BABES – BOLYAI UNIVERSITY
FACULTY OF BIOLOGY AND GEOLOGY SCIENCES

EFFECTS OF SOME BIOLOGICALLY ACTIVE SUBSTANCES ON THE ANIMAL ORGANISM AND MODULATION OF THE IMMUNE RESPONSE

SUMMARY OF DOCTORAL THESIS

SCIENTIFIC COORDINATOR
Professor Doctor CORNEANU GABRIEL

Ph.D. candidate:
EDITOIU CORNELIA DANIELA

CLUJ – NAPOCA
2011

CONTENTS:

Keywords: biologically active substances, *Aralia mandshurica*, *Nigella sativa*, immunomodulatory effect, effect of radiation protection.

Introduction

Plants are major sources of obtaining bio-products there are essential to the survival of the entire animal kingdom, accounting also sources of pharmaceutical compounds, aromatic and industrial.

Nowadays, people are bombarded with thousands of toxic substances, pollutants and unhealthy products, the level of sensitivity to the disease is very high and therefore the use of plants may be the best solution.

Recently, the World Health Organization estimated that approximately 80% of people worldwide are interested in herbal medicines, its use for medicinal purposes increasing in the last 30 years.

Medicinal plants and plant products have been used by man since ancient times, without having even the most basic notions about their chemical composition, about the mechanisms of action, without knowing that their effect is due to some constituents they contain , but knowing only that one plant or another is better to treat one or another disease.

Interdisciplinary studies conducted in recent years (botany, analytical chemistry, microbiology, pharmacognosy, pharmacology) allowed completion of a road to knowledge-producing species, pending the development of pharmaceutical forms using the species as a source of active principles.

In Romania and in many countries, plants are constantly being studied because of their therapeutic properties. It summarizes both basic compounds necessary to their survival (in the category of carbohydrates, proteins and lipids), but also a wide range of organic substances that can be extracted because of their significant importance as raw materials with various scientific, technological and commercial applications.

In preparing this paper and the experiments I worked and was guided by people with outstanding professional qualities, which I wish to sincerely thank them.

THE PURPOSE AND THE OBJECTIVES OF THE PERFORMED RESEARCHES

The experimental research conducted in this project consisted of monitoring the effects induced by administration of some extracts from species of *Aralia mandshurica* and *Nigella sativa*, analyzing the radioprotection effect and their immunostimulating on the structure and function of organs of mice *Mus muscullus* in two experimental conditions: under normal conditions, without prior involvement of the stressor and after the stressor intervention (X rays).

Objectives of research:

- the study of effects of administration of ***Aralia mandshurica*** cortex extracts on hematological parameters, immunological, cytogenetic, structural and ultrastructural under normal conditions;
- the study of the effects of administration of ***Aralia mandshurica*** cortex extracts on hematological parameters, immunological, cytogenetic, structural and ultrastructural under stress, X rays;
- the study of the effects of administration of ***Nigella sativa*** seed extracts on hematological parameters, immunological, cytogenetic, structural and ultrastructural under normal conditions;
- the study of the effects of administration of ***Nigella sativa*** seed extracts on hematological parameters, immunological, cytogenetic, structural and ultrastructural under stress, X rays;
- the study of the effects of using ***DDW*** - the animal organism in normal conditions;
- the study of the effects of using ***DDW*** - the animal organism under conditions of stress, X-rays.

THE THEORETICAL PART

1. The topicality of the investigated theme and its degree approach

Lately, a number of natural products obtained from plants have been increasingly researched and investigated from chemical and biological point of view for their effects of stimulating the body's functions. A special category of such extracts obtained from plants is that of immunomodulatory, anti-inflammatory, antioxidant qualities. In the traditional medicine of some countries they are basic concepts in therapeutic techniques and methodologies of some diseases.

Aralia mandshurica (Fam. *Araliaceae*) is used because of its stimulating properties on the central nervous system, cardiovascular and immune, possessing an antistress adaptogen activity of very good quality (Maslov L.N. and Guzarova N.V., 2007).

Nigella sativa (Fam. *Ranunculaceae*) has been used since antiquity by the Mediterranean populations, evidence of its use dating from antiquity to the Middle Ages - in the Old Testament (Isaiah 28), research conducted during the Greco-Arab Medical School (Abou Basha I.L., 1995), which Mohammed himself said that "cure any disease except death" possesses antitumoral, antibacterial, antispasmodic, immune stimulators (Bellakhdar I., 1997).

Some of the results shown in the thesis have been produced under the grant financed by CNCSIS "*Study of the effect and the mechanism of action of some immunostimulators radioprotective of plants origins (**Nigella sp., A. mandshurica**). Role of cytokines in modulating the systemic response* "Grant 2004-2006, no. 33.062/2004.06.2004, theme 16, code 292.

1.1. Presentation of the biologically active substances used

1.1.3. The active principles used

From the whole chemical composition of the two plant species used in this work, only a few active principles which provide a therapeutic interest have been chosen, namely: volatile oil, poliholoside, polyphenols and alkaloids.

*A. **The oils*** are volatile liquid substances, insoluble in water, soluble in organic solvents or fats, with a strong flavor, which evaporate completely and leave no fat spots. They are obtained either by steam distillation or by pressing.

B. Poliholosidele are macromolecular, linear or branched constituents composed from more than 10 ounces of molecules or derivatives (uronic acids). The poliholosides represent a class of active principles for which recent researches, both in vitro and in vivo showed a real immunostimulatory quality.

C. Polyphenols are a group of aromatic chemicals in which structure one or more simple or condensed aromatic nuclei are encountered; they are clear substances with antioxidant, antibacterial and antifungal agents, which are widespread in the plant kingdom. Polyphenols are natural antioxidants of great interest in the last years because they exhibit a radio protective action, together with other components (flavones, anthocyanins) (Gatea F. et al., 2006).

Flavanols (flavonoids) are natural phenolic compounds, which are predominant in higher plants; they are found in flowers, fruits, stems, roots, tree bark, being located in the vacuolar juice and in cromoplaste, too. In addition to their famous anti-inflammatory and antioxidant properties, the flavonoids are also capable of activating genes (Baker M.E., 1998), mutations of the DNA and repairs (Ferguson LR.., 2001).

D. Alkaloids are heterocyclic nitrogen organic substances of vegetable origin, with basic character, resulting from the secondary metabolism of plants. Their molecules contain carbon, hydrogen, nitrogen and, often, oxygen. These substances with nitrogen atom that forms part of the heterocycle often have very different chemical structure. It is a very heterogeneous group from the chemical point of view, and even if it is toxic in small doses it is used in the treatment of diseases, having a calming, anesthetic and healing action.

1.3. The phytotherapeutic importance of *Aralia mandshurica* and *Nigella sativa* species

1.3.1. *Aralia mandshurica Rupr. et Maxim*

A. Botanical data

The systematic plant *Aralia mandshurica* Rupr. et Maxim (Sirbu A., 1999): Phylum *Magnoliophyta,* Class *Magnolias* (*Dicotiledonatae*), *Araliales* Order, *Araliaceae* Family, Genus *Aralia*, *Aralia mandshurica* Species.

Fam. *Araliaceae* includes 55 genera, which include about 800 species (Pîrvu C., 1997), dicotyledonous plant trees and shrubs, rarely herbaceous, widespread in tropical regions, subtropical and temperate in the least (Wen J., 2001). *Aralia* genus name comes from the Indian name of the plant; mandshurica, the historical region of northeastern China - mandshurica (Manchurian, Mandschuria), called San Dong Shen - indicating places of spontaneous growth of the plant.

Figure 1. *Aralia mandshurica.*

Figure 2. *Aralia mandshurica.*

B. Phytochemical data

The roots, bark, leaves and fruits containing *A. mandshurica* saponosides triterpenoid, alkaloids, flavonoids, coumarins and tanning substances. Plant root contains triterpenoid saponosides - aralozide (4.7%), volatile oil (0.12%), flavonosids, resins and alkaloid aralin (Zagnat M., 2004, Lutomski J., 1986), separating aralozidele A, B , C

1.3.2. *Nigella sativa Linn.*

A. Botanical data

Nigella sativa L. taxonomic classification of the species (Pârvu C., 1997): Phylum *Magnoliophyta*, Class *Magnoliatae* (*Dicotiledonatae*) Order *Ranunculale*, Family *Ranunculaceae*, Genus *Nigella*, *Nigella sativa* species.

Nigella genus name comes from the Latin *"niger"* = black color due to seed's color; popularly, is called "negruşcă" or Negril.

The first references to this plant were found in the Old Testament book of Isaiah 28: 25, 27 (Bellakhdar I., 1997). Hippocrates, known as the father of medicine, said that *Nigella sativa* is a remedy for liver disease and metabolic disorders. Researches on this plant were made during the Greco-Arab Medical School (represented by Ibn Sina and Galenus) and have been continued until today. In the book "The Canon of Medicine", Ibn Sina (980-1037) talks about *Nigella seeds* as " body energy incentives those which help to regain the balance after fatigue and disappointment" (Abou-Basha I.L. et al., 1995).

Figure 3. *Nigella sativa.*

Figure 4. *Nigella sativa.*

B. Phytochemical data

Nigella sativa seeds are a good source of oil and proteins; the chemical analysis of seeds showed a composition of 20.85% protein, 38.20% fat, 4.64% moisture, ash 4.37%, 31.94% raw fiber and 31,94% carbohydrates (Al-Jassir M.S., 1992), the predominant elements being potassium, phosphorus, sodium and iron, while zinc, calcium, magnesium, manganese and copper were found at lower levels. The amino acid content is about 30% of the total protein content, while about 84% of the fatty acids are unsaturated fatty acids, linoleic and oleic acids mainly (Al-Jassir M.S., 1992, Nergiz C. and Otles S., 1993). The oily extract of seeds also contains significant amounts of sterols, β-sitosterol is the dominant (69%), while campesterol and stigmasterol represent 12% and 19% of the total sterols. It also contains polyphenols and tocopherols (Nergiz C. and Otles S., 1993).

