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# Effects of Oxfendazole on Metabolic Enzymes in Hemolymph of *Galleria mellonella* L. (Lepidoptera: Pyralidae) Larvae Reared on Artificial Diet

## *Oksfendazolün Yapay Besinde Yetiştirilen Galleria mellonella L. (Lepidoptera: Pyralidae) Larvalarının Hemolenf Dokusundaki Metabolik Enzimler Üzerine Etkisi*

Serkan Sugeçti[1,2*], Kemal Büyükgüzel[1]

[1]Zonguldak Bulent Ecevit University, Faculty of Science and Arts, Department of Biology, Zonguldak, Turkey
[2]Altınbas University, Vocational School of Health Services, Department of Electroneurophysiology, İstanbul, Turkey

**Abstract**

Oxfendazole is used as an antihelminthic drug in parasite infections of animals. Oxfendazole was added at different concentrations (0, 0.0015, 0.015, 0.15, and 1.5%) to artificial diet and the wax moth *Galleria mellonella* larvae were reared to 7$^{th}$ stage. The changes in metabolic enzymes activity in *G. mellonella* hemolymph tissue were investigated. It was found that the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was increased which are cell damage biomarkers. The highest concentration of this antihelmintic was increased the enzme activity (ALT) from 329 ± 4.33 to 529. 5 ± 3.17 U/L. Gamma glutamyl transferase (GGT) enzyme activity was increased from 0.87 ± 0.1 to 4.32 ± 0.22 U/L. Creatine kinase (CK) and amylase (AMYL) activities were significantly increased in hemolymph of *G. mellonella* larvae fed with the highest oxfendazole concentration (1.5%) when compared to the control group. Our results indicate that oxfendazole can be used as an insecticide with a well-adjusted concentrations because of its low acute toxicity to humans and other non-target organisms.

**Keywords:** Biochemical damage, *Galleria mellonella*, Oxfendazole, Transaminase enzymes

**Öz**

Oksfendazol, hayvanlarda görülen pazariter enfeksiyonlarda kullanılan antihelmintik bir ilaçtır. Yapay besine farklı konsantrasyonlarda (% 0, 0,0015, 0,015, 0,15, ve 1,5) oksfendazol ilave edilerek, büyük bal mumu güvesi *Galleria mellonella* larvaları 7. evreye kadar beslendi. *G mellonella* hemolenf dokusundaki metabolik enzim aktivitesindeki değişimler araştırıldı. Hücre hasarı biyobelirteci alanin aminotransferaz (ALT) ve aspartat aminotransferaz (AST) aktivitelerinin arttığı belirlendi. Antihelmintik maddenin en yüksek konsantrasyonda enzim aktivitesi 329 ± 4,33'den 529, 5 ± 3,17 U/L'ye yükseldi. Gama glutamil transferaz (GGT) enzim aktivitesi 0,87 ± 0,1'den 4,32 ± 0,22 U/L' ye yükseldi. Kreatin kinaz (CK) ve amilaz (AMLY) enzim aktivitelerinin, en yüksek oksfendazol konsantrasyonu (% 1,5) ile beslenen *G. mellonella* larvalarının hemolenf doksunda kontrol grubu ile karşılaştırıldığında istatistiksel olarak önemli derecede arttığı tespit edildi. Bu çalışma; oksfendazolün insan ve diğer hedef olmayan organizmalara karşı düşük akut toksisiteye sahip olması sebebiyle konsantrasyonlarının iyi ayarlanması ile insektisit olarak kullanılabileceğini işaret etmektedir.

