NEONİKOTİNOİD PESTİSİTLERİN HİSTOPATOLOJİK VE GENOTOKSİK ETKİLERİ

The Histopathological and Genotoxic Effects of Neonicotinoid Pesticides

Özlem ÖNEN[1], Pınar AKSU KILIÇLE[1], Yasemen ADALI[2], Hatice BEŞEREN[3]

[1]Kafkas Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, Kars

[2]Çanakkale Onsekiz Mart Üniversitesi, Tıp Fakültesi, Tıbbi Patoloji Anabilim Dalı, Çanakkale

[3]Kafkas Üniversitesi, Veteriner Fakültesi, Patoloji Bölümü, Kars

Özlem ÖNEN, Dr.
Pınar AKSU KILIÇLE, Dr.
Yasemen ADALI, Dr.
Hatice BEŞEREN, Dr.

İletişim:
Dr. Özlem ÖNEN
Kafkas Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, Kars
Tel: 0 (474) 225 11 50-56
e-mail:
onenozlem@gmail.com

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ÖZET

Pestisitler, toksik etkileri ve birikimleri nedeniyle insan ve çevre sağlığı için en tehlikeli kirleticilerden biridir. Bu derlemede günümüzde yaygın olarak kullanılan pestisit gruplarından neonikotinoidlerin insanlar üzerindeki etkilerine dair elde edilen verilerin değerlendirilerek gelecekteki çalışmalar için özetlenmesi amaçlanmıştır.
Mevcut literatür bilgileri, Kafkas Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Zooloji-Ekotoksikoloji ve Moleküler Biyoloji-Genetik Anabilim Dalları Laboratuvarları ve Tıp Fakültesi, Patoloji Anabilim Dalı Laboratuvarındaki çalışmalar ışığında gözden geçirilerek derleme halinde düzenlenmiştir.

Canlıda oluşan serbest radikaller, lipit, karbonhidrat, protein ve nükleik asit gibi biyomolekülleri ya da hücresel komponentleri etkileyip yeni serbest radikalleri oluşturur. Bu da hücrede yapısal hasarlar meydana getirerek metabolik değişikliklere yol açmaktadırlar. Bu histopatolojik ve genotoksik derlemede anlatılan çalışmalar çerçevesinde neonikotinoidlerin omurgalılarda son derece toksik oldukları ve omurgalılarda hızlı metabolize olamadıklarından akut toksisitelerinin yüksek olduğu ortaya konmuştur. Gelişmiş tekniklerin kullanıldığı ileri düzeydeki bütün araştırmalar, histolojik ve genotoksik düzeyde temel verilere dayanarak gerçekleştirilebilir ve histopatolojik ve genotoksik araştırma yöntemleri özel alanlardaki araştırmaların temel anahtarıdır.

Anahtar Sözcükler: *Neonikotinoid pestisitler; Memeliler; İnsan; Histopatoloji; Genotoksisite.*

ABSTRACT

Pesticides are one of the most hazardous pollutants for human and environmental health due to their toxic effects and accumulation. In this review, it was aimed to summarize the literature which can be a source for future studies by evaluating the data about histological and genotoxic effects of neonicotinoids, one of the insecticide groups commonly used today, on vertebrates.

The available literatural information arranged as compilation by revised in accordance with the works in the Kafkas University, Faculty of Arts and Sciences, Department of Biology, Zoology-Ecotoxicology and Molecular Biology-Genetics Laboratories and Medicine Faculty, Pathology Laboratory.
Free radicals occured in vivo create new free radicals by affecting biomolecules such as lipids, carbohydrates, proteins, and nucleic acids and cellular components. This also leads to metabolic changes by causing structural damage in the cell. It was demonstrated that neonicotinoids are extremely toxic to vertebrates and the acute toxicity of neonicotinoids is high because they cannot metabolize rapidly in vertebrates within the context of the studies described in this histopathological and genotoxic review. All further investigations using advanced techniques can be performed based on histological and genotoxic levels and histopathological and genotoxic research methods are the fundamental key of researches in specific fields.