THE EXPERIMENTAL PART

2. THE BIOLOGICAL MATERIAL AND THE METHODS OF INVESTIGATION

2.1. The biological material

2.1.1. *Mus muscullus*

In this case there were used young animals, healthy ***Mus muscullus*** with about 25 to 30 g/sample. Animals used for experiments were kept in laboratory conditions, being provided appropriate food and water *"ad libitum"*.

Animals were injected intraperitoneally every two days with 0.5 ml aqueous solution of plant extract, diluted in distilled or depleted in deuterium (DDW) water.

Before X-irradiation there were administered three injections and after X-irradiation, there were two injections. The slaughter of animals was performed the day after the last injection and there was collected the biological material for haematological, immunological, structural, ultrastructural and cytogenetic investigations.

Experiments were performed on 16 types of animals, as follows:

1. The non- irradiated control version:
- Control = M;
- Distilled water (AD) = M-AD;
- Deuterium depleted water (DDW) =M-DDW;

2. The irradiated control version:
- Control = M-X;
- Distilled water (AD) = M-AD-X;
- Deuterium depleted water (DDW) = M-DDW-X ;

3. The version treated with volatile oil from Aralia mandshurica, non-irradiated:
- Distilled water (AD) = UA-AD;
- Deuterium depleted water (DDW)= UA- DDW ;

4. The version treated with volatile oil from Aralia mandshurica, irradiated:
- Distilled water (AD) = UA-AD-X;
- Deuterium depleted water (DDW) = UA-DDW-X ;

5. The version treated with poliholosides from Aralia mandshurica, non-irradiated:

- Distilled water (AD) = PA-AD;

- Deuterium depleted water (DDW)= PA - DDW ;

6. The version treated with poliholosides from Aralia mandshurica, irradiated:

- Distilled water (AD) = PA-AD-X;

- Deuterium depleted water (DDW) =PA-DDW-X ;

7. The version treated with polyphenols and flavones from Aralia mandshurica, non-irradiated:

- Distilled water (AD) = PFA-AD;

- Deuterium depleted water (DDW)= PFA- DDW ;

8. The version treated with polyphenols and flavones from Aralia mandshurica, irradiated:

- Distilled water (AD) = PFA-AD-X;

- Deuterium depleted water (DDW) PFA –DDW-X;

9. The version treated with volatile oil from Nigella sativa, non-irradiated:

- Distilled water (AD) = UN-AD;

- Deuterium depleted water (DDW)=UN- DDW;

10. The version treated with volatile oil from Nigella sativa, irradiated:

- Distilled water (AD) = UN-AD-X;

- Deuterium depleted water (DDW)=UN-DDW-X;

11. The version treated with poliholosides from Nigella sativa, non-irradiated:

- Distilled water (AD) = PN-AD;

- Deuterium depleted water (DDW)=PN- DDW;

12. The version treated with poliholosides from Nigella sativa, irradiated:

- Distilled water (AD) = PN-AD-X;

- Deuterium depleted water (DDW)=PN- DDW-X;

13. The version treated with acid extract of alkaloids from N. sativa, non-irradiated:

- Distilled water (AD) = AAN-AD;

- Deuterium depleted water (DDW) =AAN-DDW;

14. The version treated with basic extract of alkaloids from N. sativa, non-irradiated:

- Distilled water (AD) = ABN-AD;

- Deuterium depleted water (DDW) ABN-DDW;

15. The version treated with acid extract of alkaloids from N. sativa, irradiated:

- Distilled water (AD) = AAN-AD-X;

- Deuterium depleted water (DDW) =AAN-DDW-X;

16. The version treated with basic extract of alkaloids from N. sativa, irradiated:

- Distilled water (AD) = ABN-AD-X;

- Deuterium depleted water (DDW) = ABN-DDW-X;

2.1.2. . Extracts from *Aralia mandshurica* and *Nigella sativa* plants

For conducting experiments I used the following extracts:

- ***Aralia mandshurica*** - volatile oil;
  - poliholosides;
  - polyphenols and flavones.
- ***Nigella sativa*** - volatile oil;
  - poliholosides;
  - alkaloids.

Each extract was diluted in distilled water and in water depleted in deuterium, then the experimental animals were injected intraperitoneally.

2.1.3. Deuterium depleted water - *DDW*

Research on the biological effects of low-deuterium water showed spectacular positive results in the sense that a decrease of deuterium in organisms (plants, animals and / or human) improves the metabolic performance (Nedelcu I. et al., 2002). This water has the appearance of colorless liquid with the taste of plain water with low ions content and low in hydrogen isotopes - deuterium - that is super light water, hypotonic hypodeuteric.

2.1.4. X-radiation stress factor

For these experiments, the stress factor was the X-rays released by a RAP-150/300 device (eg. USSR), in single dose, acute at parameters of 250 kV, 5mA, 52.8 R / min dose rate, 528 R total acute dose (5.28 Gy), df = 500 mm, 1 mm Al filter. Note that X irradiation or gamma irradiation are frequently used as the main factor of stress to which it is analyzed the immuno-stimulating or protective activity of different stressors.

2.2. Methods of investigation:

2.2.1. Blood investigations - The blood count analysis gives us information not only on the way the figurative blood cells are formed and their way of functioning but also, the degree of impairment of the hematogenous bone marrow.

2.2.2. Immunological investigations - - We can notice the involvement of immunoglobulins in neutralizing the toxins and other exogenous factors (IgG), the involvement in immune defense (IgM) and mucosal involvement, being the main effectors of the secretor immune system (IgA).

2.2.3. Histological investigation - The structural analysis of cells and tissues allows the deciphering of structural and operational status of the investigated organs.

2.2.4. Ultrastructural investigation - Study of the ultra fine sections of cells and tissues participating in understanding the ultrastructural organization of living matter and the intimate processes taking place in the material substrate of life.

2.2.5. Cytogenetic investigations - Performs the diagnosis of chromosomal diseases, the study of congenital malformations (from a genetically point of view) and the study of the genetic mutagens effect with the most serious consequences on the human species.

3. RESEARCHES MADE OF PLANT EXTRACTS TAKEN FROM THE CORTEX OF THE *ARALIA MANDSHURICA* PLANT

Investigations consisted of analysis of three extracts (volatile oil, poliholosides, polyphenols and flavones) from the cortex of *Aralia mandshurica* plant administered intraperitoneally to mice.

The substances used in research were diluted in 1% of the raw extract obtained being produced in distilled water dilutions, respectively depleted in deuterium water (DDW), then administered intraperitoneally.

For the substances used, testing was done on six types of animals in two experimental conditions: under normal conditions without prior involvement of the stress factor and after the intervention of the stressor (radiation-X). During the administration of the tested substances there were not found significant changes in animals' behavior tested with the investigational substances to those of controls' versions.

3.1. The effect of the volatile oil extract

a) Blood Investigations

In tables 1 and 2 are the values recorded for the components of blood count, to *M. musculus* for the variants treated with volatile oil extract diluted in distilled water, respectively water depleted in deuterium.

In the case of hematocrit - for the M-X the value decreases and the variants treated with distilled water, respectively DDW, there were approximately equal values for both the irradiated and non- irradiated variants. For the irradiated variants treated with volatile oil of the roots of *A. mandshurica* there is a slight decrease in red blood cells for dilution with distilled water and a slight increase in red blood cells for dilution with DDW. This increase was also recorded for the untreated control version; this is because of the bone marrow damage due to irradiation. Compared with the control non - irradiated versions, the white blood cell counts are significantly reduced in the irradiated versions. The megakaryocytes, precursors of platelets have a role in the nonspecific immunity; compared to increase of platelets for the M-X version there is a slight decrease in platelets in irradiated versions compared to the non-irradiated versions, in the case of

dilution with distilled water and a significant decrease of platelets in irradiated versions compared to the non-irradiated versions, for dilution with DDW.

It can be observed the increase in number of neutrophils in all irradiated versions compared to non-irradiated versions, a less relevant increase for the versions treated with volatile oil. The number of eosinophils is slightly increased for the M-AD-X version to the non- irradiated version; for the M - DDW-X version there are recorded the same values as for the non-irradiated versions, and if treated with volatile oil there are approximately the same values in irradiated versions compared to non- irradiated versions. The basophils are absent in all cases, normal data for experimental mice. The number of lymphocytes decreases for all the irradiated versions and a smaller decrease is registered for the UA-DDW-X compared to all the other versions (fig.5); in the case of the irradiated versions treated with volatile oil there is an insignificant decrease in the number of monocytes, especially for the UA-DDW-X version. So, the volatile oil diluted in DDW is more effective than diluted in distilled water (Corneanu C., 2006).

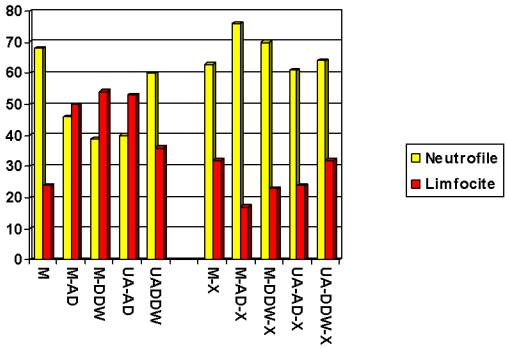

Figure 5. Chart with comparison between the control groups and those treated with volatile oil from *Aralia mandshurica* irradiated and non-irradiated for determination of neutrophils and lymphocytes.