**Anahtar Kelimeler:** Biyokimyasal hasar, *Galleria mellonella*, Oksfendazol, Transaminaz enzimleri

## 1. Introduction

Agricultural pest insects cause significant economic losses by their harmful effects on agricultural products. Insect species belonging to the Lepitoptera order are particularly harmful for agricultural areas. For this reason, studies on chemical and biological control with these species have gained importance (Büyükgüzel 2001, Büyükgüzel and Kalender 2009, Büyükgüzel et al 2013, Sugeçti et al 2016, Sertçelik et al 2018). The use of insecticides against pest insects has also been increased in order to prevent these losses and to increase product yield. The increase in the chemicals used as agricultural insecticides poses a threat to other non-target living things and the environment. Moreover, insecticides used in agricultural areas are highly toxic to environment and non-target organisms. For this reason, the use of chemicals with less toxic effects on the chemical management with pest insects is utmost importance. In

*Corresponding author: serkan.sugecti@altinbas.edu.tr

Serkan Sugeçti orcid.org/0000-0003-3412-2367
Kemal Büyükgüzel orcid.org/0000-0002-6959-8480

addition, physiology, biochemistry and nutritional ecology of insects should be known in detail in management with pests insect (Büyükgüzel 2006).

*Galleria mellonella* L. is used as an alternative invertebrate host to experimental animals which are able to determine the pathogenicity of certain chemicals, fungi or bacteria that infect humans and animals (Ramarao et al., 2012, Maguire et al., 2016). Moreover, this insect has been used as a model for evaluating the insecticidal properties of anthelmintic drugs with clinical prescription in recent years (Büyükgüzel and Kalender, 2007, Sugeçti et al., 2016, Sertçelik et al., 2018).

It is important to investigate the use of this antihelminthic substance as an insecticide in the management of pest insects in addition to investigating the effect of this substance over an eukaryotic organism, which is an important group of invertebrates. The reason why oxfendazole is preferred in this study is due to the fact that it is reliably used with minimal side effects in the treatment of parasites in mammals and this feature is intended to minimize the toxicity to humans and other vertebrate animals.

In this study, it was aimed to determine the metobolic enzymes activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and amylase (AMYL) in hemolymph tissue of model insect *G. mellonella*.

## 2. Materials and Methods

### 2.1 Insect Culture

Greater wax moth larvae and pupae were collected from infected hives in apicultural areas around Zonguldak, Turkey, and the newly emerged adults were used to maintain the stock culture. The insects were reared in 1,000 ml glass jars with an artificial diet (Bronskill, 1961) at 30 ± 1°C, 65 ± 5% relative humidity in constant darkness. The standard diet was composed of 420 g of bran, 150 ml of filtered honey, 150 ml of glycerol, 20 g of ground old dark honeycomb, and 30 ml of distilled water. Newly emerged adult females were placed in the jars and provided a piece of old honeycomb on the diet for egg deposition and feeding of newly hatched larvae. Preparing and dispensing diets into containers to obtain eggs and transferring larvae onto diets followed our standard protocol. For continuing insect culture, these newly hatched larvae were reared through seventh instar at the same artificial diets. The last larval instars were transferred into new jars to obtain pupal and adult stages (Büyükgüzel et al., 2010, Sugeçti et al. 2016).

### 2.2 Experimental Designs

Oxfendazole was directly incorporated into diets at concentrations of 0.0015, 0.015, 0.15, or 1.5 %. Our preliminary experiments showed that these concentrations enable larvae to complete their adult development with gradually increasing mortality. Larvae reared on oxfendazole -free diets were used as controls. Using standard laboratory rearing conditions, we determined the influence of dietary oxfendazole on activities of AST, ALT, LDH, CK, AMY, ALP and GGT in hemolymph of seventh-instar larvae.

### 2.3 Sample Preparation and Biochemical Assay

The larvae were chilled on ice for 5 min and surface sterilized in 95% ethanol. Larval hemolymph was collected into cold Eppendorf tubes and stored at −80°C. A few crystals of phenylthiourea (PTU) were added to each sample to prevent melanization. Analyzes were performed using the appropriate kit with Roche Hitachi Cobas c501 instrument. Used kits; ALT (Roche, Germany, Kit no. 23641701), AST (Roche, Germany, Kit no. 22310601), BILD (Roche, Germany, Kit no. 15104901), BILT Roche, Germany, Kit no: 20341501), TP (Roche, Germany, Kit no. 19727001), UA (Roche, Germany, Kit no. 24202101), UREA Germany, Kit no. 24887901), LDH (Roche, Germany, Kit no. 23767001).

