THE ADAPTOGENIC FEATURES OF THE *Nigella sativa* L. SPECIES (Ranunculaceae)

CORNEANU C. Gabriel, CORNEANU Mihaela, CRĂCIUN Constantin

Abstract. In this paper, the experimental research regarding the effect of four groups of bioactive substances extracted from the *Nigella sativa* seeds (total extract of alkaloids, polyholosides, saponins and volatile oil) are presented and analysed toward the presence of a stress factor (sublethal doses of X-rays or a quasi-null geomagnetic field). The experiments were performed on the young animals of *Mus musculus* injected or not with one of the four bioactive substances, and exposed or not at one factor of physical stress. The interaction between the bioactive substance and animal organism was established on the basis of haematological, immunological and ultrastructural investigations (the analysis of the ultrastructural features of liver or spleen). The experimental results confirm the character of adaptogenic species for *Nigella sativa*. This is in concordance with its use since Antiquity and Middle Ages. In ancient Egypt this species was used for its role of assisting the Pharaoh when he passed on into the afterlife, and at the begin of the Middle Ages, Prophet Muhammad said: **"Use the Black Seed for indeed, it is a cure for every disease except death".**

Keywords: *Nigella sativa*, adaptogen, alkaloids, volatile oil, polyholoside, saponins.

Rezumat. Caracteristici adaptogene ale speciei *Nigella sativa* L. (Ranunculaceae). În lucrare sunt prezentate şi analizate cercetările experimentale privind efectul a patru grupe de substanţe bioactive extrase din seminţele unor populaţii de *Nigella sativa* (extract total de alcaloizi, poliholoside, saponine şi ulei volatil), faţă de prezenţa unui agent de stres (doza subletală de radiaţii-X sau câmp geomagnetic cvasi-nul). Experimentele au fost efectuate pe exemplare tinere de *Mus musculus*, injectate cu una din cele patru substanţe bioactive şi expuse sau nu la un factor de stres fizic. Interacţiunea dintre substanţa bioactivă şi organismul animal, a fost stabilită pe baza investigaţiilor hematologice, imunologice sau ultrastructurale (analiza ficatului şi splinei). Rezultatele experimentale, confirmă încadrarea speciei *Nigella sativa* în grupul speciilor cu rol adaptogen. Aceasta este în concordanţă cu utilizările ei în antichitate şi în Evul Mediu. În Egiptul antic, era utilizată de faraoni care credeau ca îi ajută să treacă în altă viaţă, iar profetului Mahomed i se atribuie afirmaţia, că **„Vindecă orice boală cu excepţia morţii".**

Cuvinte cheie: *Nigella sativa*, adaptogen, alcaloizi, ulei volatil, poliholoside, saponine.

INTRODUCTION

***Nigella sativa*, medicinal plant.** *Nigella sativa* (Fam. Ranunculaceae), native of the Mediterranean region, has been used as food, aromatic or therapeutic plant since Antiquity. Its seeds were found in Pharaoh Tutankhamen's tomb (1325 BC), having the role of assisting the Pharaoh when he passed on into the afterlife (AL-BUKHARI, 1976). Species of *Nigella* genus were described and recommended for use in alimentation and medicine by the philosophers of ancient Greece and Rome. The most valuable species is *Nigella sativa*, known under different names at different peoples: git (ancient Greece); melanthion, melanthium, melaspermon (ancient Rome), black cumin, black seeds or barakka (countries from the United Kingdom) a/o. *Nigella sativa* was described in their works by Cato, Hippocrates, Dioscorides, Plinius, Palladium, and *Nigella aristata* by Dioscorides (DIOSCORIDES, 1529; PLINIUS SECUNDUS, 1668; FRAAS, 1845; LENZ, 1859).

In the Holy Quran, it is stated that Prophet Muhammad said: **"Use the Black Seed for indeed, it is a cure for every disease except death"** (AL-BUKHARI, 1976; SHARMA et al., 2009). Ibn Sina, known under the name of AVICENNA (980-1037) in "The Canon of Medicine", included this species among the natural drugs "Tibb-e-Nahavi", "Medicine of the Prophet" (MANNAN & KAHVIC, 2010). In the characterization of this species, features of the actual adaptogenic species are mentioned: "the seeds stimulate the body's energy and help recovery from fatigue and dispiritedness", the black seeds being used for healing all diseases. In traditional medicine, the barakka seeds are used for their properties: carminative, aromatic, stimulant, diuretic, anthelmintic, galactagogue and diaphoretic. They are used as a condiment in curries. Tincture prepared from seeds is recommended in indigestion cases, the loss of appetite, diarrhoea, dropsy, amenorrhea and dysmenorrhoea, as well as against worms and in cutaneous eruption. The oil obtained from seeds has antiseptic properties. To stop vomiting, roast seeds were recommended. The seeds are also used as a condiment for bread, different sorts of cheese, aromatization of some culinary products, and others.

Chemical composition of the seeds. Numerous researchers analysed the chemical composition of *Nigella sativa* seeds. They contain a yellowish volatile oil (0.5-1.6%) and a fixed oil (35.6-41.6%); proteins (22.7%) as albumin, globulin; 18 amino acids (lysine, leucine, iso-leucine, valine, glycine, alanine, phenylalanine, arginine, asparagine, cystine, glutamic acid, aspartic acid, iso-leucine, proline, serine, threonine, tryptophan and tyrosine) that are needed for many body functions to maintain good health; alkaloids (nigellidin, nigellicin, nigellimin-N-oxide), organic acids, fatty acids in particular the linoleum acid (unsaturated fatty acid) implied in the cancer prevention as well as in the improvement of cystic fibrosis and dermatitis; tannins; resins; triglycosides; saponins and an isobenzofuranone derivative; minerals; carotene and phytosterols (shown to lower cholesterol); essential oils, the main component being thymoquinone (up to 50%), p-cymene (40%), pinene (up to 15%), dithymoquinone and thymohydroquinone.

Therapeutic utilization. *Nigella sativa* has been used since Antiquity in the Mediterranean area and in Indian subcontinent. Healing of wounds is one of the important areas of clinical medicine explained in many Ayurvedic texts. The Indian traditional system of medicine Ayurveda is based on empirical knowledge of the observations and the experience over millennia. In different classical ayurvedic texts, more than 1200 diseases, more than 1000 medicinal plants (89.93%), 58 minerals, metals or ores (5.24%) and 54 animal and marine products (4.86%) are mentioned to heal wounds. Plants have the immense potential for the management and treatment of wounds (AVINASH KUMAR REDDY et al., 2012). The seeds are considered as bitter, pungent, aromatic, appetizer, stimulant, diuretic, emmenagogue, galactagogue, antihelmintic, acrid, thermogenic, carminative, anodyne, deodorant, digestive, constipating, sudorific, febrifuge, expectorant, purgative, abortifacient. They are used in ascites, cough, jaundice, hydrophobia, fever, paralysis, conjunctivitis, piles, skin diseases, anorexia, dysentery, intrinsic haemorrhage and amenorrhea. Seed oil is a local anaesthetic. Analysing the recent performances and progresses in therapeutics obtained with *Nigella sativa* bioactive extracts, SHABANA et al. (2013) reported their beneficial effect in many cardiovascular diseases, and others. Other important activities performed with total or fractioned extracts from *Nigella sativa* seeds were also pointed out, such as: antitumor activity, antidiabetic activity, cardiovascular activity, gastroprotective activity, pulmonary activity, nephroprotective activity, antihypertensive and diuretic activity, hypercholesterolemia activity, hepatoprotective activity, anti-inflammatory activity, immunomodulatory activity, central nervous system activity, anticonvulsant activity, antinociceptive activity, anxiolytic activity, antioxidant activity, antioxitocic activity, post-coital contraceptive activity, abortifacient activity, anti-implantation activity, antiurolithatic activity, antispasmolytic activity, antibacterial activity, antifungal activity, anti-schistosomiasis activity, antiparasitic activity, antihelminthic activity, apoptosis induction activity, as well as in opioid dependence treatment, experimental autoimmune encephalomyelitis, stimulation of uterine contraction, analgesic activity, a/o (MOHAMMAD YAHIYA & MOHAMMAD ISMAIL, 2009). MOHAMED et al. (2011) also established that *Nigella sativa* exerts hepatoprotective effect against liver carcinogenesis.