Table1. Analysis of complete blood cell counts on *Mus muscullus*, non-irradiated version.

Non-irradiated version	Hematocrit %	Red blood cells mil/mm³	Leukocytes nr/mm³	Thrombocytes nr/mm³	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %
M	12.5	4500000	8500	400000	68	3	0	24	5
M-AD	12.8	5000000	6000	700000	46	1	0	50	3
M-DDW	12.0	3900000	7000	850000	39	2	0	54	5
UA-AD	11.8	4000000	5000	400000	40	0	0	53	7
UA-DDW	13.0	4300000	5500	700000	60	0	0	36	4
Refference values									
C.Pârvu1982	38.7-47.1	7-9 mil	6300-15500	220000-700000	10-35	0.5-2	0	27-88	1
I.Marcus,2004	36-49	7-125.5 mil	6000-15000	160000-410000	10-40	0-4	0-0.3	55-95	0.1-3

Table2. Analysis of complete blood cell counts on *Mus musculus*, irradiated version.

Irradiated version	Hematocrit %	Red blood cells mil/mm³	Leukocytes nr/mm³	Thrombocytes nr/mm³	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %
M-X	12	5000000	8000	450000	63	2	0	32	3
M-AD-X	12,8	4300000	2000	320000	76	3	0	17	4
M-DDW-X	12.9	4800000	1500	400000	70	2	0	23	5
UA-AD-X	11.6	2900000	4500	350000	61	0	0	24	5
UA-DDW-X	13.0	5000000	3500	500000	64	1	0	32	3

b) Immunological Investigations

In the following Tables 3 and 4 there are presented the values recorded for immunoglobulins, on M. muscullus for the versions treated with volatile oil extract diluted in distilled water and in water depleted in deuterium.

Table 3. Analysis of immunoglobulin for non - irradiated versions on *Mus muscullus.*

Non-irradiated version	IgG (mg/ml)	IgM (mg/ml)	IgA (mg/ml)	Hemolysis
M	4.2	0.8	2.5	
M-AD	12.0	2.0	3.0	
M-DDW	10.0	1.7	2.6	hemolysis
UA-AD	1.8	1.9	2	
UA-DDW	4.2	0.8	2.5	Intense hemolysis
Refference values				
Anonymus	0.1-1	1-10	1-3	

Table 4. Analysis of immunoglobulin for irradiated versions on *Mus muscullus.*

Irradiated version	IgG (mg/ml)	IgM (mg/ml)	IgA (mg/ml)	Hemolysis
M-X	1.4	0.4	2	
M-AD-X	11.0	2.4	3.8	Light hemolysis
M-DDW-X	7.0	3.0	3.5	
UA-AD-X	4	2.3	3.2	
UA-DDW-X	1.4	0.4	2.0	Intense hemolysis

IgG immunoglobulins are involved in neutralizing toxins and other exogenous factors. For the control versions treated with distilled water and with DDW it is observed an increase in IgG values for both the irradiated and non- irradiated versions compared to the non-irradiated and irradiated control version; for the irradiated animals the IgG decreased value is more pronounced on M- DDW-X version. For the UA-AD-X version there is an increase of IgG value, but for the UA-DDW-X version there is recorded a lower IgG value.

3.2. The effect of the poliholoside extract

c) Cytogenetic Investigations

As a result of X irradiation, the percentage of PCD was 12% for M-AD-X version and for the version M- DDW-X there was no value. In the case of X irradiation, the main cause for the gaps was the de - condesation processes of the chromatin fiber.

Premature centromeric dividing process was accompanied by a decondensation of the centromeric region, which may be a predisposition to this phenomenon.

For animals on which aqueous solutions of poliholosides extract from *Aralia mandshurica* were administered intraperitoneally before and after irradiation of X- rays, the percentage of abnormal metaphases and acentric fragments present to 100 metaphases showed low values, highlighting the protective effect of this bioactive substance, lower values were obtained when administering the DDW poliholosides extract, from which the protective effect registered for this combination. The PCD process may be partial, when affecting several chromosomes (1-3) of the metaphase plate or may be total, when it affects all the chromosomes of the metaphase plate (Fig. 6).

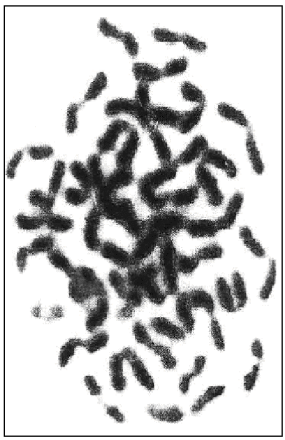

Figure 6. Metaphase with total premature centromeric dividing.

Polyploid metaphases were found, and, also, hypoploid metaphases with centromeric and extended chromosoms fusion or metaphases with most chromosomes with premature centromeric division and two acentric fragment (A) (Fig. 7).

Figure 7. Metaphase with a majority of chromosomes with premature centrometric division and two acentric fragments (A).

3.3. The effect of the extract of polyphenols and flavanols

c) Histological Investigation

Liver: PFA DDW: The liver presents granulo-vacuolar degeneration, vascular hyperemia (Fig. 8).

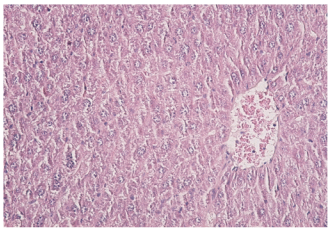

Figure 8. Mouse liver with granulo-vacuolar degeneration (oc. 10 x ob. 20).

PFA-DDW-X: The liver shows a light hypertrophy of hepatocyte nuclei with granulo-vacuolar degeneration areas (focal granular degeneration, fig. 9); also it highlights a dilation of the centrilobular veins; the hepatocyte nucleus keeps its nuclear membrane remains integrated, with a heterogeneous condensation of chromatin, some with prominent nucleoli.

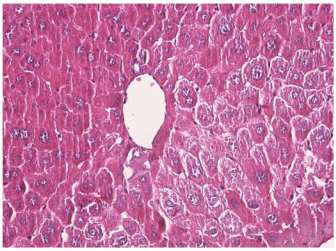

Figure 9. Mouse liver with granulo-vacuolar degeneration
(oc. 10 x ob. 40).

Spleen: PFA-DDW: The spleen presents the hypertrophy white pulp, rare cells of nuclear atypia, many follicles with germ centers (Fig. 10).

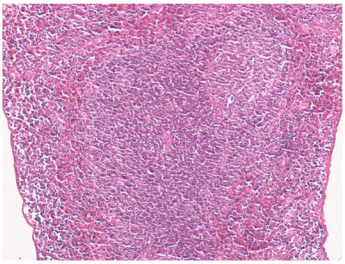

Figure 10. Mouse spleen with numerous follicles with germ centers
(10 x ob oc.. 10).

PFA-DDW-X: The spleen shows the marked atrophy of white pulp highlighted by the presence of small and rare splenic follicles (Fig. 11). It can be observed the red pulp hypertrophy marked by intense dilation of the sinusoid capillaries and the presence of numerous megakariocytes. At the interstitial level, the fibro-connective septa appear uneven, some hypertrophied, others with normal aspect.

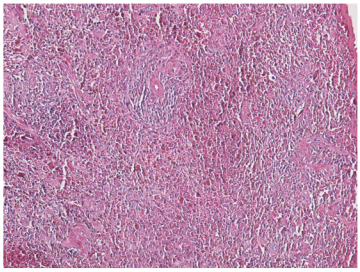

Figure11. Mouse spleen with white pulp atrophy
(oc. 10 x ob. 20).

d) The ultrastructural investigation of the liver

The M-DDW version: The hepatocytes respond to the presence of DDW, which, as an exogenous substance affects the cellular metabolism (Fig. 12) being observed some lipid droplets. The nucleus presents a wavy nuclear shell; it shows a proliferation of smooth endoplasmic reticulum with a role in detoxification and small drops of glycogen. Mitochondria present the central lysed matrix and few crystals.

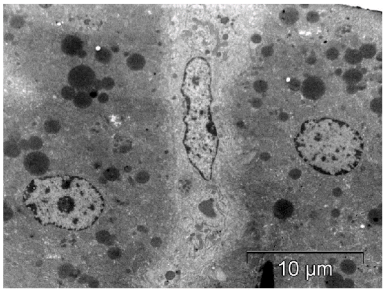

Figure 12. Hepatocytes, sinusoid capillary and the Ito cell: ultrastructural appearance.

PFA-DDW Version: The hepatocytes have normal structure (Fig. 13), with numerous lipid droplets, the glycogen is practically absent; the nucleus looks normal and the cellular organelles have normal structure. Ito cells present lipid droplets in very small number, almost absent; Kupffer cells have lisosoms, haematin and, in general, the cellular material is destroyed and the capillaries without processes of stasis.

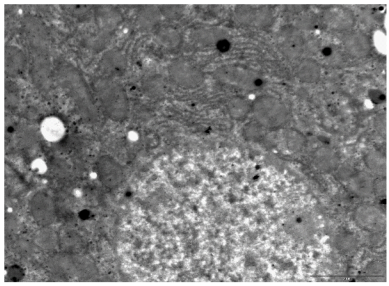

Figure 13. Hepatocytes with normal structure.

M-DDW-X Version: The lesions induced by X-rays are limited, the mitochondria has a normal aspect, and lipid droplets are observed in small numbers, because DDW acts as a trap for free radicals. The hepatocytes have lisosomi and phagocytosed debris (Fig. 14); their presence confirms the ability of polyphenols and flavones extract to annihilate the alterations produced by radiation X and to maintain normal metabolic activity in hepatocytes.

