Kocatepe Veterinary Journal

Kocatepe Vet J (2020) 13(2):214-218
DOI: 10.30607/kvj.710779

RESEARCH ARTICLE

Protective Effects of Taurine on Imidacloprid-Induced DNA Damage and Reproductive Performance in The *Drosophila melanogaster* Model

Damla ARSLAN ACARÖZ[1*], Sinan İNCE[2], Fahriye ZEMHERİ NAVRUZ[3], Nalan BAYŞU SÖZBİLİR[4]

[1]Afyon Kocatepe University, Bayat Vocational School, Afyonkarahisar, Turkey
[2]Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Afyonkarahisar, Turkey
[3]Bartın University, Faculty of Science, Department of Molecular Biology and Genetics, Bartın, Turkey
[4]Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Biochemistry, Afyonkarahisar, Turkey

ABSTRACT

Imidacloprid is a neonicotinoid group insecticide and is widely used in veterinary medicine for the control of pests such as lice and fleas in domestic animals. Insecticides are known that they induce toxic effects on living organism, causing oxidative stress and DNA damage. Taurine plays a role in many physiological and biochemical functions and provides an antioxidant effect by stabilization of biological membranes. This study investigated the effect of imidacloprid on DNA damage and reproductive performance and the possible protective effect of taurine in *Drosophila melanogasters*. Imidacloprid (0.6 μM) alone or in combination with taurine (1, 2 ve 3 mM) were given to broths for 20 days. The results of the study showed that imidacloprid application decreased reproductive performance and increased DNA damage in groups, whereas these effects decreased with taurine administration. In conclusion, it was determined that the adverse effects of imidacloprid regarding DNA and reproductive performance in *Drosophila melanogasters* were prevented by taurine application.
Keywords: DNA damage, *Drosophila melanogaster*, imidacloprid, taurine

***

Drosophila melanogaster Modelinde Imidakloprid ile İndüklenen DNA Hasarı ve Üreme Performansı Üzerine Taurinin Koruyucu Etkileri

ÖZ

İmidakloprid, neonikotinoid grubu bir insektisid olup evcil hayvanlarda bit ve pire gibi zararlı böceklerin kontrolü için veteriner hekimlik alanında yaygın olarak kullanılmaktadır. İnsektisitlerin canlılarda toksik etki gösterdiği, oksidatif strese ve DNA hasarına neden olduğu bilinmektedir. Taurin birçok fizyolojik ve biyokimyasal olayda rol almakta ve biyolojik membranlarda stabilizasyonunu sağlayarak antioksidan etki göstermektedir. Bu çalışmada imidaklopridin DNA hasarı ve üreme performansına etkisi ve buna karşın taurinin olası koruyucu etkisi *Drosophila melanogaster*'lerde araştırıldı. İmidakloprid (0,6 μM) tek başına ve taurin (1, 2 ve 3 mM) ile birlikte besi yerlerine 20 gün boyunca ilave edildi. Çalışmanın sonuçları, imidakloprid uygulamasının gruplarda üreme performansını azalttığını ve DNA hasarında artış meydana getirdiğini, buna karşın taurin uygulaması ile bu etkilerin azaldığını gösterdi. Sonuç olarak *Drosophila melanogaster*'lerde imidaklopridin DNA ve üreme performansında yol açtığı olumsuz etkilerin taurin uygulaması ile engellendiği belirlendi.
Anahtar Kelimeler: DNA hasarı, *Drosophila melanogaster*, imidakloprid, taurin

To cite this article: Arslan Acaröz D. İnce S. Zemheri Navruz F. Bayşu Sözbilir N. Protective Effects of Taurine on Imidacloprid-Induced DNA Damage and Reproductive Performance in The Drosophila melanogaster Model. Kocatepe Vet J. (2020) 13(2):214-218

Submission: 29.03.2020 **Accepted**: 25.05.2020 **Published Online**: 26.05.2020
ORCID ID; DAA: 0000-0001-9230-6725, Sİ: 0000-0002-1915-9797, FZN: 0000-0003-1744-1091, NBS: 0000-0002-2270-8312
*Corresponding author e-mail: darslan@aku.edu.tr