In experiments performed with ethanol extracts of seeds, administered in rats exposed to an exhausting physical activity (swimming endurance), ROSHAN et al. (2010) reported that this extract manifests an anti-stress activity. PAWAR VINOD & HUGAR (2012), analysing numerous experiments, consider *Nigella sativa* as being an adaptogenic species. Results construe that the wound healing effect of *N. sativa* is related to its antioxidant, antibacterial and anti-inflammatory properties, evoke the mechanism of action is at inflammatory and proliferative phase. PANDEY (2011), a reputed expert in the medicinal plants, in a synthesis about the herbs with anticancer activity, presented the classical adaptogenic plants, together with the recent plants, with adaptogenic properties, such as *Nigella sativa*, a.o. In *Nigella sativa* seeds thymoquinone and dithymoquinone are present, which determined a strong anticancer activity against various cancer types, as well as immune-enhancing and anti-inflammatory properties. Moreover, *Nigella sativa* seeds increase the immune function of the body, reduce side effects of chemotherapy and radiotherapy. STEVENSON (2012) considers that the adaptogenic character of some plant species is determined by the polyphenol presence. MICHEL et al. (2010, 2011) used aqueous extracts of *Nigella sativa* seeds, in different pharmaceutical excipients (soya lecithin/span80/sodium taurocholate, PEG 400, and Meglyol 810). The obtained products, involved in the treatment of different affections, present good effects and efficacy in treatments performed in laboratory small animals. CHOWDHURY et al. (2012) analysed the features of a toothpaste, composed of 32 herbs, as (*Capsicum annuum*, *Nigella sativa*, *Ocimum tenuiflorum*, *Withania somnifera*, *Glycyrrhiza glabra*, *Mentha spicata*, *Aloe vera*, *Camellia sinensis*, *Echinacea* sp., and others). Their investigations pointed out that this toothpaste can cure various diseases of teeth like gingivitis, tooth decay, cavity, gum bleeding, bad breath and dental-caries, and it also has antismoking and anticancer properties. Regarding *Nigella sativa*, they affirm that the thymoquinone, isolated from *N. sativa* seed oil, blocks the pancreatic cancer cell growth *in vitro* and kills the cells by enhancing the process of programmed cell death (apoptosis) acting as an HDAC inhibitor.

The doses, which produced a toxicological effect, were also established. The LD_{50} values are different, depending on species and the administration type. In mice, in an oral administration of liquid extracts, a value of 28.8 ml/kg b.w.-po, and a value of 2.061 ml/kg b.w. for the intraperitoneal administration were recorded. In the case of seeds the LD_{50} values are 104.7 mg/kg, for intraperitoneal, and 870.9 mg/kg for ingestion. DIOSCORIDES mentions, in "Peri hiules iatriches", that a dose over one drachma (about 3.4 g) produces lethality in humans. But, no health hazards or side effects are known with the proper administration of designated therapeutic dosages (SHARMA et al., 2009). From this reason, and for their effects and action of bioactive substances extracted from their seeds, described by different authors (CORNEANU et al., 2005b, 2012; PAWAR VINOD & HUGAR, 2012; ROSHAN et al., 2005), the *Nigella sativa* is a primary adaptogenic species.

In this paper, the results of some earlier research are presented and discussed (CORNEANU et al., 2000, 2004, 2005a, 2005b, 2006, 20012) considering the belonging of *Nigella sativa* species to the group of adaptogenic organisms.

MATERIAL AND METHODS

Biological material. In experiments performed on laboratory animals (*Mus musculus*), the effect of different fractioned extracts obtained through classical methods from the seeds of three *Nigella sativa* populations (originating from Morocco, Gaza and Romania) was analysed. Total alkaloid extract (0.01% concentration, in ethanol extracted), polyholosides, saponins (0.05% concentration), and volatile oils (0.1% concentration) were successively used. The

experiments were performed on an albino mice population (*Mus musculus*), two month old, individual weight about 24-25 g. The experiment took place in the biobase of the Medicine and Pharmacy University of Craiova.

Work method. For each bioactive substance tested, the experiment protocol was similar. Each animal was administered intraperitoneally five injections, every two days, with 0.5 ml solution of total extract of bioactive substance diluted in distilled water or in DDW. The solution concentration was of 0.01% in the experiment with alkaloids, 0.05% in the experiment with total extracts of polyholosides or saponins, and 0.1% in the experiment with a total extract of volatile oil. DDW (deuterium-depleted water) is distilled water with lower deuterium content. In all experiments, a DDW with 30 ppm deuterium was used versus 145 ppm deuterium in the spring water. A day after the third injection, half of animals from each variant were irradiated with a unique sublethal dose of X-rays (5.28 Gy) at the following parameters: 250 kV, 5 mA, d.f. = 500 mm, 1 mm Al filter, the dose capacity of 5.28 R. A day after the fifth injection, the animals were sacrificed by sectioning the jugular vein. The ultrastructural features of the liver and spleen, as well as some immunological parameters were analysed. Pieces of about 1 mm^3 were prefixed in a 2.5% glutaraldehide solution (2 ½ hours), post fixed in a 1% Millonig solution (1 ½ hours), infiltrated and included in EPON 812. The serrated sections of about 80-90 nm tick were contrasted with uranyl acetate and lead citrate. The analysis of the ultrastructural features was performed by a TEM JEOL JEM 10-10 apparatus (Center of Electron Microscopy, *Babeş-Bolyai* University, Cluj-Napoca).

The immunoglobulin amount and the C3 complement of cytochrome value were established by methods used in human investigation. The amount of some bioactive substances in the *Nigella sativa* seeds was established by usual methods.

RESULTS AND DISCUSSIONS

1. Experiment with a total acid alkaloid extract

1.A. Ultrastructural features

Control variant. The structural features in the control variant are characteristic for this organ and species (MACSWEEN et al., 2002). The hepatic lobule presents a centro-lobular vein covered with an epithelium. The sinusoid capillaries are disposed around the centro-lobular vein. The hepatocytes of polygonal shape are disposed in rows. Every hepatocyte presents one (two) nuclei of oval-spherical shape (Fig. 2). Numerous mitochondria are present in cytoplasm, normally structured, with an electron-dense matrix (Fig. 1). A rugose endoplasmic reticulum is dispersed among mitochondria, formed from narrow profiles, usually disposed around the mitochondria (Fig. 2). At the vascular pole, the hepatocytes present microvillus evaginated in the Disse space. In sinusoid capillary, there are Kupffer cells with numerous lysosomes and with a normal activity. Smooth endoplasmic reticule, as well as the dictyosomes, is poorly represented. The lipid drops are present in a small quantity, being represented through small drops, disposed with predilection toward the vascular pole (in transit; Fig. 2). In Control variant, and in other experimental variants, some parasite entities (viruses) were observed in hepatocytes, as well as some filamentous structures as a result of the metabolic activity (CORNEANU et al., 2005a).