Keywords: *Neonicotinoide pesticides; Mammalia; Human; Histopathology; Genotoxicity.*

INTRODUCTION

Pesticides are generally used to combat pests in agriculture, forestry, community health and veterinary medicine, but they can also negatively affect non-target organisms such as useful plants, insects, invertebrates and some vertebrates (1-4).

Exposure to pesticides in very low quantities can lead to the exposure of animals to residual concentrations of insecticides directly or through the involvement of stress mechanisms/neuroendocrine system, leading to immunosuppression (5). In recent years, too much use of neonicotinoids in agriculture has contributed to soil retention and soil retention (7) as well as contamination of groundwater (7).

It has been found that pesticides in the direction of the data obtained from the studies made are quite toxic not only for a certain living group but also for other organisms constituting the food chain (8-15).

Neonicotinoids, which form an important group of pesticides, are synthetic analogues of natural nicotine insecticides and nicotinic receptor agonists are used for the treatment of sprays, liquid medicines, seeds and soil (16-21). Neonicotinoids are the main class of insecticides developed over the last 30 years and are used as systemic insecticides to increase fertility in plants (22).

Neonicotinoid group pesticides are used alternatively as organophosphate and carbamate pesticides (23-27). In this context, a study investigating the effects of neonicotinoids on some insects has been reported to find leg tremors, rapid wing movements, stiletyline withdrawal (aids), irregular movement, paralysis and death in insects subjected to acetamiprid, clothianidin, imidacloprid, nitenpyram, nithiazine, thiacloprid and thiamethoxam (21, 28, 29).

Nicotine and nicotinoids contain a predominantly protonated nitrogen atom at physiological pH, causing a higher affinity to vertebrate nicotinic acetylcholine receptors (nAChR) and hence a higher mammalian toxicity, a weak to moderate affinity for the insect receptor. Significantly contrasted neonicotinoids contain a N-non-protonated nitroimine structure at physiological pH, which plays a critical role in high affinity and selectivity for insect nicotinic acetylcholine receptors (30).

Neonicotinoids are nAChR potent selective agonists in insects and are widely used in plant protection and animal health practices. Since the introduction of imidaclopridine in the early 1990s, neonicotinoids have become one of the most widely used pesticide classes. From the insecticides, the neonicotinoid class still represents about 15% of the global world market (11, 31 and 32).

Mechanisms of action are explained by the stimulation of nicotinic receptors of acetylcholine. It stimulates the autonomic nervous system and nicotinic receptors in skeletal muscles. It also stimulates acetylcholine receptors in the central nervous system and cause long-term depolarization. During this time paralysis occurs because the nerves cannot respond to the incoming warning. Immediate disintegration affects toxicities in the environment. Moreover, it has been reported that nicotinoids cannot be degraded by the serum acetylcholinesterase enzyme (21, 33).

Neonicotinoids have high selectivity for nicotinic acetylcholine receptors (nAChRs) in insects and thus produce selective toxicity to insects in invertebrates. Studies to date have probably contributed to the formation of hydrogen bonds and the selectivity of electrostatic interactions between neonicotinoids and insect nAChRs (34). Specific subunit combinations differentiate between acetylcholine susceptibility and pharmacological profiles among vertebrate nAChR subspecies (35).

According to the World Health Organization, around three million people in the world are poisoned annually due to pesticides, and a large proportion of these people are reported to have died. Neonicotinoid pesticides have been reported to inhibit nicotinic acetylcholinesterase receptors and thus directly affect the central nervous system and suppress cellular responses at the cellular level (10).

Due to the rapid progress in industrialization, environmental pollution has increased over time, causing living things, especially people, to be exposed to chemical and physical factors. Investigating the effects of these factors on living beings has attracted the attention of researchers. It is extremely important to identify the harmful effects of these carcinogenic, toxic, mutagenic and teratogenic agents and to take precautions. Many chemicals, especially pesticides, have increased their concern that they could enter the human body directly and indirectly, affect health, and create a potential danger in genetic material (36).

Genotoxicity is the damage caused by chemical and physical agents in the DNA material. These damages are single chain fractures, double chain fractures, alkali labile regions and DNA adducts. It is suggested to study at least two methods in genotoxicity studies instead of one method (37).

