid drops. The Kupffer cells are in activity and present lyzosomes, pinocitose vesicles, as well as the cells rests (Fig. 4). In the Ito cells are present small lipid drops.

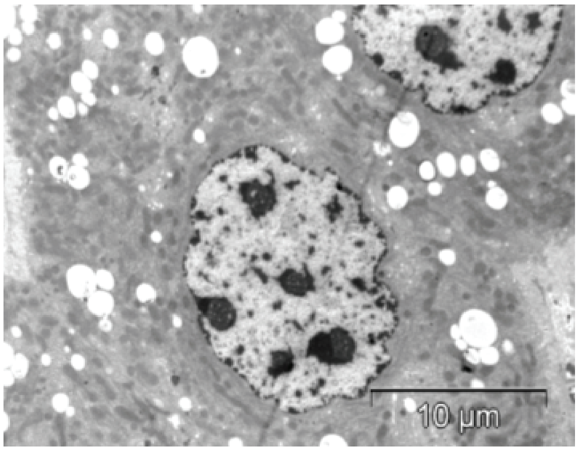

Fig. 3. Hepatocyte with many lipid drops and adulterated nucleus

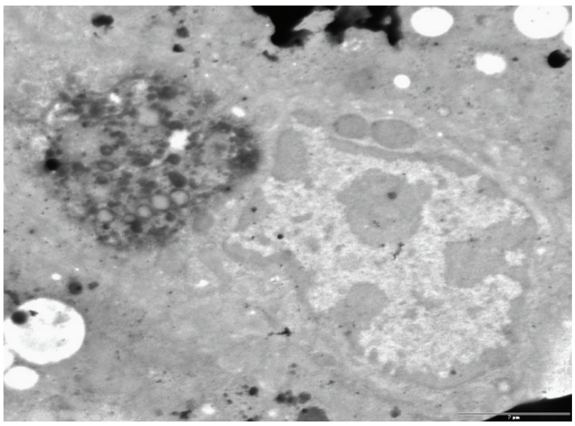

Fig. 4. Kupffer cell with lyzosomes and residual corps

3.3. DDW action

The hepatocytes present some particularly features as result of presence in its inner of an exogenous substance, respectively DDW. The main feature is the presence in hepatocyte of a big amount of lipid drops. Some researches performed by different authors, underlined that DDW can optimized the lipid consumption. Thus, DDW can improves the water metabolism, remove disorders of fatty metabolism, in cell being available a great amount of lipids, present as drops in hepatocyte (Vemuri et Darshan, 2007). In the presence of the exogenous substance (DDW), the hepatocyte react through the proliferation of the smooth endoplasmic reticulum (with a role in the cell detoxification), which there are in a great number in the hepatocyte under a vesicular shape (Fig. 5).