Control-X (the X-irradiated effect). As a result of the X-ray effect, some adulterations were induced in hepatocytes. The nuclei present an unregulated shape outline, sometimes the nuclei being hypertrophied, especially the *vacuolar* and *pars amorphous* components of the nucleolus. The smooth endoplasmic reticule is proliferated, as a reaction to the destructive action of X-rays. Rugose endoplasmic reticulum presented dilated cisterns, and little ribosomes associated, because of the reduction of the metabolic activity and of the protein synthesis. A depletion of the glycogen was also emphasised. In some hepatocytes small focuses of cytoplasm lyses are present, the nuclei from these cells being pyknotic and hyper chrome. There is also an accumulation of lipid drops of different sizes. As a result of X-irradiation the plasmalemma was destroyed, the cellular compounds being free in sinusoid capillary. In some hepatocytes, the perinuclear space is dilated. The Kupffer cell is active (Fig. 3), and in the Disse space there is an accumulation of glycogen (CORNEANU et al., 2012).

Alkaloids in distilled water do not affect the ultrastructural features of the cell, the nucleus (Fig. 4), mitochondria and endoplasmic reticulum having normal features. The Kupffer cells are in metabolic activity.

Alkaloids in DDW do not affect the normal structure of the hepatocyte. The presence of a total acid alkaloid extract intensifies the cell metabolism, the nucleus having a polymorphism (the shape and stage of the mitotic cycle), and some hepatocytes being in mitotic cycle (prophase) and having the chromosomes well structured and an accumulation of lipid drops.

Alkaloids in distilled water - X-rays. The analysis of the ultrastructural features of hepatocytes points out a hepatoprotective effect induced by the total alkaloid extract, present during the X-irradiation of animals. The nucleus and nucleolus presented a normal structure, being in intense metabolic activity (Fig. 5; from CORNEANU et al., 2012). The presence of a stress factor is signalled by some ultrastructural features of mitochondria, endoplasmic reticulum and sinusoid capillaries. Thus, in some areas of the mitochondria, the matrix is rarefied and cristae are shorter and less numerous (Fig. 6, arrow). Some canaliculi of the endoplasmic reticulum are dilated having the shape of a cistern. In some sanguine capillaries, there is cell detritus.

The experimental results regarding the hepatoprotective effect of *Zingiber officinalis* extract versus the treatment with adriamycin (SAID AHMED, 2013) was weaker in comparison with hepatoprotective effect induced by

different bioactive substances extracted from *Nigella sativa* seeds, toward X-irradiation. Similarly to the situation reported in our experiment, a treatment with adriamycin induced in rats, at the liver level, destruction of the hepatic cords, cytoplasm vacuolization, cellular degeneration, pyknotic and atrophic nuclei, damaged mitochondria, accumulation of lipid droplets in the hepatocytes and deposit of a collagen-like fibrous material in the blood sinusoids and space of Disse with dilated intercellular spaces (SAID AHMED, 2013). The dilatation of blood sinusoids and bile canaliculi, as well as atrophy of its microvilli, also took place. The damaged Kupffer cells with vacuoles were prominent.

Alkaloids in DDW – X-rays. The presence of a total acid alkaloid extract during the X-irradiation of the animal induced more ultrastructural adulterations, in comparison with cases of single X-ray action. As a defence reaction, the nucleus presents big blocks of heterochromatin disposed on the nuclear envelope. The presence of the stress factor also induces the amplification of the smooth endoplasmic reticulum (SER) with the profiles slightly dilated. In some cells, there are lyses areas in cytoplasm, granular endoplasmic reticulum (GER) has some dilated cisterns, and there are rarefied chromatin areas in the nucleus. The collagen fibbers and liposomes are well represented.

ABDEL SALAM et al. (2012) analysed the structural and ultrastructural features of the liver in rats in Control, as well as the result of a treatment with cisplatin and silymarin (a protector), administered single or together. In Control animals, the liver ultrastructural features are similar to the features recorded at mouse in the present study. The oral administration of silymarin ameliorated the deleterious hepatic structural and ultrastructural toxic effects induced by cisplatin. In comparison with alkaloids extract which in the present study induced an adaptative effect at the liver level (annulated the deleterious effects of X-rays), the silymarin administration induced a partial restoration of the hepatic architecture.

In experiments performed on hyperlipidemic rats, DAVID RAJ et al. (2012), there were analysed the effects of six aqueous or ethanol extracts from six medicinal plants (*Cassia angustifolia, Nigella sativa, Phyllanthus amarus*, *Emblica officinalis, Zingiber officinale* and *Terminalia chebula*), using different investigation parameters (biochemical and cytological). The alcoholic extract (1.7 g %) from seeds of *Nigella sativa*, manifested hepatoprotective, neuroprotective and antioxidant effects. BAGHDADI & AL-MATHAL (2011) evaluated *in vivo* the anti-coccidian effect of *Nigella sativa* L. seeds in aqueous suspensions and oil emulsions in rabbits, through morphological and histological investigations at the liver level. Both treatments had anti-coccidian effects as demonstrated by reduced faecal oocyst shedding, significant body weight gain, recovery of normal liver weights and remarkable improvement of liver tissue histopathology. This improvement includes disappearance of bleeding between hepatic lobules and severe reduction in the infiltration of the inflammatory cells in the portal area, reduction in the various stages of parasites in the bile ducts, return of the natural hepatocytes radial arrangement, disappearance of all severe symptoms and the heading of the tissue structure towards full recuperation.

In an experiment, performed on Swiss albino mice, KAUSHIK et al. (2012) established that a 50% alcoholic extract from *Mentha piperita* manifest a radioprotective effect ($LD_{50/30}$ = 8.052 ± 0.115 Gy), in comparison with the variant without radioprotective extract from *M. piperita* ($LD_{50/30}$ = 5.598 ± 0.090 Gy). In this 50% alcoholic extract, all the bioactive substances from this species were (probably) present.

1.B. Spleen ultrastructural features

Although at the single application, DDW affects the normal activity of the lymphocytes, the treatment with a total acid alkaloid extract from *Nigella sativa* seeds, diluted in DDW, maintained a normal aspect of the cell. The lymphocytes from the white pulp of the spleen are numerous and densely packed, without rarefaction, having a normal structure. Most of them contain in their cytoplasm numerous ribosomes and Golgi complexes, very well developed, an aspect which means an intense metabolic activity. Moreover, some of them present a stimulation of the metabolic activity. Lymphoblasts are also present, some of them being in amitotic division. Lymphocytes with pyknotic nuclei are also present, situated in a normal stage of aging (apoptosis), the structural modifications recorded especially at the nucleus level (heterochromatinisation of different degrees, a/o) being similar to those reported by other authors in case of apoptosis (CUMMINGS et al., 1997). The analysis of the cytogenetic modifications recorded in animals that were intraperitoneally injected with alkaloid diluted in distilled water or in DDW, or with DDW alone, recorded similar values: 94.0% normal metaphases and 6.0% PCD (premature centromeric division; CORNEANU et al., 2004). The previous experiments in mice showed that the premature centromeric division (total or partial) tends to reduce the time of the mitotic chromosomal cycle, having a predisposition to aneuploidy because of an unequilibrated segregation of the chromosomes (ROGOZ et al., 2002). As DDW does not manifest its destructive effect in the alkaloid presence, it can be asserted that it has a radioprotective effect (CORNEANU et al., 2005b).

Alkaloids are present in many plants. The species *Punica granatum* is native of Himalaya and northern India to Iran and naturalized since ancient times in the entire Mediterranean region. At present, this species is cultivated in Southeast Asia, East Indies and tropical Africa. Being a medicinal plant, with therapeutic usage, different researchers analysed the alkaloid content in different plant organs. MOHAMMAD & KHASANI (2012) detected that saturated alkaloids present in the root and body rinds are not present in the leaves, whereas 2-(2-propenyl)-piperidine of unsaturated alkaloids was present in the leaf extract.