DNA sequence changes, chromosomal aberrations, multiple nucleotide changes, resulting in mutation, aging, tissue damage, and cancer can occur when genetic material is not repaired. Molecular studies have shown that genotoxicity is associated with mutations (37, 38).

They have to be very resistant to environmental conditions, repeated applications and widespread use of ecosystems to increase the pollution of nicotinoids and adversely affect systems. It has been reported that invertebrates are particularly adversely affected (11, 21). For this reason, legal restrictions have been introduced, in part, on production and use in some countries (39-41). Misuse and widespread use of invertebrates can lead to poisoning in animals (18). Pesticides that absorbed through the digestive system reach the nervous system (6, 42 and 43). Metabolized and expelled from the organism by urine and bile (44, 45).

In this review, it was aimed to summarize the histological changes and genotoxic effects of certain tissues of mammals from model organisms in order to determine the effects of neonicotinoids on humans which are non-target organisms.

MATERIALS AND METHODS

The available literatural information arranged as compilation by revised in accordance with the works in the Kafkas University, Faculty of Arts and Sciences, Department of Biology, Zoology-Ecotoxicology and Molecular Biology-Genetics Laboratories and Faculty of Medicine, Faculty of veterinary medicine.

RESULTS

Histological changes can be used as sensitive tools to detect the direct toxic effects of various compounds and are considered good indicators of environmental stress (46, 47). Histopathological studies play a supporting role by providing more information about possible mechanisms of action of pesticides on biomarkers at the cellular and molecular level and on non-target organisms (48).

Histopathological Changes

Imidacloprid was given orally to female albino mice (Mus musculus) at different doses (37.5, 75 and 112.5 mg/kg) to determine the effects of imidacloprid on mammals from highly preferred neonicotinoid pesticides. There was a significant increase in total protein ($P<0.01$), acetylcholinesterase ($P<0.001$) and DNA ($P<0.05$) with dose increase compared with the control group, although there was a significant increase in the RNA due to mild dose at the end of the administration. It is noted that the change in DNA and RNA for low dose imidacloprid is insignificant and that the maximal damage is observed in high dose imidacloprid exposure. In the liver, hepatocyte degeneration, dilation in the sinusoids, irregularity in hepatocyte alignment, leukocyte infiltration, necrosis and hemorrhage were recorded (Figure 1, 49). In another study imidacloprid was conducted in female rats with doses of 0, 5, 10, 20 mg/kg/day. The brain, liver and kidney of rats had showed mild pathological changes in high dose imidacloprid group. It was reported that it is conspicuous that imidacloprid has induced toxicological effects at 20 mg/kg/day to female rats, based on the histological studies in this research (50).

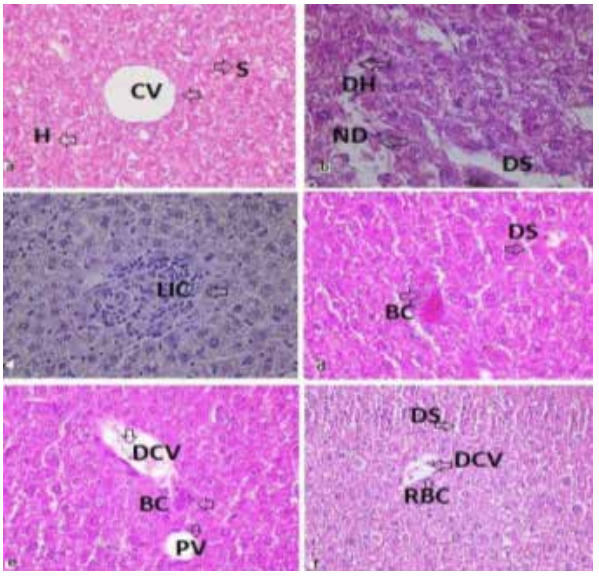


Figure 1. Liver transection. a. Control group; CV: vena centralis, H: hepatocytes, S: sinusoids; b. Positive control group (after cyclophosphamide exposure); DH: degenerated hepatocytes, DS: dilation in sinusoids, ND: necrotic residues; c. After 37.5 mg / kg imidacloprid exposure, leukocyte infiltration is observed; d. After 75.5 mg/kg imidacloprid exposure; BC: congestion, DS: dilation in sinusoids; e-f. After 112.5 mg/kg imidacloprid exposure; DCV: vena centralis corruption, congestion in dilated sinusoidal, accumulation of erythrocytes in vena centralis (49).