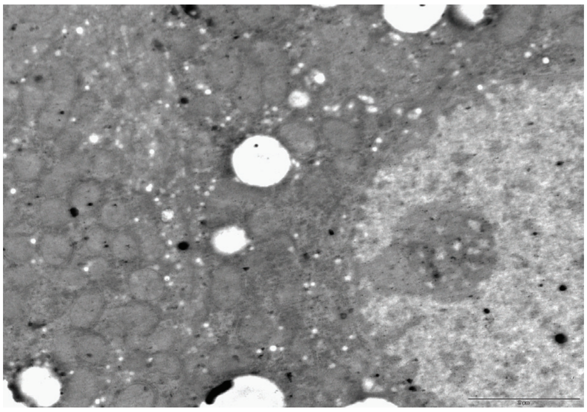

Fig. 5. Nucleus surrounded by a proliferate smooth endoplasmic reticulum

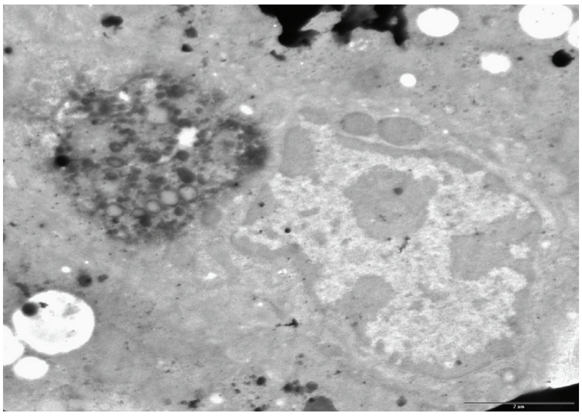

Fig. 6. Kupffer cell with lyzosomes and residual matter

The Ito cells can have a small lipid drops amount, or can be without lipid drops. The nucleus present a normal shape, with heterochromatin discrete dispersed in inner. The vacuolar component of the nucleoli is enhanced, the metabolic activity of the cell being intense (Fig. 6). Also, the rugous endoplasmic reticulum is proliferating. The Kupffer cell is in metabolic activity, having many lyzosomes and many degraded material (Fig. 6).

The glycogen is present in a small amount in hepatocyte. Mitochondria of elongated or spherical shape are in a great number, having a normal structure. Sometimes, the mitochondria present a decondensed matrix.

3.4. Irradiated DDW

The X-irradiation of the animals in the DDW presence reduced the amount of the lipid drops from hepatyocyte, because the DDW acts as scavering of the free radicals induced by X-rays. In support of this claim are the ultrastructural aspects of the cell, as well as the presence of the lipid drops in the Ito cells (Fig. 7).

The DDW presence during X-rays irradiation, conduct to limits of the lesions induced by X-rays. A small number of mitochondria are destroyed, as result of the free radicals produced by X-rays in cell. The mostly of the mitochondria present the two membranes and a normal matrix.

The rugous endoplasmic reticulum is well represented, the glycogen in a small quantity, and collagen is absent. The nucleus is well structured, and the vacuolar component of the nucleoli evidently, they being in high metabolic activity (Fig. 8). The Kupffer cell present lysosomes and phagocytes' products.

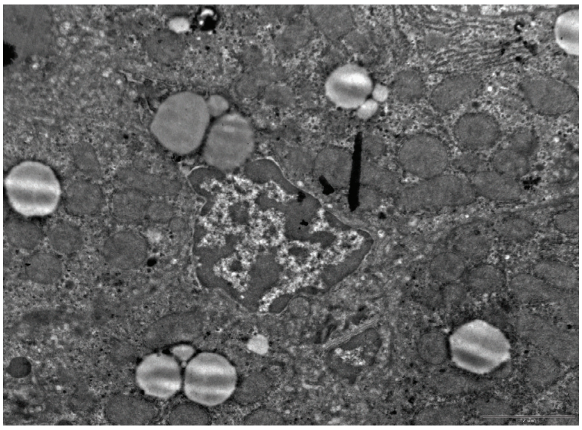

Fig. 7. Ito cell with lipid drops

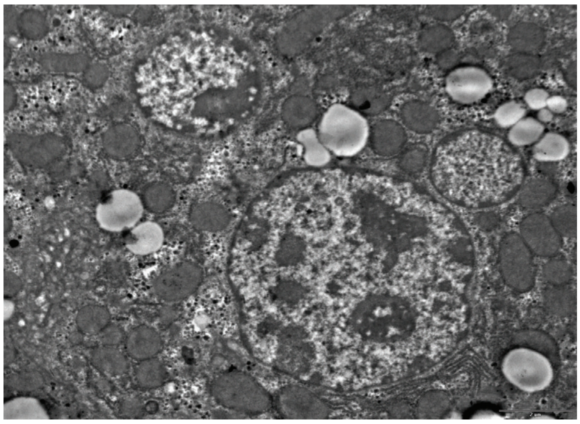

Fig. 8. Hepatocyte with almost normal ultrastructure

3.5. Polyphenols in DDW

The hepatocytes present a normal structure, with numerous lipid drops in its inner, the glycogen being practically absent. In experiments performed with mice, treated with polyphenols, Hou et al. (2008)