SLUNSKA et al., (2010) established that the two alkaloids from the *Symphytum officinale* leaves (sanguilutine and chelilutine) and present in tea manifest potent antiproliferative activities against three tumour cells (HeLa, H-L60 and A-02780) and are efficient inductors of apoptosis. Also, the quaternary protoberberine alkaloids (QPA) and some

related compounds exhibit considerable biological activities (GRYCOVÁ et al., 2007). The QPA interact with nucleic acids predominantly as intercalators or minor groove binders. In many laboratories, the current investigations are focused on the antibacterial and antimalarial activity, cytotoxicity, and potential genotoxicity of QPA.

2. Experiment with total polyholosides extract

Polyholosides (polyosides) result from the association of a big number of O-glycosides. They can present linear or ramified chains. They are normal constituents of plants, the main representatives being: starch, glycogen, cellulose, agar-agar, dextrans, and hyaluronic acid. They are a group of substances with a structural role, deposits of organic matter, energetic role (starch) and informational role, being capable of recognizing some characteristic structural elements. They can be homogeneous (neutral or acid) or heterogeneous (extracts from algae or extracts from superior plants). In the last group, there are pectin, gums and mucilages. In this experiment, the effect of the presence, during the irradiation of animals, of a total extract of polyholosides from *Nigella sativa* seeds, Morocco population, was analysed.

Polyholosides from *Nigella sativa*, unirradiated animals. The optical microscopy analysis of the hepatic lobule evidenced some reversible modifications. A stasis process in the centrolobular vein and a slight stasis process in the sinusoid capillary are present. In the nuclei, agglomerations of heterochromatin are present at the periphery. Nucleoli are hypertrophied.

Polyholosides from *Nigella sativa*, irradiated animals. The polyholoside presence during the irradiation of the animals has a hepatoprotective effect. The liver presents structure characteristics for an intense metabolic activity. In some cells, two nuclei are in an adjacent position, suggesting a mitotic division activity. The chromatin fibbers are finely dispersed, the NAB (*nucleolus-associated body*) structure being also present, which indicates an intense metabolic activity as a result of the animal adaptation to the action of this stress factor. The values recorded for some immunoglobulins (IgM, IgG and IgA) and C_3 component of cytochrome, are presented in Table 2.

Table 2. The amount of immunoglobulin and C_3 component of cytochrome in *Mus musculus* (after CORNEANU et al., 2004).

Experimental variant	IgM	IgG	IgA	C_3 component of cytochrome
Unirradiated animals				
Control	190	200	90	76
Saponins	180	100	62	54
Polyholosides	130	96	71	72
Irradiated animals				
Control	100	115	50	125
Saponins	170	84	68	100
Polyholosides	60	77	62	76

In the *Punica granatum* (Fam. Punicaceae) fruit, there are free oses (fructose, glucose and raffinose in a small amount), pectin substances, hemicelluloses A and B, as well as polyholosides soluble in water (2.58%). The glucides from fruit (mannose, galactose, rhamnose, arabinose, glucose and galacturonic acid) are implied in the calcium pectate formation in lamella. Their reducing properties represent the main properties of polyholosides: reduction of the metallic ions, reduction of organic compounds, and oxidation by enzymatic way, diverse hydrolysis types, and degradation (MOHAMMAD & KASHANI, 2012).

3. Experiment with total saponin extract

Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin), which may be triterpenoid in nature. The aglycone may contain one or more unsaturated C–C bonds. The great complexity of saponin structure arises from the variability of the aglycone structure, the nature of the side chains and the position of the attachment of these moieties on the aglycone. Experiments demonstrating the physiological, immunological and pharmacological properties of saponins have provoked considerable clinical interest in these substances (FRANCIS et al., 2002). Saponins occur constitutively in ~~a great~~ many plant species, in both wild plants and cultivated crops. In cultivated crops, the triterpenoid saponins are generally predominant, while steroid saponins are common in plants used as herbs or for their health-promoting properties. TAŞKIN et al., 2005, isolated three triterpene glycosides from *Nigella sativa* seeds, from which one is a derivate of hederagenin, signalled for the first in the *Nigella* genus.

Several biological effects have been ascribed to saponins. Saponin mixtures present in plants and plant products have diverse biological effects when present in the animal body. Extensive research has been carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoa and molluscs, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral agents. These compounds can thus affect animals in a host of different ways both positive and negative. Saponins also show wide-ranging cytostatic effects against cancer cells. LUO et al. (2011) also demonstrated that garlic

saponins manifest antioxidant effects and protect PC12 cells from hypoxia-induced damage, in mice. The oat saponins (avenacosides A and B) also affect the permeability of the rat intestine (ÖNNING et al., 1996).

In this experiment the effect of the presence of a total saponin extract from *Nigella sativa* seeds, Morocco population, during the irradiation of animals, was analysed.

Saponins from *Nigella sativa*, unirradiated animals. Slight, reversible modifications are induced, such as: a dilatation of the centrolobular vein and erythrocytes disposed on the endothelium. Around the centrolobular vein, the hepatocytes are disposed in order. A stasis process is also present at the centrolobular vein level. In the nucleus from the pericentrolobular hepatocytes, the heterochromatin is pulverized.

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

MOHAMMED et al. (2008) tested the antimicrobial saponins extracted from *Nigella sativa* seeds, for a wide range of microorganisms (*Staphylococcus aureus, Bacillus subtilis*, *Salmonella typhi, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa*). In comparison with control samples (treated with commercial antibiotics – gentamicine, tetracycline), the saponin compounds showed significant inhibiting effect on all the bacteria.

In the irradiated animals, the value of some immunoglobulins (IgM, IgG and IgA) generally recorded smaller values, and higher values for C_3 component of cytochrome, in comparison with the same variant of bioactive substances, but without the presence of the X-ray stress (Table 2; CORNEANU et al., 2004).

NALBANTSOY et al. (2011) analysed the haemolytic activities of two saponins extracted from *Astragalus oleifolius* (Mac B) and *A. trojanus* (Ast VII), and their adjuvant potentials on the cellular and humoral immune responses of Swiss albino mice against bovine serum albumin (BSA). They established that the two analysed saponins (Ast VII and MacB) generate important specific antibody and cellular response against BSA in mice, proving their potentials as a new class saponins adjuvant.