### 2.4 Statistical Analysis

Data on the total protein amount and AST, ALT, CK, LDH, AMY, ALP and GGT activity were analyzed by one-way analysis of variance (ANOVA). To determine significant differences between means, the least significant difference (LSD) test (SPSS, 1997) was used. When the F estimate exceeded the probability of 0.05 the differences were considered significant. Regression analysis was used to analyze possible correlations between dietary (SPSS, 1997).

## 3. Results

This study shows that the transaminase enzymes activitis were increased statistically in the hemolymph of the larvae of *G. mellonella* which fed with the highest oxfendazole concentration (1.5%). The AST enzyme activity was found to be 81.5 ± 1.03 U/L in the control group, which increased to 155.8 ± 3.29 U/L at this highest concentration. Activity of ALT enzyme was also increased significantly in the hemolymph of *G. mellonella* larvae fed with artificial nutrient containing 1.5% oxfendazole compared to control group. The GGT enzyme activity was recorded as 0.87 ± 0.10 U/L in the control group, which increased to 4.32 ± 0.22 U/L in the hemolymph of *G. mellonella* larvae fed artificial diet

containing 1.5% oxfendazole. It was found that the CK activity and the AMLY activities, the metabolic enzymes were increased significantly in hemolymph of the larvae which fed the highest oxfendazole concentration (1.5%) when compared with the control group. LDH enzyme activity was significantly decreased in hemolymph *G. mellonella* larvae which fed the highest oxfendazole concentration (1.5 %) in comparison to the control group. The lowest dietary concentration of oxfendazole significantly increased ALP activity whereas other lower concentrations resulted in decreased enzyme activities in larval hemolymph of *G. mellonella*. However, there was no statistically significant change between ALP enzyme activity in the control group and the highest dietary oxfendazole concentration (Table 1).

## 4. Discussion

This study presented that oxfendazole exerted different effects according to its concentrations on the metabolic enzymes activities in of hemolymph in *G. mellonella* larvae. Insects need important nutrients (proteins, lipids and carbohydrates) to continue their vital activities. These biomolecules are directly influenced by many factors such as changes in dietary ecology, food quality and external chemical and physiological effects (Büyükgüzel 2006). Insecticides cause oxidative stress in insects which results in the formation of free radicals. It is known that the reactive molecules are significantly effective on protein, lipid, carbohydrate, nucleic acids and enzymes (Damien et al., 2004). In addition, reactive molecules that are formed cause oxidative damage to insects. Insects try to eliminate the oxidative damage by producing antioxidant enzymes. If the antioxidant defense system is inadequate, increased damage will result in cellular aging, cancer, or cell death (Büyükgüzel et al., 2010). Metabolic enzymes such as; AST, ALT, CK, GGT and AMYL, were found to be significantly increased in high concentrations of oxfendazole. Chemical and biological agents cause tissue damage in organisms, result in the release of cellular enzymes, leading to an increase in the enzyme concentration (İçen et al., 2005).

It is known that additives in diets would damage the quality of food and the resulting oxidative stress affects the insect physiology (Timmermann et al., 1999). Oxfendazole, which is added to the artificial diet, may cause changes in insect consumption behavior by affecting the quality of the diet.

Activities of transaminase enzymes are used as a clinical diagnosis for testing organ dysfunctions in mammals and for detecting functional defects if present (Rosenthal, 1997). However, these tests have also begun to be used by researchers who are particularly interested in environmental biology and the effects of toxic chemicals on insects. Especially, increased amounts of AST and ALT are the most important biomarkers of progressive tissue and cell damage (Sidlova et al., 2003). It has been reported that AST and ALT activity are significantly increased in *Culex fatigans* (Wiedemann) type flies which were exposed to different insecticides (Verma and Rahman 1984). In another study, AST and ALT enzyme activities of the larvae of *Philosamia ricini* (Boisduval), that fed with tetracycline, were doubled (Eid et al., 1989). In another study investigating the toxic effect of organophosphorus insecticides on *G. mellonella* larvae, AST and ALT activity were found to be increased significantly (İçen et al., 2005). In the highest concentration of oxfendazole (1.5 %) added to artificial nutrient, AST and ALT cell damage biomarkers' activity was found to be significantly increased compared to the control group. (Table 1).