recorded also a great number of lipid drops in hepatocyte. They underline that polyphenol-activated SIRT1 acts upstream of AMPK signaling and hepatocellular lipid metabolism. Polyphenols increase SRT1 deacetylase activity, LKB1 phosphorylation at Ser^{428} and AMPK activity. The nucleus present the heterochromatin disposed in fine blocks in inner or near the nuclear envelope (Fig. 9).

The nucleoli present the vacuolar component well represented, being in high metabolic activity. The cytoplasm presents the numerous cellular organelles with normal structures. Numerous mitochondria of elongated or spherical shape present crista well represented and a dense matrix. The rugous endoplasmic reticulum with narrow profiles is disposed between mitochondria. The smooth endoplasmic reticulum is poor represented. In the Ito cells, the lipids are practical absent though they are present in hepatocyte. In the Kupffer there are lyzosome, hematin and destroyed material. In the sinusoid capillaries and in billiar canalicles are not evidenced the stasis processes (Fig. 10).

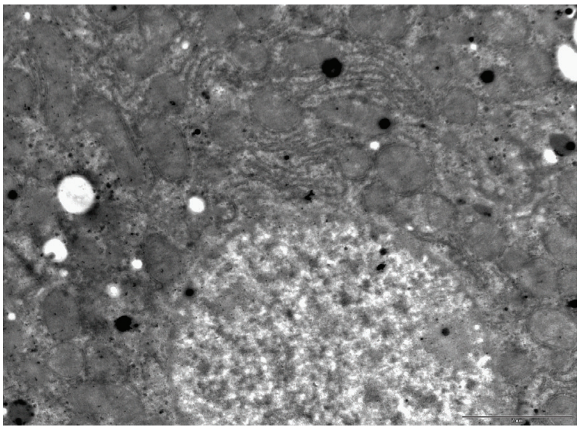

Fig. 9. Hepatocyte with a few lipid drops

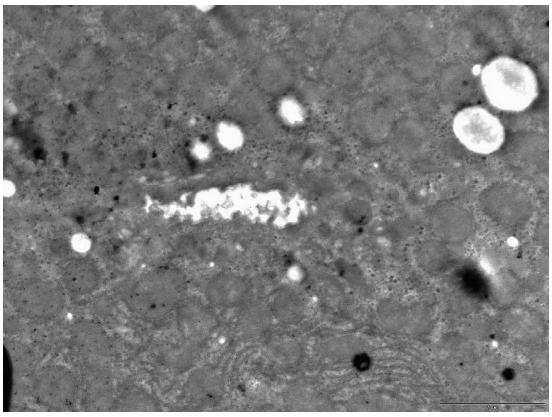

Fig. 10. Biliar canalicle without stasis process

3.6. Polyphenols in DDW, irradiated

The animal irradiated in the presence of a total polyphenols extracts diluted in DDW, assure the total protection towards the X-irradiation.

It was not found the lesions at the level of the cellular organelles, the hepatocytes presenting the similar ultrastructural features at the Control animals (Figs. 11, 12). The nucleus having the heterochromatin disposed in fine blocks in its inner, present evidently nucleoli. Around the nuclei there are profiles of smooth endoplasmic reticulum, rough endoplasmic reticulum and numerous mitochondria. Sometime is evidently a dyctiosome (Fig. 11).

Mitochondria of normal shape, present an evidently matrix and crista. In cytoplasm are present a few lipid drops. The presence of the stress factor at the cell level is evidenced by the sinusoid capillaries and Kupffer cell. In the Kupffer cell are present lyzosomes, primary and secondary (Fig. 12) with residual corps, as result of the phagocyte action of the cellular rests destroyed. Also, some sinusoid capillaries are slightly congestion, being full with hematies, while other is not congestion, being presents the microvillus which proeminet in the Disse space.