4. Experiment with total volatile oil/essential oil extract

In the fixed and volatile oils from *Nigella sativa* seeds from Iran, NICKAVAR et al. (2003) identified eight fatty acids (99.5%), and thirty-two compounds (86.7%), respectively. The main fatty acids of the fixed oil were linoleic acid (55.6%), oleic acid (23.4%), and palmitic acid (12.5%). The major compounds of the volatile oil were *trans*-anethole (38.3%), *p*-cymene (14.8%), limonene (4.3%), and carvone (4.0%). In a review paper, SALEM (2005) underlined that both the oil from *Nigella sativa* seeds, and its active constituents, especially thymoquinone, manifest antioxidant effects, by increasing the oxidant scavenger system. The oil and thymoquinone also manifest potent anti-inflammatory effects on several inflammation-based models including experimental encephalomyelitis, colitis, peritonitis, oedema, and arthritis through suppression of the inflammatory mediators, prostaglandins and leukotrienes. The oil and certain active ingredients also showed beneficial immunomodulatory properties, increasing the T cell and natural killer cell-mediated immune responses. Most important, both the oil and its active ingredients expressed antimicrobial, anti-tumoural and anticarcinogenic properties against different microbes and cancers. Previously, MANSOUR et al. (2001) in experiments performed on mice, established that thymoquinone and two related constituents (its precursors p-cymene and a-pinene) present in volatile oil extracted from *Nigella sativa* seeds, manifest an important role as antioxidants and may efficiently act as protective agents against chemically-induced hepatic damage. The antimicrobial activity of volatile oils from *Nigella sativa* seeds was established by different researchers (ARA et al., 2005), toward some Gram-positive and Gram negative bacteria; GERIGE et al., 2009, toward 4 Gram positive and 8 Gram-negative bacteria, 5-fungi, one yeast and one dermatophyte, a/o). ASADUZZAMAN et al., (2011), summarized the action of thymoquinone and crude oil from *Nigella sativa* seeds, against different cancers as well as their molecular mechanisms. Research performed by different authors on blood cancer (leukaemia), the cancer of breast, colon, pancreatic, hepatic, lung, skin, fibrosarcom, renal cancer, prostate cancer and cervical cancer, is mentioned. Similarly, BABAZADEH et al., 2011, investigated the genoprotective effects of *Nigella sativa* and thymoquinone on DNA damage of PC12 cells under conditions of serum/glucose deprivation (SGD). The results of the investigations indicate that *Nigella sativa* extract (NSE) and thymoquinone have genoprotective property, as revealed by the comet assay, under SGD condition in PC12 cells. EL-TAHIR & BAKEET (2006) underlined also the importance of the clinical evaluation of the volatile oil effects. Analysing the recent performances and progresses in therapeutics obtained with *Nigella sativa* bioactive extracts, SHABANA et al., 2013, report their beneficial effect in many cardiovascular diseases, or in other diseases as: diabetes mellitus, insulin resistance syndrome, total lipid profile and cardiovascular system, as well as antiplatelet sections. The pre-treatment with *Nigella sativa* oil also decreased cyclosporine A, cyclophosphamide, and doxorubicin injury in animal heart. HADJZADEH et al. (2007) also established that a treatment of the rats with an ethanol extract of *Nigella sativa* seeds conducts to the reduction of the number of calcium oxalate deposits, and diminished the concentration of calcium oxalate in urine.

The analysis performed on three *Nigella sativa* populations originating from different geographical areas (Morocco, Gaza and Romania), but developed in the same area (the Botanical Garden ”Prof. Alexandru Buia”, Craiova, Romania), identified a different component number depending on the considered population (CORNEANU et al., 2006): 53 compounds in Craiova population, 55 in Morocco population and 73 compounds in Gaza population.

Table 3. The thymoquinone and its precursor content in the seeds of three *Nigella sativa* populations (after CORNEANU et al., 2006).

Component from	% concentration	in *Nigella sativa*	Populations
Volatile oil	Morocco	Romania	Gaza
Thymoquinone	6.62	3.63	0.12
p-**Cymene**	39.61	53.98	33.17
Thymol	0.05	Traces	0.09
Carvacrol	2.61	1.99	6.20

The main substance with a role in immunity present in the *Nigella sativa* seeds was thymoquinone, together with its precursors, thymol, p-cymene and carvacrol (Table 3). The thymoquinone amount recorded high values in Morocco population (6.62%), medium in population from Europe (3.63), and low in population from Minor Asia (0.12%), but their precursors are in a big amount, especially carvacrol.

The effect of volatile oil extracted from the seeds belonging to two *Nigella sativa* populations (Morocco and Romania), diluted in distilled water or in DDW, toward a stress factor (a sublethal dose of X-rays, applied in a single dose) was tested on *Mus musculus*, in an experiment. The experiment stages were similar to the other experiments performed with alkaloids, saponins or polyholosides. Control variants were with animals not injected, or injected with distilled water or DDW, non-irradiated or X-irradiated. The amount of some immunoglobulins was established at the experiment end (animal sacrificed) after protocols used in human medical laboratories.

The intraperitoneal administration of total volatile oil extract, from the *Nigella sativa* seeds, Morocco or Romania populations, lead to the increase of the IgG, IgM and IgA immunoglobulins (Table 4). The immunoglobulin amount recorded higher values in Morocco population, with a higher amount of thymoquinone, in comparison with Romania population. The best values were recorded for IgG at volatile oil from Morocco population, and for IgA and IgM at volatile oil from Romania population.

In the irradiated variants, at animals treated with volatile oil diluted in DDW, the immunoglobulin amount was generally higher, in comparison with non-irradiated variants, for all the three types analysed. Thus, the volatile oil presence during animal irradiation induces a protective effect and an adequate response.

Table 4. The immunoglobulin values (mg/ml) in some experimental variants in *Mus musculus* (from CORNEANU et al., 2006).

Variant	IgG	IgM	IgA
Control			
Absolute	4.2	0.8	2.5
Absolute-X	1.4	0.4	2.0
H_2O	12.0	2.0	3.0
H_2O-X	11.0	1.4	3.8
DDW	10.0	1.7	2.6
DDW-X	7.0	3.0	3.5
Volatile oil *Nigella sativa* Morocco population			
H_2O	9.0	1.2	3.2
H_2O-X	11.1	1.9	2.8
DDW	10.0	1.5	2.7
DDW-X	10.0	2.0	3.0
Volatile oil *Nigella sativa* Romania population			
H_2O	2.0	4.5	5.0
H_2O-X	12.0	1.6	3.5
DDW	10.0	1.2	3.0
DDW-X	10.0	2.4	3.0
Reference	Values		
Anonymous	0.1 – 1.0	1.0 – 10.0	1.0 – 3.0

Fatty oil and quasi-null geomagnetic field

Some features of the extra-terrestrial space constitute stress factors: quasi-null geomagnetic and geoelectrical field, gravitational force, a/o. In an experiment, the effect of the fatty oil from *Nigella sativa* seeds over the hepatocyte features in animals maintained in a normal geomagnetic field (about 47,000 nT) or in a quasi-null geomagnetic field (200 nT) for 22 days was analysed. The ultrastructural analysis of the hepatocytes and some features of the haemoleucogram and blood glycaemia evidenced a slight stress protective effect induced by the presence of the fatty oil (CORNEANU et al., 2000).

In experiments performed with an electromagnetic field, some ultrastructural adulterations were also reported. Thus, EL-HADY EL-DESOKY & MOHAMADY (2011) analysed the ultrastructural adulterations induced in *Rattus norvegicus* liver, under the action of an electromagnetic field (static or alternating of 2 mT), of long term exposure (1 hour per day, 3 days weekly for 4 weeks). The results revealed some ultrastructural irregularity in the hepatocyte, especially at nucleus level (unregulated outline, widening of the nuclear pores and heterogeneous distribution of nuclear chromatin), mitochondria (swelling and clumping as well as deformation), rough endoplasmic reticulum (dilatation) or lysosomes (some being distorted).

CONCLUSIONS

The analysis of the ultrastructural features of hepatocytes from animals exposed to a stress factor (X-rays), in the presence or not of a bioactive substance extracted from *Nigella sativa* seeds indicates a hepatoprotective effect of the total acid alkaloid extract.

The polyholoside presence during the irradiation of the animals has also a **hepatoprotective effect**.

The polyholosides can recognize characteristic structural elements and can be implied in the recognition of the normal and adulterated structures under the impact of the stress factors. Thus, they are informational molecules, being implied in modelling the organism response and in its adaptation to different environmental conditions.

The presence of a total extract of volatile oil during the X-irradiation induces an increase of the IgG, IgM and IgA immunoglobulins.

The immunoglobulin amount recorded higher values in Morocco population, with a higher amount of thymoquinone, in comparison with Romania population.

The best values were recorded for IgG at volatile oil from Morocco population, and for IgA and IgM at volatile oil from Romania population.

In the irradiated variants at animals treated with volatile oil diluted in DDW, the immunoglobulin amount was generally higher in comparison with non-irradiated variants, for all the three types analysed.