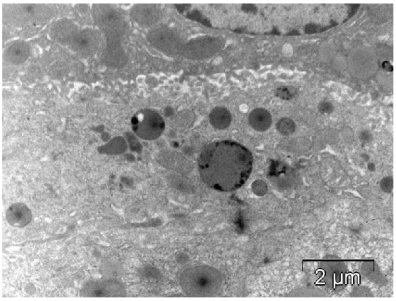

Figure14. Hepatocytes with lisosoms and phagocytosed debris.

PFA-DDW-X Version: The hepatocytes (Fig. 15) present a structure similar to the control version. In the Kupffer cells there are present primary and secondary lisosoms with cellular residues (phagocytes with destroyed cellular debris). Some sinusoid capillaries are slightly congested sinusoids; prominent microvilli are highlighted in Disse space. The polyphenols and flavones extract offer total protection against X-rays.

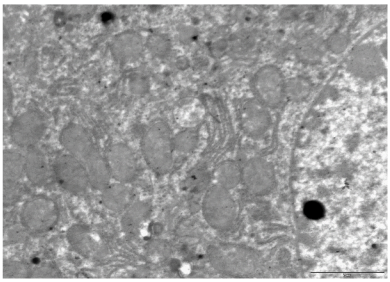

Figure 15. Normal hepatocyte, the endoplasmic reticulum and mitochondria.

4. RESEARCHES MADE ON EXTRACTS TAKEN FROM SEEDS OF *NIGELLA SATIVA*

4.1. The effect of volatile oil extract

a) Blood Investigations

In Tables 5 and 6 are shown the recorded values for the blood cell count components , at *Mus muscullus* for the versions treated with volatile oil extract of *Nigella sativa* seeds, diluted in distilled water, respectively, in water depleted in deuterium.

For the versions treated with volatile oil and irradiated, the hematocrit registers an insignificant increase compared to the non-irradiated one, for both populations of plants; for the UN-AD-X version the number of red blood cells increases and for the A-DDW-X version the number of red blood cells show a decrease, for plants from Morocco's population and a decrease in red blood cells in the two versions for Craiova's population. The leukocyte count significantly decreases for UN-AD-X version (in both populations of plants) and less for the UN-DDW-X version. In the case of thrombocytes in irradiated versions there is a decrease in thrombocytes count, a most significant decrease for Craiova's population; the number of neutrophils greatly increases in the irradiated versions, the number of lymphocytes greatly decreases and the number of monocytes slightly decreases for Morocco's population (Fig. 83) and an increase in the number of monocytes in a UN-DDW-X variant, the population of Craiova.

The volatile oil can be used as a natural radiation protection agent against oxidative and immunosuppressive effects of ionizing radiation (Assayed M.E., 2010).

The results obtained from different experimental systems suggest the radio protective ability of Nigella sativa ethanolic extract involving oxidative damage prevention induced by radiation (Rastogi L., 2010).

Table 5. Analysis of complete blood cell counts in *Mus musculus*, non- irradiated version.

Non – irradiated version	Hematocrit %	Red blood cells mil/mm³	White blood cells nr/mm³	Thrombocytes Nr/mm³	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %
M	12.5	4500000	8500	400000	68	3	0	24	5
M-AD	12.8	5000000	6000	700000	46	1	0	50	3
M-DDW	12.0	3900000	7000	850000	39	2	0	54	5
Population of Morocco									
UN-AD	12.0	4500000	7000	900000	7	0	0	90	3
UN-DDW	12.3	5100000	6000	650000	19	0	0	79	2
Population of Craiova									
UN-AD	11.9	4900000	6500	750000	50	1	0	44	5
UN-DDW	12.8	5000000	6000	780000	56	0	0	40	4

Table 6. Analysis of complete blood cell counts in *Mus musculus*, irradiated version.

Irradiated version	Hematocrit %	Red blood cells mil/mm³	White blood cells nr/mm³	Thrombocytes nr/mm³	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %
M-X	12	5000000	8000	450000	63	2	0	32	3
M-AD -X	12.8	4300000	2000	320000	76	3	0	17	4
M-DDW-X	12.9	4800000	1500	400000	70	2	0	23	5
Population of Morocco									
UN-AD-X	13.0	5000000	2000	800000	70	1	0	27	2
UN-DDW-X	12.8	4300000	4000	500000	55	0	0	44	1
Population of Craiova									
UN-AD-X	13.2	4800000	1600	600000	58	1	0	39	3
UN-DDW-X	12.6	4200000	2000	280000	56	2	0	34	6
Refferece values									
C.Pârvu, 1982	38.7-47.1	7-9 mil	6300	220000-700000	10-35	0,5-2	0	27-88	1
I.Marcus, 2004	36-49	7-12.5 mil	6000-15000	160000-410000	10-40	0-4	0-0.3	55-95	0.1-3.5

c) Histological Investigation

UN-AD: The liver presents a partially preserved architecture, with vascular swellings, hyperaemia (Fig. 16) and nuclear changes more pronounced than in other substances.

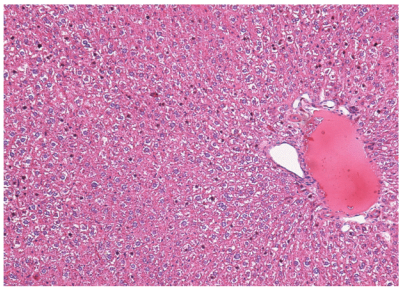

Figure 16. Mouse liver with vascular swelling and hyperaemia (oc. 10 x ob. 10).

UN-DDW: The liver shows a scratchy appearance, with dilated portal spaces, alternating with areas of narrowing portal spaces and nuclear preapoptotic changes (Fig. 17), areas with proliferation of interstitial vessels, with forked looking, dilated vessels and forked areas, like "antlers".

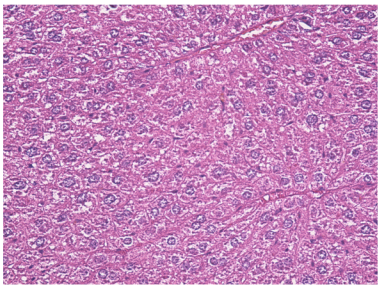

Figure 17. Mouse liver with nuclear preapoptotic changes (oc. 10 x ob. 20).

UN-AD-X: The liver presents severe nuclear changes at the level of hepatocytes , with vascular hyperemia (Fig. 18).

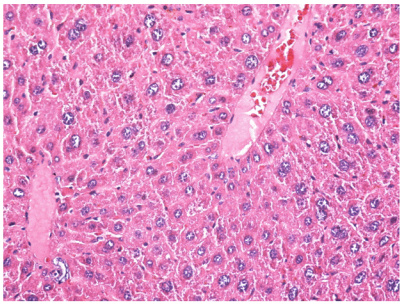

Figure 18.Mouse liver with vascular hyperaemia

(oc. 10 x ob. 20).

UN-DDW-X: The liver presents not only moderate and severe nuclear changes, but also areas with dilatation of the portal spaces and vascular hyperaemia.(Fig. 19).

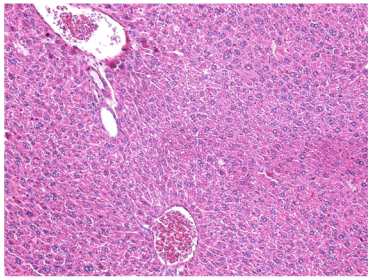

Figure 19. Mouse liver with areas of dilatation of the portal spaces

(oc. 10 x ob. 10).

Spleen: UN-AD: At the level of spleen it is observed a hypertrophy of the fibrous connective septa; a hypertrophy of the white pulp, a hyperplasia of the red pulp with the proliferation of megakaryocytes. (Fig. 20).

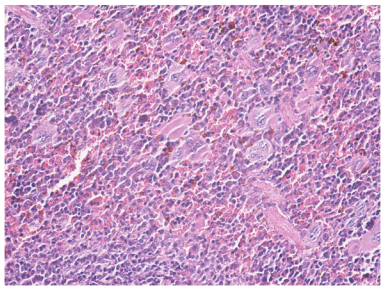

Figure 20. Mouse spleen with red pulp hyperplasia
(oc. 10 x ob. 20).

UN-DDW: The spleen shows hypertrophy and hyperplasia of the white pulp (Fig. 21), interstitial fibrosis, the red pulp is slightly dilated and the presence of rare megakaryocytes.

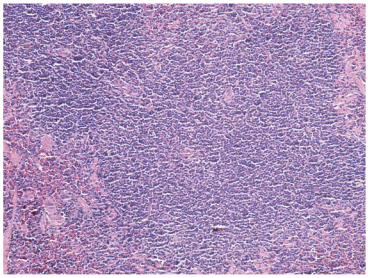

Figure 21. Mouse spleen hypertrophy and hyperplasia of the white pulp
(oc. 10 x ob. 10).

UN-AD-X: The spleen shows intense interstitial fibrosis, with red pulp expansion and changes in the inhomogeneous white pulp; normal-looking areas alternating with areas of white

pulp hypertrophy, hyperplasia of reticular cells (Fig. 22), areas with dilated capillaries of the red pulp.

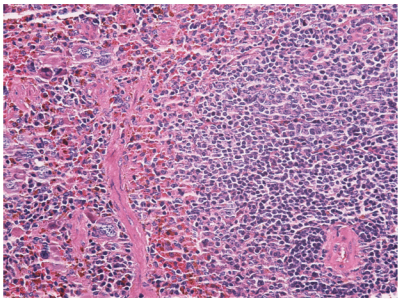

Figure 22. Mouse spleen with hyperplasia of reticular cells
(oc. 10 x ob. 20).