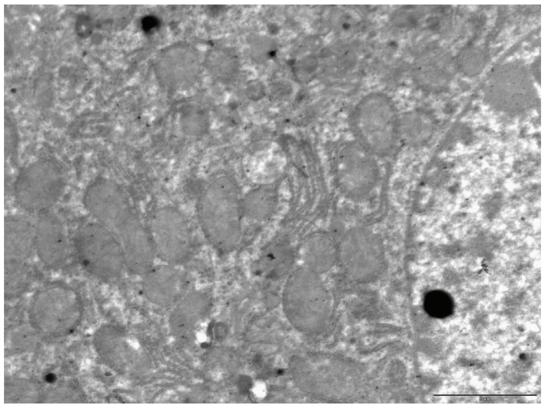

Fig. 11. Hepatocyte with normal structure: nucleus, dyctiosome, endoplasmic reticulum, mitochondria

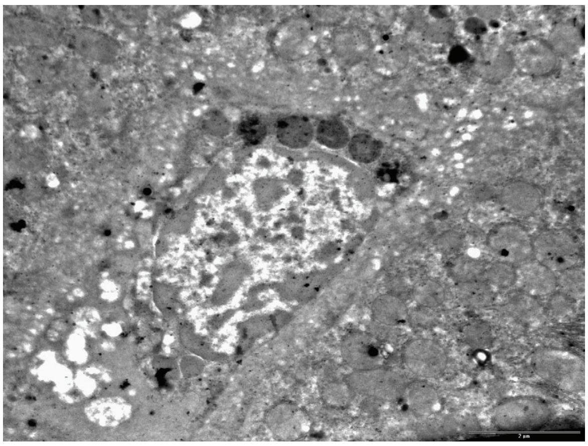

Fig. 12. Kupffer cell with secondary lyzosomes and residual corps

4. Conclusions

According to the results obtained the following conclusion must be noticed:

- a dose of 0.01% total polyphenols extract from cortex of *Aralia mandshurica* did not induced cytotoxic effects;
- the presence in organism of a single exogenous factor (X-rays, DDW or polyphenols), alter the lipid metabolism and induce the presence of a great number of lipid drops in hepatocytes;
- the total polyphenols extract (0.01%) administered in DDW, does not manifest a cytotoxic effect;
- the DDW presence in the time of X-irradiation of the animals, reduce significantly the injuries produced by X-irradiation at the liver level, the ultrastructural features being appropriate to Control animals;
- the X-irradiation of the animals in the presence of DDW and a total polyphenols extract from cortex of *Aralia mandshurica*, manifest a strong stressprotector effect;
- these results can be further applied in the radiotherapy treatment in the human public health.

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References

Begnescu R., (1980), *Liver cell*, In: *Normal and patological cytology at animals*, Manolescu N. (Ed.), (in Romanian), Ceres Press, Bucharest, Romania, 149-161.

Bild W., Năstasă V., Hăulică I., (1999), Researches concerning the radioprotective and immunostimulating effects of deuterium-depleted water, *Romanian Journal of Physiology*, **36**, 205-218.

Borchardt J.R., Wyse D.L., Sheafter C.C., Kauppi K.L., Fulcher G.R., Ehlke N.J., Biesboer D.D., Bey R.F., (2008), Antioxidant and antimicrobial activity of seed from plants of the Mississippi river basin, *Journal of Medicinal Plants Research*, **4,** 081-093.

Cărpinişan L., Petcu M.D., Petrovici S., Chis C., Ghişe A., Zehan R., (2010) The influence of deuterium depleted water on the hematocrit and leukocyte formula in rats intoxicated with chromium, *Scientific Papers: Animal Science and Biotechnologies*, **43**, 464-468.