ACKNOWLEDGEMENTS

This research was sponsored by two grants from CNCSIS, Ministry of Research and Education, Bucharest, Romania. We also thank Prof. Dr. Gheorghe-Ştefan Drăgoi (University of Medicine and Pharmacy, Craiova) for the optical microscopy investigations; Assoc.-Prof. Dr. Marin Zagnat (UMF Gr.T. Popa Iaşi) and Lecturer Dr. Paul Atyim (Vasile Goldiş, West University, Satu Mare Branch) for the bioactive substances extracted from seeds. We also thank to other members of these grants for the performed investigations and analyses, which were included in printed papers elaborated from these investigations and which are used in this review paper: Prof. Dr. Suzana Rogoz (Medicine and Pharmacy University from Craiova) for the immunological analysis; Prof. Dr. Ion Rogoz (Medicine and Pharmacy University from Craiova) for the cytogenetic investigations; Assoc.-Prof. Nicoleta Hădărugă (University of Agricultural Sciences and Veterinary Medicine of Banat from Timişoara) and Lecturer Dr. Daniel Hădărugă (Politehnica University from Timişoara) for the chemical analysis of seeds; Prof. Dr. Ioan Ştefănescu (Institute of Cryogenic and Isotopic Separation from Râmnicu Vâlcea) for the DDW put at our disposal and Dr. Marin Popa (S.C. Electroputere S.A. Craiova) for the X-irradiation of the animals.

The authors also express their sincere gratefulness to Acad. Prof. Marian Traian GOMOIU, the Romanian Academy, Ovidius University from Constanţa, Romania, and Prof. Ivan ILIEV, Forestry University, Sofia, Bulgaria, for the critical analysis of this paper, which conducted to its improvement.

REFERENCES

ABDEL SALAM G., HEGAZY A. M. S., ALI A. M., RIZK A. H. 2012. *Silymarin ameliorates hepatotoxic effect of cisplatin: a structural and ultrastructural study of adult albino rats*. Journal American Science. **8** (12): 490-498.

AL-BUKHARI M. I. 1976. *Division [71] on medicine*. In: Sahih. Al-Bukhari (Eds.). The collection of authentic sayings of Prophet Mohammad [peace be upon him] (2nd ed.). Ankara. Turkey: Hilal Yayinlari.

ARA N., CHOUDHURY S. A. R., AMIN R. 2005. *In vitro antimicrobial activity of the volatile oil of Nigella sativa Linn seeds. TAJ (J. Teachers Ass.)*. Rajshahi Medical College. Rajshahi. **18** (2): 109-112.

ASADUZZAMAN K., HAN-CHUN C., MOUSUMI T., DIANZHENG Z. 2011. *Anticancer activities of Nigella sativa (Black Cumin).* African Journal of Traditional, Complementary, and Alternative Medicines. AJTCAM, PUBMED/MEDLINE, Health System Trust. South Africa. **8**(5S): 226-232.

ATIF AHMED SAID A. M. 2013. *The protective effect of ginmger (Zingiber officinale) against adriamycin-induced hepatotoxicityy in rats: histiological study*. Life Science Journal. **10**(1): 1412-1422.

AVINASH KUMAR REDDY G., PRIYANKA B., SAI SARANYA G. H., ASHOK KUMAR C. K. 2012. *Woundheating potential of Indian medicinal plants*. Intern. J. Pharm. Rev. Res. **2**(2): 75-87.

BABAZADEH B., SADEGHNIA H. R., KAPURCHAL E. S. 2011. *Protective effect of Nigella sativa and thymoquinone on serum glucose deprivation-induced DNA damage in PC12 cells.* Avicenna Journal Phytomedicine. **2**(3): 125-132.

BAGHDADI H. B. & AL-MATHAL E. M. 2011. *Anti-coccidial activity of Nigella sativa L.* J. Food, Agric. Environ., WFL Publish Science & Technology. Helsinki. Finland. **9**(2): 10-17.

CHOWDHURY B. R., GARAI A., DEB M., BHATTACHARYA S. 2013. *Herbal toothpaste a possible remedy for oral cancer*. J. Nat. Prod. **6**: 44-55.

CORNEANU C. G., CRĂCIUN C., ROGOZ S., GRIGORESCU EM., IACOB M., CRĂCIUN V., CORNEANU M., ZAGNAT M. 2000. *The effect of the fatty oil from Nigella sativa seeds at Mus musculus I.* The ultrastructural modifications of the

hepatocytes and of some blood features. Acta Phytotherapica Romanica. Societatea de Fitoterapie din Romania. Biertan. **6**(1-2): 87-88.

CORNEANU C. G., DRĂGOI S. G., ROGOZ S., ŞUTRU C., CORNEANU M., ROGOZ I., ZAGNAT M., GRIGORESCU E., ATYIM P., MELINTE P. R., POPA M. 2004. *The single or combined action of X-rays and saponins or polyholosides in Mus musculus L. (spleen structure and immunoglobulins amount).* Buletin USAMV-CN. Edit. USAMV. Cluj-Napoca. **61**: 50-55.

CORNEANU C. G., CIUPINA V., PRODAN G., CORNEANU M., STEFANESCU I., ATYIKM P., IACOB M., POPA M. 2005a. *Efectul detoxifiant la nivelul ficatului si splinei la Mus musculus sub actiunea unor factori externi*. Analele SNBC. Edit. Risoprint. Cluj-Napoca. **10**: 285-290.

CORNEANU G., CRĂCIUN C., CIUPINĂ V., PRODAN G., CORNEANU M., ATYIM P., ŞTEFĂNESCU I., IACOB M., CORNEANU L.-M., 2005b. *The effect of Nigella sativa L. alkaloids and of deuterium-depleted water on Mus musculus L. spleen.* Rev. Cytol. Biol. Végét.-Le Botaniste. Edit. H. Couderc. Paris. **28** (s.i.): 84-91.

CORNEANU G. C., SILOSI I., ROGOZ S., CORNEANU M., HĂDĂRUGĂ N., HĂDĂRUGĂ D., ZAGNAT M., ŞTEFĂNESCU I., CORNEANU L. M., POPA M. 2006. *Efectul radioprotector al DDW si al uleiului volatil din semintele de Nigella sativa L. sau din cortexul de Aralia mandshurica L.* Analele SNBC. Edit. Risoprint. Cluj-Napoca. **11**: 487-493.

CORNEANU G., CORNEANU M., CRĂCIUN C., CIUPINĂ V., ZAGNAT M., ATYIM P., PRODAN G., DRĂGOI G. ŞT., ŞTEFĂNESCU I. 2012. *Bioactive substances from Nigella sativa seeds*. An. Acad. Rom. Sc., Agron. Forest and Vet. Med. Sc. Edit. Academiei Oamenilor de Ştiinţă din România. Bucureşti. **1**(1): 13-28.

CUMMINGS M. C., WINTERFORD C. M., WALKER N. I. 1997. *Apopthosis (Review).* Am. J. Surg. Pathol. Lippincott, Williams & Wilkins. Charlottensville, USA. **21**: 88-101.

DAVID RAJ C. JAYANTHI V., NANASWINI V.S., GAYATHRI C., BRINDHA P. 2012. *Effect of polyherbal formulation (OB-6) on high fat diet induced hyperlipidemia in rats*. Intern. J. Pharm. Pharm. Sc. **4** (suppl. 2): 31-35.

DIOSCORIDE PEDANIOS FROM ANAZARBA. 1529. *Peri huiules iatriches (De materia medica).* Edition of Marcelus Virgiliu, Köln.

EL-HADY EL-DESOKY M. & MOHAMADY M. 2011. *Ultrastructural studies on the effect of electromagnetic field on the liver of albino rats (Rattus norvegicus)*. Journal of American Science. **7**(2): 154-165.