UN-DDW-X: The spleen shows dilatation of the sinusoids spaces at the level of the red pulp; the white pulp looks normal, but has interstitial fibrosis (Fig. 23).

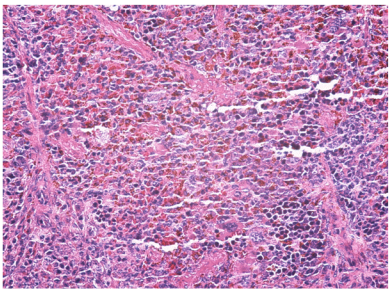

Figure 23. Mouse spleen with interstitial fibrosis
(oc. 10 x ob. 20).

4.2. **The effect of the poliholosides extract**

b) Immunological Investigations

In the following tables 7 and 8, there are shown the values recorded for immunoglobulins, at *Mus musculus*, in the versions treated with the poliholosides extract of *Nigella sativa* seeds, diluted in distilled water, respectively in water depleted in deuterium.

Table 7. Analysis of immunoglobulins in non- irradiated variants of *Mus musc*ulus.

Non-irradiated version	IgG (mg/ml)	IgM (mg/ml)	IgA (mg/ml)	Haemolysis
M	4.2	0.8	2.5	
M-AD	12.0	2.0	3.0	-
M-DDW	10.0	1.7	2.6	haemolysis
Poliholoside from seeds of *Nigella sativa*, population of Morocco				
PN-AD	13	2.4	2.8	haemolysis
PN-DDW	13.1	1.2	2.5	haemolysis
Anonymus	0.1-1	1-10	1-3	

Table 8. Analysis of immunoglobulins in irradiated variants of *Mus musculus.*

Irradiated version	IgG (mg/ml)	IgM (mg/ml)	IgA (mg/ml)	Haemolysis
M-X	1.4	0.4	2	
M-AD-X	11.0	1.4	3.8	light haemolysis
M-DDW-X	7.0	3.0	3.5	-
Poliholoside from seeds of *Nigella sativa*, population of Morocco				
PN-AD-X	12	1.7	3	
PN-DDW-X	8	10	2	

IgG Immunoglobulin – in the control versions treated with distilled water, respectively DDW is observed an increase in IgG values for non- irradiated and irradiated versions; for the irradiated animals the decreasing of the IgG value is more pronounced in M-DDW-X version.

IgM Immunoglobulin - fits within the reference range, a slight increase is registered for M-DDW-X version.

IgA Immunoglobulin - shows a slight increase for irradiated control options.

For versions of Nigella sativa treated with poliholosides, the IgC values register a slight decrease in for PN-AD-X version and a significant decrease in the PN- DDW-X version. There is a slight decrease in IgM values for PN-AD-X version and a significant increase in PN-DDW-X version.

There is an insignificant increase in PN-AD-X version and a slight decrease for PN-DDW-X version (Fig. 24).

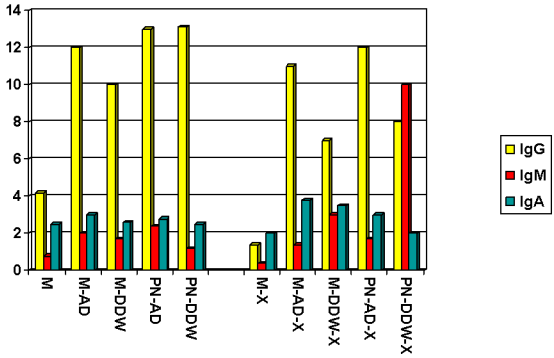

Figure 24. Graphic comparison between the control groups and the one treated with poliholosides of *Nigella sativa* irradiated and non- irradiated for immunoglobulin analysis.

4.3. The effect of alkaloid extract

c) Ultrastructural investigations

M-AD version: The hepatic lobe shows a centrilobular vein covered with epithelium;the sinusoids capillaries are arranged around the centrilobular vein. The hepatocytes are in polygonal shape and they are arranged in rows; each hepatocyte shows two oval-ball nuclei. In the cytoplasm, numerous mitochondria are present, with normal structure with dense matrix. Among mitochondrias there are dispersed the rough endoplasmic reticulum which consists of narrow sections, usually arranged around mitochondrias (Fig. 25).

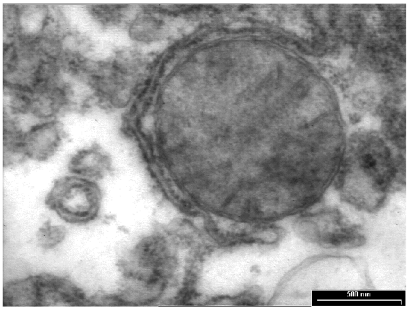

Figure 25. Mitochondria with slightly dilated crystals.

M-DDW Version: The hepatocytes show some slight changes that do not significantly affect cell metabolism. The rough endoplasmic reticulum has a high activity, compared to the control version, being arranged in parallel ducts, ready for protein synthesis, but it has some slight dilation. Also, smooth endoplasmic reticulum is hypertrophied compared to the control version treated with distilled water, being involved in the detoxification process (Fig. 26).

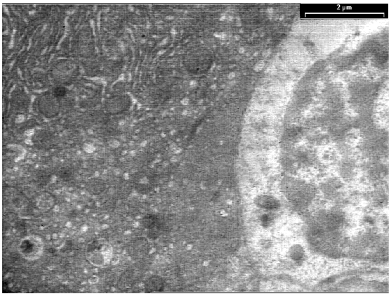

Figure 26. The hypertrophied smooth endoplasmic reticulum.

AN-AD Version: The cell ultra structure is not affected when used alkaloid extract dissolved in distilled water. The core has a normal structure, with clusters (blocks) of

heterochromatin dispersed inside or nearby its nuclear contents. Also, the mitochondrias have a normal structure, with a compact matrix, long and numerous inward crystals. The rough endoplasmic reticulum and the smooth endoplasmic reticulum are well represented (Fig. 27).

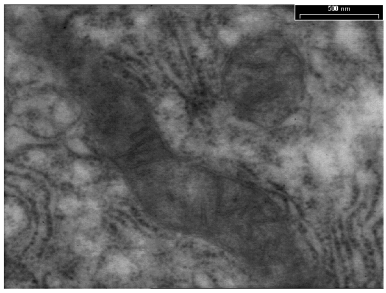

Figure 27. Mitochondrias and the rough endoplasmic reticulum

AN-DDW Version: The extract alkaloids of Nigella sativa seeds diluted in DDW does not affect the normal structure of hepatocytes. The core has a polymorphism in terms of their shape and cell cycle stage. In some cells, chromosomes are well structured, and the cells are in an intense metabolic activity (Fig. 28).

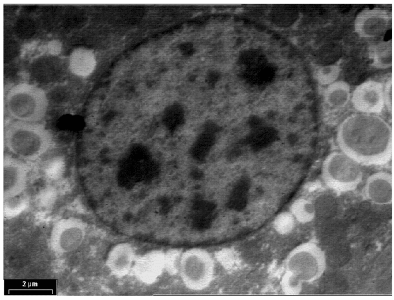

Figure 28. Core with structural chromosomes

M-AD-X Version: Under the action of irradiation, there were induced some changes: hepatocytes nuclei have an irregular contour; some nuclei are hypertrophied (Fig. 29). Also, vacuoles and other amorphous components are hypertrophied. The smooth endoplasmic reticulum is proliferated in response to the destructive action of X-rays. The rough endoplasmic reticulum shows dilated cisterns and small ribosomes which reduce the metabolic activity and the associated protein synthesis.

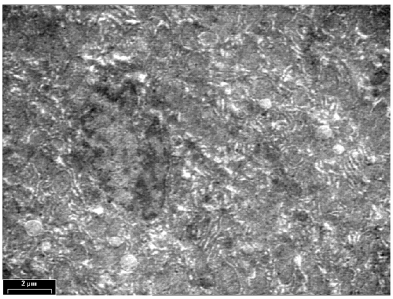

Figure 29. Pyknotic and hyperchrome nuclei; endoplasmic reticulum with dilated cisterns.

M-DDW-X Version: In this version, the rough endoplasmic reticulum is well represented in the cell, compared with the irradiated animals. Also, for the combined action of DDW and stress factor (X-ray), the lipid droplets are in a lower cell number compared with the non-irradiated or irradiated control version. The nucleus has irregular shape and the chromatin is tenuous. The nucleoli are hypertrophied and the vacuolar and amorphous components are quantitatively improved or have an altered structure. On the vascular pole of hepatocytes, the plasmalemma of some cells is dense and many compounds migrate to sinusoids (Fig. 30).

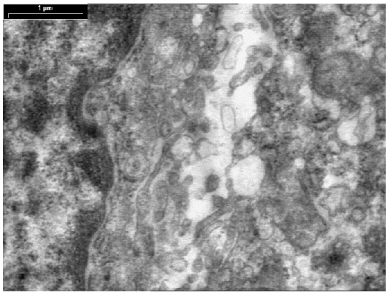

Figure 30. Prominents of hepatocytes in the Diss space

AN-AD-X Version: The presence of alkaloids extract diluted in distilled water has a radiation protection effect in comparison with the ultra structural characteristics recorded on the presence of alkaloids diluted in DDW. The nucleus has a normal structure with heterochromatin placed in or near the nuclear membrane. The nucleoli also have an intense metabolic activity. Some mitochondria have a normal structure (Fig. 31) while others have an altered structure. In this case, their matrix presents some rarefied regions with crystals there are a few and short.