INTRODUCTION

Pesticides can be defined as preparations or substances that are employed to control, repel, destroy pests including weeds, insects, rodents, fungi and molds. They possess many different groups and are classified based on their target pests (Costa 2013; Richardson 2019). Pesticides are essential both in agriculture and public health. However, their molecular targets are mainly found to be in pests and also in non-target species such as humans. This situation especially involves neurotoxic pesticides (Bonner and Alavanja 2017, Richardson et al. 2019). Neonicotinoid pesticides have been firstly introduced at the beginning of the 1990s, and widely employed as insecticides throughout the world in many areas including veterinary, agriculture, and residential environment due to their ease of application and high efficacy against insect controls (Zhang et al. 2018). Imidacloprid appeared in the global market as the first representative of neonicotinoids and became best-selling insecticide worldwide (Amjad et al. 2018). Imidacloprid exhibits its effect by ingestion or contact and shows its mode of action by interfering with many nicotinic acetylcholine receptors of the nervous system. This insecticide binds irreversibly to receptors, discharges nerve impulses, and causes failure of a neuron. Imidacloprid has a lower binding affinity towards the nicotinic receptors of mammals than that nicotinic receptors of insects (Kumar et al. 2013).

Taurine is a sulphur containing amino acid that is abundantly found intracellular in humans. This amino acid plays important role in many physiological and biological functions such as cholestasis prevention, bile acid conjugation, osmoregulation, membrane stability, neuromodulation of the central nervous system, metabolic properties and antioxidant effects (Lourenço and Camilo 2002, Ince et al. 2017). Taurine deficiency may be resulted in some pathological conditions such as renal dysfunctions, cardiomyopathy, loss in retinal photoreceptors and dysfunctions in pancreatic β cells (Ince et al. 2018). Also, some studies showed protective effect of taurine against genotoxic damage (Türkez and Geyikoğlu 2010, Alam et al. 2011).

Drosophila melanogaster, a eukaryote, has a very short and rapid reproductive rate and its many biological, physiological and neurological features are similar to that of mammals. (Pandey and Nichols 2011, Miguel-Aliaga et al. 2018). It is employed as a model organism in oxidative stress (Soares et al. 2017), genotoxicity (Mukhopadhyay et al. 2004) studies.
The present study aimed to investigate the effect of taurine against imidacloprid-induced DNA damage and reproductive performance in a *Drosophila melanogaster* model.

MATERIALS and METHODS

Imidacloprid was provided by Biyoteknik A.Ş. (Istanbul, Turkey) while taurine was purchased from Sigma-Aldrich (St. Louis, MO, USA). In the preparation of culture medium 6 g agar, 94 g sugar, 104 g cornflour, 9 g beer yeast, 6 ml acid mixture (7.83 ml orthophosphoric acid + 8.36 ml propionic acid + 1081 ml distilled water) and 1020 ml distilled water were used.

Drosophila melanogaster cultures were allocated into five groups: Group I served as a control, Group II was given only imidacloprid at the dose of 0.6 μM, Group III, IV and V were given both same doses of imidacloprid with Group II and different doses of taurine (1, 2 and 3 mM, respectively). The dose of imidacloprid was determined based on the previous study (Charpentier et al. 2014). Each experimental group possessed 10 male and 10 female unpaired and mature *Drosophila melanogaster* inoculated in glass culture flasks. These flasks contained 50 ml of broth and the incubation period was 20 days. *Drosophila melanogaster* cultures were incubated at 60-70% humidity and 24±1°C under laboratory conditions.
The present study evaluated pupa numbers and reproductive performance of Drosophila melanogaster groups. The data obtained for pupa numbers and reproductive performances in *Drosophila melanogasters* were expressed as numbers and percentages. Also, DNA damage (Olive and Banáth 2006) was determined by Comet analysis and expressed as an arbitrary unit (AU). Duncan post-hoc test was performed with one-way analysis of variance (SPSS 20.0) in DNA damage assessment. Statistically, $p < 0.05$ value was considered significant.

RESULTS and DISCUSSION

The effects of imidacloprid alone and in combination with taurine on pupa development and reproductive performance in *Drosophila melanogasters* were given Table 1 while their effects on DNA damage findings were shown in Figure 1. According to the results of the present study, imidacloprid application caused a decrease in pupa development and reproductive performance. However, the concomitant application of taurine increased development and reproductive performance compared to the alone application of imidacloprid. Also, DNA damage in *Drosophila melanogasters* was found to be higher in the imidacloprid treated group compared to the control group ($p<0.05$) whereas the taurine application reduced imidacloprid-induced DNA damage ($p <0.05$).