EL-TAHIR H. & BAKEET D.M. 2006. *The black seed Nigella sativa Linnaeus - A mine for multi cures: a plea for urgent clinical evaluation of its volatile oil*. J. T. U. Medicine Science. **1**(1): 1-19.

FRAAS Č. 1845. *Synopsis plantarum florae classicae.* München.

FRANCIS G., KEREM Z., MAKKAR H. P. S., BECKER K. 2002. *The biological action of saponins in animal systems: a review.* British Journal of Nutrition. Cambridge University Press. Cambridge. U.K. **88**: 587-605.

GERIGE S. J., GERIGE M. K. Y., RAO M., RAO R. 2009. *GC-MS analysis of Nigella sativa seeds and antimicrobian activity of its volatile oil*. Braz. Arch. Biol. Technology BABT-Curitiba-PR. Brazil. **52**(5): 1189-1192.

GRYCOVÁ L., DOSTÁL J., MAREK R. 2007. *Quaternary protoberberrine alkaloids*. Phytochemistry. Elsevier. Amsterdam. **68**: 150-175.

HADJZADEH M. A. R, KHOEI A., HADJZADEH Z., PARIZADY M. 2007. *Ethanolic extract of Nigella sativa L. seeds and kidney calculus in rats*. Urology Journal. **4**(2): 86-90.

KAUSHIK P., MATHUR M., RAWAT N., DUTT P. 2012. *Radioprotective effect of alcoholic extract of Mentha piperita (Linn) on Swiss albino mice exposed to whole body gamma irradiation: a preliminary study*. International Journal of Pharma and Bio Sciences. Elsevier. Amsterdam. **3**(3): 598-610.

LENZ H. O. 1859. *Botanik der alten Griechen und Römer*. Gotha.

LUO H., HUANG J., LIAOP W.-G., HUANG Q.-Y., GAO Y.-Q. 2011. *The antioxidant effects of garlic saponins protect PC12 cells from hypoxia-induced damage.* British Journal of Nutrition |Cambridge University Press. U. K. **105**: 1164-1142.

MACSWEEN R. N. M., DESMET V. J., ROSKAMS T., SCOTHORNE R. J. 2002. *Developmental anatomy and normal structure*. In: MacSween, Burt A. D., Portman B. C., Isak K. G., Scheurer P. J., Antony P. P. 2002. *Pathology of the liver fourth ed.*). Churchill Livingstone. Edinburgh. **1**. 66 pp.

MANNAN A. & KAHVIC M. 2010. *Ibn Sina: A tribute.* The Gulf Journal of Oncology. The Gulf Federation for Cancer Control. Kuweit. **7**: 60–63.

MANSOUR M.A., GINAWI O.T., EL-HADIYAH T., EL-KHATIB A.S., AL-SHABANAH O.A., AL-SAWAF H.A. 2001. *Effects of volatile oil constituents of Nigella sativa on carbon tetrachloride-induced hepatotoxicity in mice: evidence for antioxidant effect of thymoquinone*. Res. Commun. Mol. Pathol. Pharmacol. British Pharmacol. Soc. **110** (3 + 4): 239-251.

MICHEL C. G., EL-DINE N. S., FAHMY S. M., AZZAT S. M., NESSEEM D .I., EL-ALFY T. S. 2010. *Phytochemical and biological investigation of the extracts of Nigella sativa L. seed waste.* Drug Test. Analysis. John Wiley & Sons Ltd. 10 pp.

MICHEL C. G., NESSEEM D. I., EL-SAYED N. S., EL-ALFY T. S. 2011. *Bioactive aqueous extract of Nigella sativa* L. *seed waste: formulation and adaptation*. Journal Chem. Pharm. Res. **3** (2): 213-225.

MOHAMED H. A., EL-SAYED I. H., MOAWAD M. 2011. *Protective effect of Nigella sativa seeds against dimethylaminoazobenzene (Dab) induced liver carcinogenesis*. Cancer Biology. **1** (1): 43-50.

MOHAMMED M. J., MAHMOOD M. T., YASEEN J. M. 2008. *Biological effect of saponins isolated from Nigella sativa (seeds) on growth of some bacteria.* University of Mosul. Iraq: 1-30.

MOHAMMAD YAHEYA & MOHAMMAD ISMAIL. 2009. *Therapeutic role of prophetic medicine Habbat El Baraka (Nigella sativa L.) - a review*. World Appl. Sci. J. Cornell NY Applied Sciences. New York. **7**(9): 1203-1208.

MOHAMMAD S. M. & KASHANI H. H. 2012. *Chemical composition of the plant Punica granatum (Pomegranate) and its effect on heart and cancer.* J. Med. Plants Res. Springer J. Life Sciences. Muenster. **6** (40): 5306-5310.

MOHAMED ATIF AHMED SAID. 2013. *The protective effect of ginger (Zingiber officinale) against adriamycin-induced hepatotoxicity in rats: histological study*. Life Science Journal. **10** (1): 1412-1422.

NALBANTSOY A., NESIL T., ERDEN S., ÇALIŞ İ., BEDIR E. 2011. *Adjuvant effects of Astragalus saponins Macrophyllosaponin B and Astragaloside VII.* J. Enthopharmacology. Elsevier. Amsterdam. **134**: 897-903.

NICKAVAR B., MOJAB F., JAVIDNIA K., ROODGAR AMOLI M. A. 2003. *Chemical composition of the fixed and volatile oils of Nigella sativa L. from Iran*. Z. Naturforsch. Vlg der Zeitschrift für Naturforschung, Tübingen. **58c**: 629-631.

ÖNNING G., WANG Q., WESTRÖM B. R., ASP N.-G., KARLSSON B. W. 1996. *Influence of oat saponins on intestinal permeability in vitro and in vivo in the root.* Brit. J. Nutr. **76**: 141-151.

PAWAR VINOD S. & HUGAR S. 2012. *A current status of adaptogens, natural; remedy to stress.* Asian Pacific J. Tropical Diseases Elsevier, Amsterdam: S480-S490.

PANDEY G. 2011. *Some important anticancer herbs: a review*. Intern. Res. J. Pharmacy, IRJP. http://www.irjonline.com. **2** (7): 45-52.

PLINIUS CAIUS SECUNDUS. 1668. *Naturalis Historia* (Ed. Hackios). Lugdum Batavolum. Roterdami.

ROGOZ I., HERZOG Z., ROGOZ S., CORNEANU G., SABETAY C., CORNEANU M., DOBRESCU A. 2002. *Separarea prematura a cromatidelor surori.* Primul Congres Naţional de Genetică Medicală. Rezumate. Universitatea Oradea. 54 pp.

ROSHAN S., ABDULLAH K., TAZNEEM B., SADATH A. 2010. *To study the effect of Nigella sativa on various biochemical parameters on stress induced in albino rats.* Int. J. Pharm. Sci., Elsevier, Amsterdam. **2**(4): 185-189.

SALEM M. L. 2005. *Immunomodulatory and therapeutic properties of the Nigella sativa L. seed*. Int. Immunophma
cology. Elsevier. Amsterdam. **5**(13-14): 1749-1770.

SHABANA A., EL-MENIAR A., ASIM M., AL-AZZEH H., AL THANI H. 2013. *Cardiovascular benefits of black cumin (Nigella sativa)*. Cardiovascular, Toxicol. Elsevier. Amsterdam. **19**: 9-21.

SHARMA N. K., AHIRWAR D., JHADE D., GUPTA S. 2009. *Medicinal and pharmaceutical potential of Nigella sativa.* Etnobotanical Review. Polish Botanical Soc. J. **13**: 946-955.