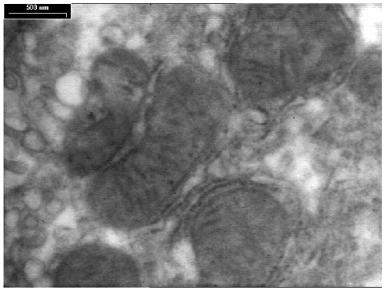

Figura 31. Mithocondrias.

AN-DDW-X Version: the ultra structural changes induced by X-rays in the presence of DDW were important as compared with the changes under the action of a single X-ray. In some cells, the nucleus has a normal shape with heterochromatin placed in blocks (inside the nucleus) and on the inner part of the nuclear membrane (fig. 32).

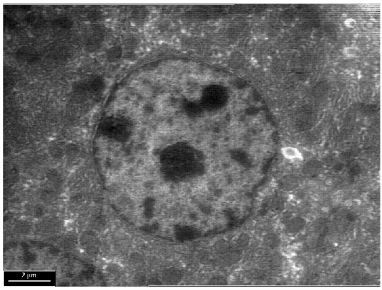

Figure 32. The layout of the heterochromatin in dense blocks within the nucleus.

a) Cytogenetic investigations

In Tables 9 and 10 there are the results of cytogenetic analysis on *M. muscullus*, for versions treated with extract of *Nigella sativa* seeds alkaloids, diluted in distilled water, respectively water depleted in deuterium.

Table 9. The characteristics of metaphases for non - irradiated version of Mus musculus.

Non-irradiated versio	Normal metaphases %	Gaps %	Polyploid metaphases %	PCD %	Metaphases with fragments	Fragments/ 100 metaphases
M-AD	92	-	-	-	-	-
M-DDW	94	-	-	6	-	-
AAN-AD	94	-	-	6	-	-
AAN-DDW	94	-	-	6	-	-
ABN-AD	90	4	-	6	-	-
ABN-DDW	94	-	-	6	-	-

Table 10. The characteristics of metaphases for irradiated variants of *Mus musculus.*

Irradiated version	Normal metaphases %	Gaps %	Polyploid metaphases %	PCD %	Metaphases with fragments	Fragments/ 100 metaphases
M-AD-X	65	-	12	12	11	16.7
M-DDW-X	85	15	-	-	-	-
AAN-AD-X	80	6	4	10	-	6
AAN-DDW-X	86	-	1	8	2	3
ABN-AD-X	79	5	5	8	3	4
ABN-DDW-X	86	-	4	8	2	3

The main cause of the gaps was the processes of decondensation of the chromatin fiber. For X irradiation, the chromosomes decondensation was also found (ABN-AD-X version) or their depolymerisation (AAN-DDW-X version) and cases of hipoploidy.

For animals on which aqueous solutions of total alkaloid extract of *Nigella sativa* seeds was administered intraperitoneally before and after X irradiation, the percentage of abnormal metaphases and acentric fragments per 100 metaphases showed lower values, highlighting the protective effect of the two types of bioactive substances. These values were significantly lower when given the two types of alkaloids in DDW and so it comes the registered protective effect of this combination.

5. DISCUSSION ON THE IMMUNE RESPONSE

Generally speaking, the harmful effects of X-rays on bone marrow are observed by decreasing the number of red blood cells, leucocytes, thrombocytes, and the percentage of lymphocytes and by increasing the percentage of monocytes; for the performed tests there is a greater decrease in the percentage of lymphocytes in irradiated versions, especially for those not treated with bioactive substances.

For our researches it is difficult to sustain which are the acting mechanisms of the plant extracts. However, based on bibliographical data we can assume that active principles are involved, which protects against the action of free radicals and thus prevent the harmful effects of radiation. These compounds belong to various chemical species (vitamins, carotenes, phenols, glycosides, polyphenols, flavonoids, saponins, etc.). And they are called chemo preventives (Stavric, 1993). The mechanisms of action of chemo preventives are complex and heterogeneous and their antioxidant capacity has a significant role in the hepatoprotective effects, for which may be included in the first line of cellular defense against xenobiotics (Rusu M.A. et al., 2005). At the intracellular level, these active substances are real traps for electrophilic groups of free radicals, neutralizing them in this way. They are also able to reduce the activity of enzymes involved in the formation of free radicals and to increase the activity of enzymes responsible for detoxification of free radicals (Stavric , 1993).

Administration of vegetal extracts of *Aralia mandshurica* cortex and of *Nigella sativa* seeds to *Mus musculus* mice for a period of 13 days, shows no toxic side effects in the studied organs: spleen, liver, bone marrow, and has protective action on the liver, spleen and bone marrow. Structural-functional investigations revealed that the tested plant extracts stimulates in some way the proliferation, differentiation and maturation of cells in the liver and spleen.

The toxic action is directed mainly to the organs with intense cell division, the most affected being bone marrow, liver and spleen. These organs are characterized by a profound disruption of the cellular and vascular components.

During experiments, in case of X-irradiation, the percentage of neutrophils increases and by activation of oxidative metabolism it participates to formation of toxic oxygen free radicals (RLO), and other reduced oxygen species, which, if formed in excess can cause not only phagocyte material destruction but also serious vascular injury. Increasing the number of neutrophils causes inflammation directly by tissue damage because of the generation super oxide

radical. For treatments with the used extracts it can be observed the radiation protection effect on the figurative elements of blood. Although they have a special extension, their structural and ultra structural changes induced by radiation are reversible, as evidenced by the resumption functionality of the studied organs - bone marrow; 5 days after administering the radiation dose, hematological parameters are inferior to the control.

The volatile oil from the cortex of *A. mandshurica* has immunomodulatory and anti-inflammatory properties, significant for the non-irradiated versions (dilution with DDW) and for the irradiated versions (diluted with distilled water). For the irradiated versions (diluted with distilled water), there is an inflammatory process initiated as a defense response against the stress factor, noted both by hypertrophy of hepatocytes and hyperplasia and hypertrophy of Malpighi lymph follicles. For the irradiated variants (dilution with DDW) a reduced inflammatory infiltrate is observed, which indicates a hepatoprotective effect, but immunostimulating too, observed by dilation of the red pulp and by hyperplasia of white pulp follicles.

The administration of poliholosides extract from the cortex of *A. mandshurica* both diluted in distilled water and in DDW, determined the stimulation of immunity by stimulating both the polymorphonuclear and mononuclear phagocyte system. Also, for the irradiated versions it caused a stimulation of blood repopulation with figurative elements, probably due to stimulation of the granulocitopoieza, monocitopoieza, lymphopoieza at the level of the bone marrow. The poliholoside extract shows the protective effect both before and after X irradiation, which is observed by subtracting the percentage of abnormal metaphases and of acentric fragments registered per 100 metaphases.

Administration of polyphenols and flavones extract from the cortex of *A. mandshurica* has a radiation protection, anti-inflammatory and antioxidant effect with a positive influence for regeneration of red blood cells and leukocytes and can be used with spectacular results in treating anemia and leukemia. The diluted extract of polyphenols and flavanols in both distilled and in DDW, has immunostimulating properties for irradiated versions, which is observed by the increase in IgG, IgM and IgA values. It also has antioxidant and hepatoprotective effects, through alterations annihilation produced by X radiation (maintaining normal metabolic activities in hepatocytes and helping to regenerate them), and at the spleen level an acceleration of the structural recovery processes, disturbed by the action of the X radiation.

The volatile oil from Nigella sativa seeds has radio protective, immunostimulating and antioxidant properties, involving the prevention of oxidative damage induced by radiations. The administration of volatile oil (diluted with DDW) has the action of stimulating the recovery process for structural variants of irradiated bone marrow, compared with the extract diluted in distilled water. The extract of volatile oil (Morocco's population and Craiova's), leads to increased values of IgG, IgM and IgA immunoglobulin, the best values obtained are for IgG when using volatile oil - Morocco's population and for IgA and IgM - population of Craiova. For irradiated versions, the volatile oil determined a structural recovery process of spleen (due to toxic action of X-rays), a phenomenon observed in the splenic follicles, which are well represented with a developed germinal center.

The extract of poliholoside from *Nigella sativa* seeds is able to protect the hematopoietic cells by stimulating mononuclear phagocyte and polymorphonuclear system, with better values recorded for the extract diluted in DDW. Administration of poliholoside extract increased the level of immunoglobulins, activated the NK cells and some of the T lymphocyte populations. Thus, the harmful effects of radiation X are reduced when administering the extract of poliholoside (especially for the one diluted in DDW). For irradiated versions, the poliholoside extract caused a hepatoprotective action; the liver architecture was partially preserved, with some nuclear preapoptotic changes. For non- irradiated versions, the poliholoside extract diluted in distilled water has an immunostimulating effect on the spleen, being observed in the white pulp hypertrophy and in the presence of numerous follicles with germ centers, while the extract diluted in DDW has immunosuppressive effects, observed in the atrophy of the white pulp. For irradiated versions, the extract shows an immunosuppressive action observed by a hypotrophy of white pulp. Also, the administration of the poliholoside extract reduced the percentage of the premature division of centromere and the PCD rate is increased only in action of X radiation.

The extract of alkaloids from Nigella sativa seeds, diluted in distilled water, has a radiation protective effect compared to the ultra structural characteristics recorded in the presence of alkaloids diluted in DDW; and five days after the experiment was finished, the analysis of the white blood cell counts suggested an adaptation process of the enzyme systems of the body to the action of stress factors used. For the irradiated versions, the extract of alkaloids has slightly immunosuppressive action. The alkaloid extract diluted in distilled water or DDW did not affect the normal structure of hepatocytes, for non- irradiated versions, while for

irradiated versions induced major structural changes of hepatocytes, compared with the single action of X-rays. Also, the extract of alkaloids administration has the protective effect of the two types of bioactive substances, and so the percentage of abnormal metaphases and acentric fragments present in 100 metaphases recording lower values. These values were insignificantly lower when given the two types of alkaloids in DDW, and so the registered protective effect of this combination.