Toxic and genotoxic effects of imidacloprid were evaluated in different models. Feng et al. (2005) assessed the genotoxic effects of imidacloprid in human peripheral blood lymphocytes by sister

chromatid exchanges (SCE) and micronucleus tests (MN) and comet assay. They reported that imidacloprid significantly affected the frequencies of SCE and MN ($P<0.05$) compared to negative controls. Also, comet assay results showed that DNA damage was significantly different in imidacloprid (0.05, 0.1 and 0.5 mg/l) treated group than the control ($P<0.01$). Zhang et al. (2000) evaluated genotoxicity of imidacloprid for the earthworm, *Eisenia fetida* by the comet assay, sperm deformity assessment, *V. faba* micronucleus tests, and a mouse bone-marrow micronucleus test. Sperm deformity test showed that imidacloprid levels of more than 0.5 mg/kg dry soil significantly induced sperm deformity ($P<0.01$). *V. faba* micronucleus tests and the mouse bone-marrow micronuclei test did not show significant differences ($P>0.05$) compared to the control group until they reached to a concentration of 100 mg/ml. However, the results of the comet assay indicated that the imidacloprid induced significant DNA damage ($P<0.01$) in earthworms. In another study, Feng et al. (2004) performed an acute toxicity test, MN test and comet assay of imidacloprid on amphibian, *Rana N. Hallowell*, which is a potent the bio-indicator of agricultural and aquatic ecosystems.LC_{50}-48 h of imidacloprid were determined as 165 mg/l and 219 mg/l for tadpoles of *Rana limnocharis* and *Rana N. Hallowell*, respectively. A significant difference ($P< 0.05$) was found to be in the MN frequencies at the 8 mg/l concentration of imidacloprid compared to the control group. Comet assay results demonstrated significant differences ($P<0.01$) in the distributions of DNA damage grades between the negative controls and imidacloprid (0.05, 0.1, 0.2 and 0.5 mg/l) treated groups. A recent study conducted by Yucel and Kayis (2019) investigated imidacloprid-induced changes in *Galleria mellonella L.* (Lepidoptera: Pyralidae) by evaluating genotoxic, immunotoxic biochemical, and oxidative stress biomarkers at sublethal doses (0.25, 0.50, 0.75, and 1.00 mg) and at different time periods (24, 48, 72, and 96 h). They reported dose-dependent increases in MDA levels and activities of SOD and CAT. Also, they indicated that all imidacloprid doses significantly increased micronucleus frequency while significantly decreased total hemocyte count as immunotoxic biomarker 24th, 48th, and 72nd hours. Charpentier et al. (2014) studied lethal and sublethal effects of Imidacloprid on Drosophila melanogaster and illuminated the effects induced by this neonicotinoid at very low concentrations. It was also reported that imidacloprid did not exhibit mutagenic or recombinogenic activity at the concentration of $5X10^{-5}$ based on Somatic Mutation and Recombination Test (Frantzios et al. 2008).

Some studies have indicated that substances with antioxidant and anti-mutagenic properties showed protective effects against harmful effects caused by chemical agents on *Drosophila melanogaster* model (Uysal and Agar 2005, Prakash et al. 2014). Uysal and Agar (2005) investigated the protective activity of selenium on Aflatoxin B1-induced adverse effects on *Drosophila melanogaster*. They applied AFB1 and $Se4^{+}$ during the developmental period for egg, larva and pupae and reported that AFB1 extended metamorphosis process and decreased offsprings number. However, these adverse effects of AFB1 were reversed by selenium application (4.0 ppm and 8.0 ppm). The results of their study showed that selenium effectively inhibited abnormalities of *Drosophila melanogaster* during their developmental stages. Prakash et al. (2014) evaluated anti-mutagenic properties of caffeine on mutation rate induced by ethyl-methanesulfonate (EMS) in *Drosophila melanogaster* using wing mosaic assay and they found that EMS (0.5 mM and 1.0 mM) both 48 ± 4 and 72 ± 4 h exhibited an increased mutation rate. Nonetheless, caffeine application significantly reduced the EMS-induced genotoxicity.