SLUNSKA Z., GELNAROVA E., HAMMEROVA J., TÁBORSKÁ E., SLANIONOVA I. 2010. *Effect of quaternary benzo[c]phenanthrine alkaloids sanguilutine and chelilutine on normal and cancer cells.* Toxicol *in vitro*. Elsevier B.V. Amsterdam. **24**: 697-706.

STEVENSON. D. E. 2012. *Polyphenols as adaptogens - the real mechanism of the antioxidant effect?* In: I RASOOLI
(Ed.). Bioactive compounds in phytomedicine. In: Tech Europe, Rijeka, Croaţia. www.intechopen.com. 143-162. 218 pp. (Accesed: February 22, 2013).

TAŞKIN M. K., ALANKUŞ ÇALIŞKAN Ö., ANIL H., ABOU-GAZAR H., KHAN I. A., BEDIR E. 2005. *Triterpene saponins from Nigella sativa L.* Turk J. Chem. Tübitak. Izmir. **29**: 561-569.

Corneanu C. Gabriel
University of Craiova, Genetics Lab., A.I. Cuza 13,
200585-Craiova, Romania.
E-mail: gabicorneanu@yahoo.com

Corneanu Mihaela
University of Agricultural Sciences and Veterinary Medicine of Banat,
Genetics Laboratory,
Calea Aradului 119, 300615-Timişoara, Romania.
E-mail: micorneanu@yahoo.com

Crăciun Constantin
Babes-Bolyai University,
Electron Microscopy Center,
Clinicilor Street, 5-7,
400007-Cluj-Napoca, Romania.
E-mail: ccraciun@hasdeu.ubbcluj.ro

Received: March 29, 2013
Accepted: Jun 6, 2013

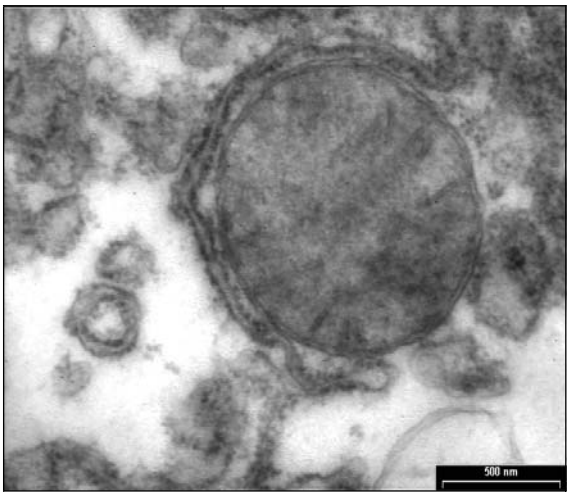

Figure 1. Control. Normal structure of mitochondria (after CORNEANU et al., 2012).

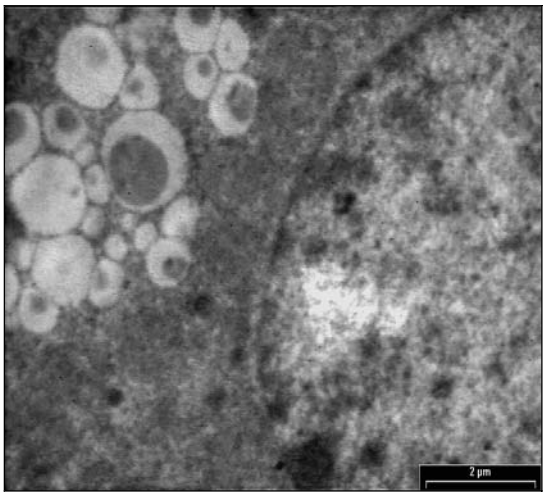

Figure 2. Control. Normal structure of nucleus (after CORNEANU et al., 2012).

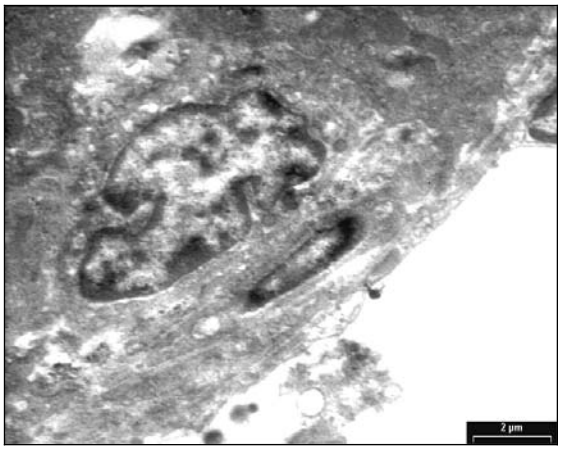

Figure 3. X-rays action. Kupffer cell in activity. (after CORNEANU et al., 2012).

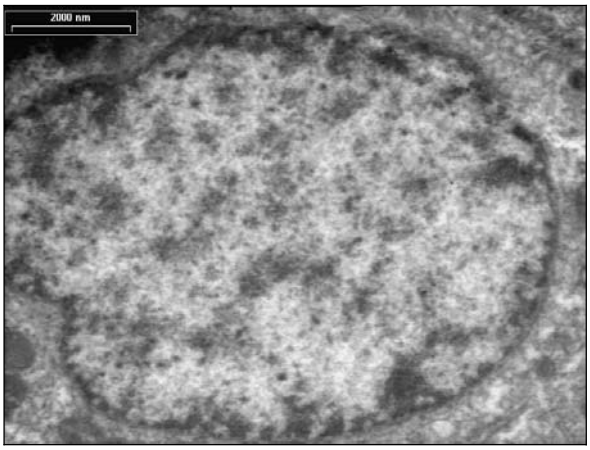

Figure 4. Alkaloids in distilled water. Nucleus ultrastructure.

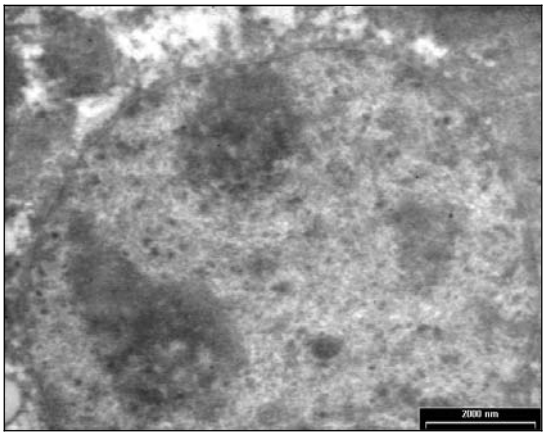

Figure 5. Alkaloids in distilled water, X-rays. Nucleus with normal structure (after CORNEANU et al., 2012).

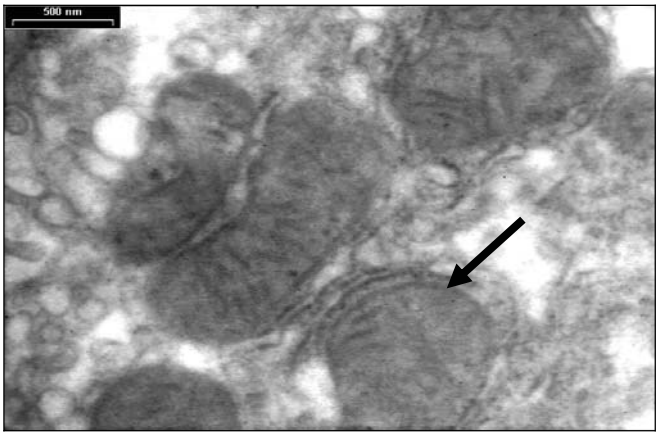

Figure 6. Alkaloids in distilled water, X-rays. Mitochondria with normal or metabolic adulterate structure (arrow).