In conclusion, the structural and functional recovery of mice *Mus muscullus'* organs affected by the X radiation of destructive action is faster under the action of extracts of *Aralia mandshurica* and *Nigella sativa* compared to their own recovery; also, the bioactive substances in the two plants show antioxidant radio protective and immunostimulating strong activities,. Comparing these results to those obtained from other clinical trials of other researchers, I may suggest a possible application of these vegetal extracts as nutritive adjuvants or supplements, helping to maintain proper homeostasis of the body.

6. CONCLUSIONS

The experimental researches conducted to determine the radioprotective and immunostimulating effect of some vegetal extracts obtained from *Nigella sativa* and *Aralia mandshurica* species - led to the following conclusions:

1. Under normal conditions, the extracts from the cortex of *Aralia mandshurica* caused stimulation of immunity by stimulating the polimorphonuclear and mononuclear phagocyte system and by increasing the values of immunoglobulins. The extract of polyphenols and flavones diluted in DDW shows strong immunomodulatory effects observed by the hypertrophy of the white pulp, by cells with rare nuclear atypia and by many follicles with germ centers. In the liver, the hepatocytes have normal structure and the Kupffer cells have lisosoms, haematin and, in general, cellular material destroyed.
2. Under stressful conditions with X-rays, the administration of extracts from the cortex of *Aralia mandshurica* has a protective effect that is more pronounced for dilution with DDW on the structure of the spleen, observed by the hypertrophy and hyperplasia of the red pulp, and immunomodulatory, observed by the increased number of megakaryocytes. In the liver, the extracts show antioxidant and hepatoprotective properties, as evidenced by hypertrophy of hepatocytes, and in the Kupffer cells primary and secondary lisosoms are present, with cellular waste. The protective effect is emphasized by the decrease in the percentage of abnormal metaphases and acentric fragments registered in 100 metaphases.
3. The extracts from seeds of *Nigella sativa* – especially the volatile oil extract – demonstrates a strong antioxidant effect and acts as a universal protector of cells; stimulates immunity by stimulating B lymphocytes and by increasing the levels of immunoglobulin. In spleen and liver , the immunostimulating effect was observed by the hypertrophy of the white pulp, in the hyperplasia of the red pulp with the proliferation of megakaryocytes, and it was also observed in the partially preserved architecture of the liver, in the normal structure if hepatocytes and areas with proliferation of interstitial vessels.
4. For the irradiated versions, the extracts from seeds of *Nigella sativa* have properties to protect hematopoietic cells and to stimulate the processes of structural recovery of the

bone marrow; five days after the experiment, analysis of white blood cell counts suggest a process of adaptation for the enzyme systems of the body to the action of the stress factors used. The immunostimulating effect is observed in spleen with normal aspect areas alternating with areas of white pulp hypertrophy, hyperplasia of reticular cells; in the liver there were found moderate and severe nuclear changes of hepatocytes, areas with dilation of port spaces and vascular hyperaemia.

5. For non- irradiated versions, DDW has a protective effect. The haematological and immunological tests showed slightly increased values compared to the untreated and non-irradiated control. In spleen, the immunostimulating effect is highlighted by hypertrophy and hyperplasia of white pulp, with the presence of large splenic follicles, some with germ centers and numerous lymphocytes and in the red pulp, by hepatocytes with slight changes, mitochondria have dense matrix, and the smooth endoplasmic reticulum is hypertrophied.
6. For the irradiated versions, DDW has protective effect for the parameters of the leukocyte formula and a immunostimulating effect evidenced by an increase of the values of immunoglobulins; there are observed the hypertrophy and hyperplasia of the white pulp, numerous lymphocytes in the red pulp too, and in hepatocytes there are recorded changes observed by the irregular contour of the nuclei , the tenuous cytoplasmic matrix and a big amount of rough endoplasmic reticulum.

SELECTED REFERENCES

Abbas A.T., Abdel-Aziz M.M, Zalata K.R., Abd Al-Galel T.D. (2005). Effect of dexamethasone and ***Nigella sativa*** on peripheral blood eosinophil count, Ig G1 and Ig G2, cytokine profiles and lung inflammation in murine model of allergic asthma. *Egypt Journal Immunology*, 2: 95-102.

Abdel-Salam I.M., Amira R., Hassan A.M. (1998). Radioprotective effects of *Nigella sativa* oil on the liver of *Swiss albino* mice. *Journal Egypt Biochemistry*, 16: 167-176.

Abidov M.T., del Rio M.J., Ramazanov T.Z., Klimenov A.L., Dzhamirze Sh., Kalyuzhi8n O.V. (2006). Effects of *Aralia mandshurica* and *Engelhardtia chrysolepis* extracts on some parameters of lipid metabolism in women with nondiabetic obesity. *Bulletin Experimental Biology and Medicine*, 141(3): 343-6.

Ali B.H., Blunden G. (2003). Pharmacological and toxicological properties of *Nigella sativa, Phytotheraphy Research.* 17(4): 299-305.

Al-Ghamdi M.S. (2001). Anti-inflamatory, analgesic and anti-pyretic activity of *Nigella sativa*. *Journal Ethnopharmacology*, 76: 45-8.

Al-Ghamdi M.S. (2003). Protective effect of *Nigella sativa* seeds against carbon tetrachoride-induced liver damage. *American Journal Chinese Medicine*, 31(5): 721-8.

Assayed M.E. (2010). Radioprotective effects of black seed (*Nigella sativa*) oil against hemopoietic damage and immunosuppression in gamma-irradiated rats. *Immunopharmacology and Immunotoxicology*, 32(2): 284-296.

Badary O.A., Taha R.A., El-Din A.M.G., Abdel-Wahab M.H. (2003). Thymoquinone is a potent superoxide anion scavenger. *Drug and Chemical Toxicology*, 26: 87-98.

Badary O.A., Abd-Ellah M.F., El-Mahdy M.A., Salama S.A., Hamada F.A. (2007). Anticlastogenic activity of thymoquinone against benzo(a) pyrene in mice. *Food Chemichal Toxicology*, 45: 88-92.

Baker, M. E. (1998). Flavonoids as hormones. A perspective from an analysis of molecular fossils. *Advances in Experimental Medicine and Biology*, 439, 249-276.

Ballenger L. (1999). *"Mus musculus"* (On-line), Animal Diversity Web. (2009). <http://www.animaldiversity.ummz.umich.edu/site/accounts/information/Mus_musculus.html>

Bamosa A., Ali B.A., Sowayan S. (1997). Effect of oral ingestion of *Nigella sativa* seeds on some blood parameters. *Saudi Pharmacology Journal*, 5: 126-9.

Bild W., Ştefănescu I., Hăulică I., Lupuşoru C., Titescu G., Iliescu R., Năstasa V. (1999). Research concerning the radioprotective and immunostimulating effects of deuterium-depleted water. *Romanian Journal Physiology*, 36 (3-4):205-218.

Bowen R. (2004). Arhitecture of the liver, <http://www.*biology.about.com/library/.../bldigestliver.html*>.

Bravo L. (1998) Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutritional Reviews*, 56: 317-333.

Brekhman I.I. (2002). A Review of the Clinical Trials and Evaluations of *Prime One* The following is a retrospective analysis of Prime One and its active ingredients, AMS Health Sciences, 1(1), <http://www.amsonline.com/Edocs/Physicians_Reference_Guide.pdf>.

Caceci T. (2004). (VM 8054) Veterinary Histology Exercise 13 Lymphatic System: <http://www.*Liver and Other Organs education.vetmed.vt.edu/.../labs/...Lab13.html*>.

Caceci T. (2004). (VM 8054) Veterinary Histology Exercise 20 Digestive System: <http://www.*Spleen and Other Organs education.vetmed.vt.edu/.../labs/...Lab20.html*>.

Cemek M., Büyükokuroğlu M.E., Bayiroğlu F., Koç M. (2008). Irradiation, Radioprotection and *Nigella sativa,* Herbal Radiomodulators. Applications in Medicine, Homeland Defence and Space, p. 47-62, http://www.bookshop.cabi.org/Uploads/Books/PDF/

Coman N. (2003). Genetică. vol. I, Universitatea "*Babeş-Bolyai*", Facultatea de Biologie-Geologie Catedra de Ecologie-Genetică, Cluj-Napoca, p. 78-87.

Corder C, Benghuzzi H, Tucci M, Cason Z. (2003). Delayed apoptosis upon the treatment of Hep-2 cells with black seed. *Biomed. Sci. Instrum.*, 39:365-70.

Corneanu G. (1989). Elemente de radiobiologie vegetală. Edit. Ceres, Bucureşti, 37-44.

Corneanu, C.G., Rogoz I., Rogoz S., Corneanu M., Zagnat M., Grigorescu E.M., Popa M.,. (2003). Testarea efectului radioprotector şi imunostimulator al unor substanţe biologic active (poliholoside şi saponine) extrase din *Nigella sativa* şi *Aralia mandshurica*. Analele SNBC, Ed. Risoprint Cluj-Napoca, VIII: 315-322.

Corneanu G.C., Rogoz I., Ediţoiu C., Corneanu M., Atyim P., Zagnat M., Grigorescu M., Ştefănescu I., Corneanu L.M. (2004). Efectul protector al apei superuşoare şi a două substanţe

bioactive (extract total alcaloizi şi poliholoside) în cazul iradierii-X la *Mus musculus.* Analele SNBC, Ed. Risoprint Cluj-Napoca, IX: (1): 260-265.