Chang H.-C., Huang G.-J., Agrawal D.C., Kuo C.-L., Wu C-R., Tsay H.-S., (2007), Antioxidant activities and polyphenols content of six folk medicinal ferns used as "Gusuibu", *Botanical Studies*, **48**, 397-406.

Corneanu G., Crăciun C., Ciupină V., Prodan G., Corneanu M., Atyim P., Ştefănescu I., Iacob M., (2006), *Ultrastructural effects of Nigella sativa total alkaloids extract at liver level* (*Mus musculus*), In: *Proceedings of 4th Conference on Medicinal and Aromatic Plants of South-East European Countries*, Ghiorghiţă G., Stănescu U., Toma C. (Eds.), Alma Mater Publishing House, Romania, Iasi, 379-384.

Frei B., Higdon J.V., (2003). Antioxidant activity of tea polyphenlos *in vivo*: evidence from animal studies, *Journal of Nutrition*, **133**, 3275S - 3284S.

Gironi F., Piemonte V., (2010), Temperature and solvent effects on polyphenol extraction process from chestnut tree wood, *Chemical Engineering Research and Design*, doi:10.1016/j.cherd.2010.11.003.

Gokhberg S.L., Rasuler N.I., Arslanova N.A., (1978), Effect of some external factors on the submicroscopic organization of the liver in rats poisoned with carbon tetrachloride, *Bulletin of experimental biology and medicine*, **86**, 498-501.

Hou X., Xu S., Maitland-Toolan K.A., Sato K., Jiang B., Ido Y., Lan F., Walsh K., Wierzbicki M., Verbeuren T.J., Cohen A.R., Zang M., (2008), Regulated hepatocyte lipid metabolism through activating AMP-activated protein kinase, *The Journal of Biological Chemistry*, **283**, 20015-20026.

Krempels K., Somlyai I., Jánosi I., Szakácz, Somlyai G., (2006), *A retrospective study to evaluate the effect of deuterium depletion on the survival of metastatic breast cancer patients*, 13th Symp. Analytical & Environmental Problems, Szeged, 43-47.

Manolescu N., Valeca S.C., Anghel R., Balanescu I., Traicu R., Marculescu D., Stefanescu I., Panait M., Balint E., Encut I., Militaru M., Pop A., Cinca S., Comisel V., Fugaru V., Mateescu C., Gruia I., Moraru V., Nistoroiu M., Begu D., Dumitrescu I., Ghita M., (2006), Deuterium depleted water (DDW) using as adjuvant in cancer therapy for cytostatic toxicity reduction, WIPO Patent Application *WO/2006/019327*.

Rahman I., (2008), Dietary polyphenols mediated regulation of oxidative stress and chromatin remodeling in inflammation, *Nutrition Reviews*, **66**, 42-45.

Somlyai G., (2001), *Defeating Cancer! The biological effect of deuterium depletion*, Conphys Publishing House, Râmnicu Vâlcea, Romania, 213.

Somlyai G., Jascsó G., Jákly Gy., Berkényi T., Szabó M., Molnár M., Gyöngyi Z., Ember I., (2004), Deuterium depletion in cancer treatment and prevention, 10th Conf. Progress in Cryogenics and Isotopes Separation, Căciulata, 38-39.

Ştefănescu I., Tămâian R., Tiţescu G., (2004), Depleted-deuterium water. Short history and news, *Progress of Cryogenics and Isotopes Separation*, **13-14**, 7-10.

Vemuri M., Kelleu S.D., (2007), *The Effects of Dietary Fatty Acids on Lipid Metabolism*, In: *Fatty Acids In Foods And Their Health Implications*, Ching Kuang Chow (Ed.), Third ed., CRC Press, 591-630.

Zagnat M., (2004), *Study of medicinal plants used in folk medicine in Moldova and Romania, plant products with saponin* (in Romanian), PhD Thesis, Kishinev, 202.