And also, rats were orally administered with imidacloprid (45 and 90 mg/kg b.w.) for the period of 28 days. It was noticed that, brain damage was occured as result of imidacloprid administration in rats (51).

Similarly, in another study to determine the adverse effects of imidacloprid, is a systemic insecticide related to the tobacco toxin nicotine, frequently used into plant pests and other creatures that can pose problems for agriculture. As a result of this study, liver and kidney histology notice high levels of hepatotoxicity and nephrotoxicity in imidacloprid. And also, it was reported that based on histopathological analyzes, it is clear that imidacloprid induces toxicological effects at 15 mg / kg / day in rats (52).

Mice were exposed intraperitoneally to the concentrations of these two pesticides (acetamiprid and propineb) for 24 and 48 hours in order to determine the effects of neonicotinoid pesticides and their co-exposure to dithiocarbamate pesticides. For the histopathological examinations, an experimental setup consisting of a total of fifteen groups consisting of three positive control groups, three negative control groups and nine exposure groups was established. The effects of mice on liver tissue were determined by light microscopy. Significant vacuolar degeneration was reported to occur at 24 and 48 hours after and sinusoidal dilatation after 48 hours of injection of acetamiprid of at low concentration (0.625 µg/ml). At 24 and 48 hours after the injection of the acetamiprid and propineb mixture, sinusoidal dilatation in the parenchyma and vacuolar degeneration in the hepatocytes were observed, unlike the negative control. Moreover, when these pesticides were compared with their individual applications at the same concentrations, it was found that the acetamiprid and propineb mixture resulted in synergistic effects in several dimensions. The results obtained led researchers to conclude that acetamiprid and propinebin mice had devastating effects on liver tissue. In this respect, the use of these pesticides in agricultural areas should be under control (53).

Genotoxic Changes

In a study conducted to determine acetamipridine genotoxicity from commonly used neonicotinoid group insecticides, in vitro cytotoxicity and genotoxicity of acetamipridine in human intestinal Caco-2 cells was studied. As a result, under experimental conditions, acetamipridine reported cytotoxic and genotoxic potential in human intestinal cells (54).

To determine the toxic effects of neonicotinoid pesticides on humans in Mexico, human peripheral lymphocytes were exposed to different concentrations of thiacloprid, clothianidin and imidacloprid from neonicotinoids; Genotoxic and cytotoxic effects were assessed by assessing the data obtained from the Alkaline Comet test and the Trypan blue exclusion test. DNA damage was assessed using genotoxicity parameters such as tail length and Comet frequency. The reported neonicotinoid pesticides have a marked increase in DNA damage occurring in parallel with increasing concentration after two hours of exposure. The toxicity was found to be the most toxic pesticide of imidacloprid from the investigated pesticides and the presence of cytotoxic and cell death was mentioned.

This study is important because it is the first report of in vitro exposure of human peripheral lymphocytes to neonicotinoid pesticides (9).

In a study to determine the genotoxic effects of imidacloprid from widely used neonicotinoid pesticides in agriculture, the human peripheral lymphocytes from healthy non-exposed volunteers were exposed to different concentrations of imidacloprid and imidaclopridine commercial formulations (0.2, 2 and 20 µM). The obtained data were evaluated in vitro using Comet and micronucleus test in the context of formulation, metabolic activation and exposure level. Commercially, it is formulated with dimethyl sulphoxide, methylpyrrolidone, propylene carbonate and mineral oil, which can change the bioavailability and toxicological profile for humans following occupational exposure. High concentrations of imidacloprid exposure were reported to significantly increase micronucleus frequency and Comet score in the results obtained. Although not evident by metabolic activation, it was reported that the effect of imidaclopridine commercial formulation led to slightly more severe DNA damage. Although the formation of reactive oxygen species is not considered a mechanism of genotoxicity, this result can be explained by the insufficient sensitivity of the 2α, 7a-dichlorofluorescein diacetate test at test concentrations of imidacloprid. These results indicate that imidacloprid is not genotoxic in vitro for human lymphocytes at concentrations lower than 20 µM. However, it is commented that the presence of other chemicals involved in the commercial formulation of imidacloprid, poor safety procedures and occupational exposure increases risk of DNA fragmentation and chromosomal aberration (55).