This study determined that taurine, whose antioxidant effects are proven by several studies (Ince et al. 2017, İnce et al. 2018) reversed imidacloprid-induced DNA damage and ameliorated productive performance of *Drosophila melanogaster*.

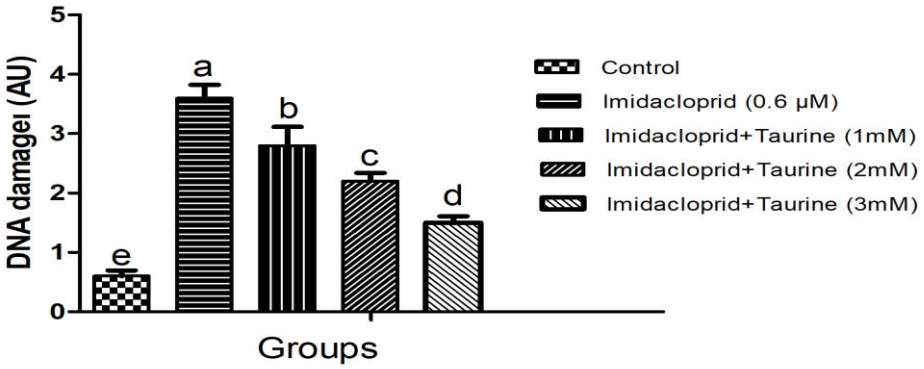

Figure 1. The effect of taurine on DNA damage in *Drosophila melanogasters* exposed to imidacloprid. Values with different letters show statistically significant differences ($p<0.05$)

Table 1. The effect of taurine on reproductive performance in *Drosophila melanogasters* exposed to imidacloprid.

Groups	Mature					Pupa	
	Female	%	Male	%	Total	Development	%
I Control	23	100	38	100	61	186	100.00
II Imidacloprid (0.6 μM)	5	21.74	6	15.79	11	30	16.13
III Imidacloprid+Taurine (1 mM)	7	30.43	9	23.68	16	49	26.34
IV Imidacloprid+Taurine (2 mM)	14	60.87	16	42.11	30	88	47.31
V Imidacloprid+Taurine (3 mM)	15	65.22	22	57.89	37	115	61.83

CONCLUSION

Consequently, it was determined that imidacloprid negatively affected the reproductive performance and pupa development of *Drosophila melanogasters* and caused DNA damage. In contrast, taurine application with imidacloprid has been found to increase reproductive performance and pupa development. Also, taurine application prevented DNA damage in *Drosophila melanogasters*.

Conflict of Interest: The authors declare that they have no conflict of interest.

REFERENCES

Alam SS, Hafiz NA, Abd El-Rahim AH. Protective role of taurine against genotoxic damage in mice treated with methotrexate and tamoxfine. Environ. Toxicol. Pharmacol. 2011;31(1): 143–52.

Amjad A, Azam I, Sarwar MK, Malik MF, Sattar A. A review of imidacloprid toxicity in coccinellids. Arthropods. 2018;7(1): 1–10.

Bonner MR, Alavanja MCR. Pesticides, human health, and food security. Food Energy Secur. 2017;6(3): 89–93.

Charpentier G, Louat F, Bonmatin JM, Marchand PA, Vanier F, Locker D, Decoville M. Lethal and sublethal effects of imidacloprid, after chronic exposure, on the insect model drosophila melanogaster. Environ. Sci. Technol. 2014;48(7): 4096–102.

Costa LG Toxic Effects of Pesticides. In: Klaassen CD (ed) Casarett & Doull's toxicology: the basic science of poisons. 2013. McGraw-Hill Education, New York.

Feng S, Kong Z, Wang X, Peng P, Zeng EY. Assessing the genotoxicity of imidacloprid and RH-5849 in human peripheral blood lymphocytes in vitro with comet assay and cytogenetic tests. Ecotoxicol. Environ. Saf. 2005;61(2): 239–46.

Feng S, Kong Z, Wang X, Zhao L, Peng P. Acute toxicity and genotoxicity of two novel pesticides on amphibian, Rana N. Hallowell. Chemosphere. 2004;56(5): 457–63.