Corneanu G.C., Drăgoi S.G., Şutru C., Corneanu M., Zagnat M., Grigorescu E., Atyim P., Melinte P.R., Popa M. (2004). The single or combined effect of the X-rays and of two bioactive substances (saponins and polyholosides) at the liver level in *Mus muscullus L.*. Proceedings (Eds. Ivana Maksimović, Maja Čuvardić and Simonida Đurić), E.S.N.A. XXXIV, Annual Meeting, Ed. Monograf., Novi Sad: 82-85.

Corneanu G.C., Crăciun C., Ciupină V., Prodan G., M. Corneanu , Atym P., Ştefănescu I., Iacob M., Corneanu L-M. (2005). The efect of *Nigella sativa L.*alkaloids and of deuterium-depleted water on *Mus musculus L.* splin. Revue de Cytologie et Biologie végétales-Le Botaniste, 28 (s.i.), 84-91.

Corneanu G.C., Ciupină V., Prodan G., Corneanu M., Ştefănescu I., Iacob M. (2005). Efectul antibacterian şi antiviral al ASDR. A XXIII-A Sesiune Ştiinţifică Anuală a Societăţii Naţionale de Biologie Celulară, Sibiu, 33: 159.

Corneanu G.C., Ciupină V., Prodan G., Corneanu M., Ştefănescu I.,Atyim P., Iacob M., Popa M. (2005). Efectul detoxifiant la nivelul ficatului şi splinei la *Mus musculus*, sub acţiunea unor factori externi. Analele SNBC, Edit. Risoprint, Cluj-Napoca, X: 285-290.

Corneanu G.C., Crăciun C., Ciupină V., Prodan G., Corneanu Mihaela, Atym P., Ştefănescu I., Iacob M. (2006). Ultrastructural effects of *Nigella sativa* total alkaloids extract at liver level (*Mus musculus*). 4th Conference on Medicinal and Aromatic Plants of South-East European Countries, Iaşi, p. 105.

Corneanu G.C., Siloşi I., Rogoz S., Hădărugă N., Hădărugă D., Corneanu M., Ediţoiu C., Rogoz I., Zagnat M., Ştefănescu I. (2006a). Testing of immunostimulatory effect of the volatile oil *(Nigella sativa* and *Aralia mandshurica)* in *Mus musculus.* Analele Universităţii din Craiova, Biologie, Horticultură, Tehnica Prelucrării Produselor Agricole, Ingineria Mediului, XI (XLVII): 229-234.

Corneanu G., Siloşi I., Rogoz S., Corneanu M., Hădărugă N., Hădărugă D., Zagnat M., Ştefănescu I., Corneanu L-M., Popa M.. (2006b). Efectul radioprotector al DDW şi al uleiului volatil din seminţele de *Nigella sativa* sau din cortexul de *Aralia mandshurica.* Analele S.N.B.C., Edit. Risoprint, Cluj-Napoca, XI: 487-493.

Corneanu C.G., Siloşi I., Rogoz S., Hădărugă N., Hădărugă D., Ediţoiu C., Rogoz I., Zagnat M. (2006c). The radioprotective effect of the volatile oil, estimated on the immunoglobulines quantity in mice (*Mus musculus L.*). University of Agricultural Sciences and Veterinary Medicine, Annual Meeting, Iaşi, XXXVI: 25.

Corneanu C. G., Corneanu M., Atyim P., Crăciun C. (2007a). The COX-2 gene implication in the immune response (experiments on animals, *Mus musculus L.*). Analele Universităţii din Craiova, Agricultură-Montanologie-Cadastru, vol. XXXVII/A: 525-535.

Corneanu C.G., Corneanu M. (2007b). Genetica. In: Enciclopedie de Biologie (Coord.Gh. Mohan, A. Ardelean), pp. 611-763, Edt. All, Bucureşti.

Corneanu C.G., Corneanu M., Crăciun C., Zagnat M., Ştefănescu I., Popa M. (2010). The radioprotective effect of deuterium depleted water and polyphenols. Environm.Eng. & Managem. J., 9 (11): 1509-1514.

Corneanu M., Corneanu C.G. (2005). Genetică generală şi evoluţia genomului. Edit. Universitaria Craiova.

Decker K., (1990). Biologically active products of stimulated liver macrophages *(Kupffer cells). European Journal Biochemistry*, 192: 2245-261.

Demir H., Kanter M, Coskun O., Hulya U.Y., Koc A., Yildiz A. (2006). Effect of black cumin *(Nigella sativa)* on heart rate, some hematological values, and pancreatic β-cell damage in cadmium-treated rats. *Journal Biological Trace Element Research*, 10(2): 151-162.

El-Naggar A.M., El-Deib A.M. (1992). A study of some biological activities of *Nigella sativa* (black seeds) *"Habat El Baraka." Journal Egypt Societies Pharmacology, Experimental Therapy,* 11: 781-99.

Enomoto, S., Asano R., Iwahori Y., Narui T., Okada Y., Singab A.N., Okuyama T. (2001). Hematological studies on black cumin oil from the seeds of *Nigella sativa* L. *Biologycal Pharmacology Bulletin*, 24: 307-310.

Ghiorghiţă G., Corneanu G. (2002). Radiobiologie, Edit. Alma Mater, Bacău.

Goodall K.B. (2003). In search of the fountain of youth. Preliminary analysis of deuterium's role in DNA degradation,
<http://www.dd-water.ru/properties/in_**search_of_the_fountain_of_youth.pdf**.>.

Kanter M. (2008). *Nigella sativa* and Derived Thymoquinone Prevents Hippocampal Neurodegeneration After Chronic Toluene Exposure in Rat. *Journal Neurochemical Research,* 33(3): 579-588.

Maslov L. N., Guzarova N. V. (2007). Cardioprotective and antiarrhythmic properties of preparations from *Leuzea carthamoides*, *Aralia mandshurica*, and *Eleutherococcus senticosus. Eksp. Klin. Farmakol.,* 70(6): 48-54.

Paşca C., Crăciun C., Ardelean A. (2000). Efecte secundare ale unor citostatice asupra organismului, Ed. Risoprint, Cluj-Napoca.

Roitt I., Brostoff J., Male D. (2001). Immunology, Sixth edition, Mosby, W.B. Saunders, London.

Rusu, M.A., Crăciun, C., Rusu, M.L., Puică, C. (2005). Xenobiotice şi radicali liberi cu tropism hepatic: Hepatectomia chimică. Ed. Eikon, Cluj-Napoca.

Smith L.E., Nagar S., Kim G.J., Morgan W.F. (2003). Radiation-induced genomic instability: radiation quality and dose response. Health Physiology, 85: 23-9.

Stavric B. (1994). Antimutagens and anticarcinogensin foods. *Food and Chemical Toxicology,* 32(1): 79-90.

Ştefănescu I., Manolescu N., Comişel V., Tamaian R., Titescu G. (2008). Deuterium depleted water effects on Walker tumours. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine*, 65(1):443-450;

Titescu G., Iliescu R., Nastasa V., Bild W., Stefanescu I., Haulica I., Lupusoru C. (1999). *Apa echilibrată în deuteriu*, Research concerning the radioprotective and immunostimulating effects of deuterium-depleted water. *Romanian Journal Physiology*, 36(3-4) : 18-205.

Zagnat M. (2003). *Aralia mandshurica Rupr et Maxim.* – a plant with a realy perspective for Roumania. „*Ovidius*" University Annals of Medical Science – Pharmacy, 1(2): 18- 21.

COMPOSED AND PRINTED WORKS FROM THE THEMES OF THE DOCTORATE THESIS

1. Ediţoiu C., Popescu C., Ispas G., Corneanu G.C., Zagnat M., Ştefănescu I. (2010). The effect of biologically active substances of *Aralia mandshurica* and deuterium depleted water on the structure of spleen in *Mus musculus*. Annals of the Romanian Society for Cell Biology, Cluj-Napoca, Vol. XV (2): 212-216.

2. **Ediţoiu C., Corneanu G.C., Popescu C., Ispas G., Zagnat M., Atyim P., Ştefănescu I. (2010).** The hepatoprotective and immunostimulating effect of the volatile oil and polyholosides in *Nigella sativa*. Scientific Conferences with International Participation „Durable Agriculture – Agriculture of the Future" – the 6th edition; The National Mycology Symposium – the 22nd edition, Craiova, Vol. XL(2): 300-305.

3. Corneanu G.C., Rogoz I., Ediţoiu C., Corneanu M., Atyim P., Zagnat M., Grigorescu M., Ştefănescu I., Corneanu L.M. (2004). Efectul protector al apei superuşoare şi a două substanţe bioactive (extract total alcaloizi şi poliholoside) în cazul iradierii-X la *Mus musculus*. Analele SNBC, Ed. Risoprint Cluj-Napoca, IX: (1): 260-265.

4. Corneanu G.C., Siloşi I., Rogoz S., Hădărugă N., Hădărugă D., Corneanu M., Ediţoiu C., Rogoz I., Zagnat M., Ştefănescu I. (2006a). Testing of immunostimulatory effect of the volatile oil *(Nigella sativa* and *Aralia mandshurica)* in *Mus musculus*. Analele Universităţii din Craiova, Biologie,Horticultură, Tehnica Prelucrării Produselor Agricole, Ingineria Mediului, XI (XLVII): 229-234.

5. Corneanu C.G., Siloşi I., Rogoz S., Hădărugă N., Hădărugă D., Ediţoiu C., Rogoz I., Zagnat M. (2006c). The radioprotective effect of the volatile oil, estimated on the immunoglobulines quantity in mice (*Mus musculus L.*). University of Agricultural Sciences and Veterinary Medicine, Annual Meeting, Iaşi, XXXVI: 25.