Thiacloprid was investigated in vitro for potential genotoxic effects on human peripheral blood lymphocytes, from chromosomal aberrations, sister chromatid exchanges and cytokinesis-block micronucleus analyzes, from the neonicotinoids commonly used to control plant pest insect species. Chromosomal aberrations, sister chromatid exchanges and micronucleus counts were observed in peripheral blood lymphocyte cells treated with 75, 150 and 300 µg/mL thiacloprid. Thiacloprid has been reported to significantly reduce mitotic index, proliferation index, and nuclear division index in all concentrations (56).

The cytotoxic effect potentials of the acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam from neonicotinoid group insecticides were investigated using MTT and NRU test results of 24 hours and 48 hours exposure to human hepatocellular carcinoma (HepG2) and neuroblastoma (SH-SY5Y) cells from high vertebrates. IC50 values of neonicotinoids were determined as 0.65→4 mM in SH-SY5Y cells after 24 and 48 hours exposure, while IC50 values in HepG2 cells were determined as 0.40→4 mM. As a result, it has been noted that neonicotinoids have a higher cytotoxic effect on HepG2 cells compared to SH-SY5Y cells (57).

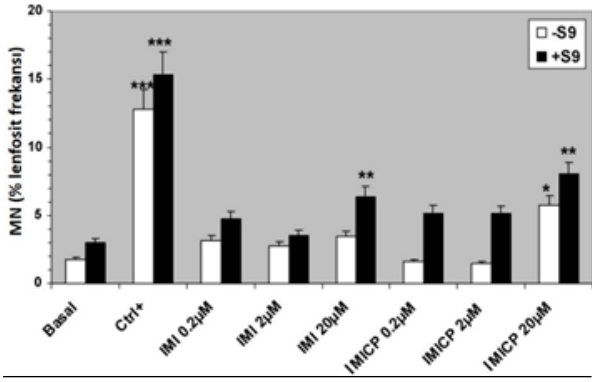
Imidacloprid and Metalaxyl are two pesticides commonly used either separately or in combination in agriculture. In one study, the effects of these chemicals on the micronucleus and sister chromatid exchange frequencies in vitro in human peripheral lymphocytes and on the micronucleus ratio in polychromatic erythrocytes in rat femur bone marrow cells in vivo were investigated. It has been observed that there is no significant difference in in vitro micronucleus frequency and sister chromatid exchange ratio compared with control in co-administered and separately applied chemistries. A significant increase in the number of micronuclei in polychromatic erythrocytes was reported in bone marrow of rats (58).

In another research, neonicotinoids have been reported to cause especially sister chromatid exchange in the molecular sense (59), DNA damage (60), micronucleus formation (61), chromosomal abnormalities (62), DNA adducts (63). And also acetamiprid causes increase in micronucleus frequency, sister chromatid exchange and chromosomal anomalies in human peripheral blood lymphocytes (64). In addition to thiamethoxam, reported to be carcinogenic, causes liver tumors in male and female rats (65).

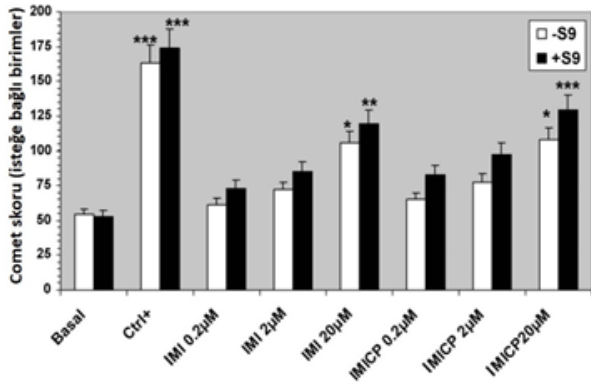
Genotoxicity tests can be used to determine whether a variety of chemical substances and environmental pollutants cause mutations in a short time.