Frantzios G, Paptsiki K, Sidiropoulou B, Lazaridis I, Theophilidis G, Mavragani-Tsipidou P. Evaluation of insecticidal and genotoxic effects of imidacloprid and acetochlor in Drosophila melanogaster. J. Appl. Entomol. 2008;132(7): 583–90.

İnce S, Acaröz U, Arslan-Acaröz D, Varol N, Gürler Z, Küçükkurt İ, Demirel HH, Eryavuz A. Sıçanlarda Akrilamid ile İndüklenen Oksidatif Strese Karşı Taurinin Koruyucu Etkisi. Kocatepe Vet. J.2018;11: 1–12.

Ince S, Arslan-Acaroz D, Demirel HH, Varol N, Ozyurek HA, Zemheri F, Kucukkurt I. Taurine alleviates malathion induced lipid peroxidation, oxidative stress, and proinflammatory cytokine gene expressions in rats. Biomed. Pharmacother. 2017;96 (May): 263–8.

Kumar A, Verma A, Kumar A. Accidental human poisoning with a neonicotinoid insecticide, imidacloprid: A rare case report from rural India with a brief review of literature. Egypt. J. Forensic Sci. Forensic Medicine Authority; 2013;3(4): 123–6.

Lourenço R, Camilo ME. Taurine: A conditionally essential amino acid in humans? An overview in health and disease. Nutr. Hosp. 2002;17(6): 262–70.

Miguel-Aliaga I, Jasper H, Lemaitre B. Anatomy and Physiology of the Digestive Tract of Drosophila melanogaster. Genetics. 2018;210(2): 357–96.

Mukhopadhyay I, Chowdhuri DK, Bajpayee M, Dhawan A. Evaluation of in vivo genotoxicity of cypermethrin in Drosophila melanogaster using the alkaline Comet assay. Mutagenesis. 2004;19(2): 85-90.

Olive PL, Banáth JP. The comet assay: A method to measure DNA damage in individual cells. Nat. Protoc. 2006;1(1): 23-29.

Pandey UB, Nichols CD. Human Disease Models in Drosophila melanogaster and the Role of the Fly in Therapeutic Drug Discovery. Barker EL, editor. Pharmacol. Rev. 2011;63(2): 411–36.

Prakash G, Hosetti B, Dhananjaya B. Protective effect of caffeine on Ethyl methanesulfonate-induced wing primordial cells of Drosophila melanogaster. Toxicol. Int. 2014;21(1): 96–100.

Richardson JR, Fitsanakis V, Westerink RHS, Kanthasamy AG. Neurotoxicity of pesticides. Acta Neuropathol. 2019;138(3): 343–62.

Soares JJ, Rodrigues DT, Gonçalves MB, Lemos MC, Gallarreta MS, Bianchini MC, Gayer MC, Puntel RL, Roehrs R, Denardin ELG. Paraquat exposure-induced Parkinson's disease-like symptoms and oxidative stress in Drosophila melanogaster: Neuroprotective effect of Bougainvillea glabra Choisy. Biomed. Pharmacother. 2017;95(July): 245–51.

Türkez H, Geyikoğlu F. The Anti-Genotoxic Effect of Taurine on Aluminum Sulphate-Induced DNA Damage in Human Peripheral Lymphocytes. Eur. J. Biol. 2010;69(1): 25–32.

Uysal H, Agar G. Selenium protective activity against Aflatoxin B1 adverse affects on Drosophila melanogaster. Brazilian

Arch. Biol. Technol. 2005;48(2): 227–33.

WHO. 2005. The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification. IPCS, Geneva 2004, Switzerland.

Yucel MS, Kayis T. Imidacloprid induced alterations in oxidative stress, biochemical, genotoxic, and immunotoxic biomarkers in non-mammalian model organism Galleria mellonella L. (Lepidoptera: Pyralidae). J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes 2019;54(1): 27–34.

Zang Y, Zhong Y, Luo Y, Kong ZM. Genotoxicity of two novel pesticides for the earthworm, Eisenia fetida. Environ. Pollut. 2000;108(2): 271–8.

Zhang Q, Li Z, Chang CH, Lou JL, Zhao MR, Lu C. Potential human exposures to neonicotinoid insecticides: A review. Environ. Pollut. 2018;236: 71–81.