Determination of whether a chemical substance is cytotoxic to the genotoxic side; chromosome aberration, micronucleus, and sister chromatid exchange tests are used (66-68). These tests, which have been in use since the 1970s, help physical and chemical agents measure and evaluate carcinogenic effects on all living organisms (69).

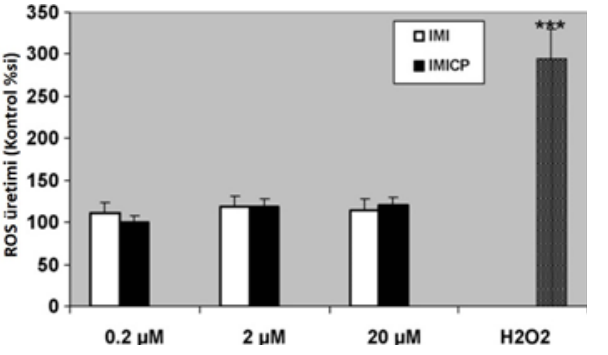
Graphic 1. Effects of pure imidacloprid (IMI) concentrations on human peripheral lymphocytes blocked by metabolic biotransformation (+S9) and without (-S9) on cytokine-blocked lymphocytes and micronucleus frequency (mean ± standard deviation) of commercial preparation (IMICP). Ctrl+: 56 µg/ml CPA (+S9) or 0.17 µg/ml MMC (−S9). *=p < 0.05, ** = p<0.01, *** = p<0.001 (Significant differences between culture treated with Dunn's test and Basal, 52)



Graphic 2. The effects of imidacloprid (IMI) and commercial product (IMICP) concentrations as pure compounds on Comet score (mean ± standard deviation) in human peripheral lymphocytes with (+S9) and without (-S9) metabolic biotransformation. Ctrl+: 100 µM H2O2. *=p < 0.05, ** = p< 0.01, *** = p<0.001 (Significant differences between culture treated with Dunn's test and Basal, 52)



Graphic 3. Effects on imidacloprid concentrations (IMI) as pure compound and on reactive oxygen species production (ROS) with human T lymphocytes (Jurkat cell line) after 24 hours exposure to commercial product (IMICP). Ctrl+: 100 µM H2O2. *** = p < 0.001 (Significant differences between culture treated with Tuckey's test and Basal, 52)



DISCUSSION AND CONCLUSION

The findings of hyperplasia and hypertrophy reported in the gills are in a barrier state that prevents the passage of exposed neonicotinoids into the circulatory system and in this context the respiratory surfaces that have been contracted by the veins may thicken and the passage of the pesticide to the blood tissue may be prevented in part. Energy metabolism in the context of enzyme activities and changes in the gill structure of teleosts with exposure to neonicotinoids are considered together and it is clear that the reduction in the amount of oxygen required for physiological functions will lead to adverse effects on behaviors such as movement, nutrition, defense and escape. The most similar histological change observed in the liver and kidney structures of the teleosts as a result of neonicotinoid administration, besides being a steatosis in the appearance of vacuolization, besides the fact that it does not have a specific character to a particular chemical as emphasized many times; and it is clear that the necrotic formations reported in liver and kidney structures are typical variations due to toxicity in the organs that are aforementioned. It is well known that tissue damage as an initial step is due to the enzymatic inhibition of cell membrane integrity and this can severely degrade both the detoxification mechanisms and the liver and other metabolic functions such as protein-carbohydrate synthesis. It is evident that degenerations reported in teleosts, both in interrenal cells and renal tubules, primarily due to the pesticide

group, may lead to individual and more massive deaths in fish populations, with subsequent disruption of renal function and subsequent increase in exposure time and concentration. In this direction, it is beneficial to monitor the ever-increasing pesticide use and the changes that have taken place in the organisms in the researches done to protect biodiversity. Advanced studies can be carried out based on the baseline data obtained at the histological level and at the level of practical genotoxic investigations, and histopathology and baseline gentoxicity markers; the starting line of research in special areas. It is a universal responsibility in the context of continuity of the environment that agricultural governments in developed countries control the use of neonicotinoid compounds as pesticides and more importantly encourage the use of organic pesticides over chemical pesticides.

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