LDH is a metabolic enzyme and its activity is increased when cell and tissue damage occurs. It was observed that the activity of LDH enzyme was changed according to the concentration of hemolymph at the larval of *G. mellonella* fed with oxfendazole which added artificially. In the highest oxfendazole concentration (1.5%), LDH enzyme activity was found to be statistically insignificant compared to the control group. The activity of LDH in hemolymph of *G. mellonella* larvae fed with artificial diet supplemented with 0.0015% oxfendazole was increased (Table. 1). In a study of the oxidative levels of the antiparasitic drug fluazuron on *Rhipicephalus* (Boophilus) *microplus*; LDH activity in haemolymph and fatty tissue increased with the chemical exposure time (Gaudêncio et al., 2016). In another study, a significant increase in LDH enzyme activity in hemolemph tissue of the insect was detected after 8 hours of the biological agent *Klebsiella oxytoca* injection to the larvae of *G. mellonella* (Sugeçti et al., 2017). Mirhaghparast (2015) reported that; 4th stage larvae of *Chilo suppressalis* was exposed to hexafluoride at different concentrations; LDH activity was statistically decreased after 6 hours and statistically increased after 24 hours (Mirhaghparast et al., 2015).

ALP is an important hydrolase enzyme; that plays a role in the mechanism of dephosphorylation of many molecules such as nucleotides, proteins and alkaloids, in alkaline medium (Ramzia et al. 2014). This enzyme has been shown to be an important role in digestive activity of insects for transporting of nutrients in midgut, hemolymph and

**Table 1:** Effects of oxfendazole on some metabolic enzyme activities in hemolymph of 7th instar *G. mellonella* larvae.

| Oxfendazole Concentrations (%) | AST (U/L) (Mean*± S.E)† | ALT (U/L) (Mean*± S.E)† | CK (U/L) ( Mean*± S.E)† | LDH (U/L) (Mean*± S.E)† | GGT (U/L) (Mean*±S.E)† | ALP (U/L) (Mean*±S.E)† | AMYL (U/L) (Mean*±S.E)† |
|---|---|---|---|---|---|---|---|
| 0.0000§ | 81.5±1.03a | 329 ±4.33a | 396±3.74a | 199±6.22a | 0.87±0.10a | 14.5±0.46a | 170.15±1.11a |
| 0.0015 | 119.25±2.72b | 438±9.11b | 514±2.23b | 231±3.84b | 2.5±0.43b | 21.85±0.27b | 1 64.8±1.08b |
| 0.015 | 118.5±2.16b | 381±8.87c | 451±2.95c | 138±2.23c | 3.55±0.26c | 6.27±0.18c | 170.22±1.63c |
| 0.15 | 80.1±1.27a | 294.5±2.38d | 502.75±4.02b | 112.5±3.63d | 2.5±0.29b | 10.65±3.71d | 127.15±1.42d |
| 1.5 | 155.8±3.29c | 529.5±3.17e | 659.25±3.43d | 139±2.95c | 4.32±0.22c | 13.4±0.58a | 196.36±1.55e |

**Mean of four replicates per treatment with 15 larvae per replicate. †Means within a column followed by the same lowercase letter are not significantly different (P > 0.05). §Control Diet (Oxfendazole free).*

fat body tissue (Zibaee et al., 2011, Ramzia et al., 2014). In another research; the effect of pyriproxyfen on the *C. suppressalis* larvae was tested and it was determined that ALP activity was significantly increased in high concentrations of the chemical when compared to the control group (Mirhaghparast et al., 2015). It has been observed that the activity of ALP enzyme in the hemolymph of *G. mellonella* larvae which was fed with oxfendazole supplemented with artificial nutrient significantly increased in the concentration of 0.0015%, although the ALP activity in the highest concentration decreased, there was no statistical difference. Changes in ALP enzyme activity in the hemolymph of *G. mellonella* larvae fed with oxfendazole supplemented with artificial nutrients may be due to changes in nutrition and metabolism physiology. The decrease in ALP enzyme activity may be due to the fact that oxfendazole inhibits alimentation of the insect by altering the physiological structure of the diet.

Enzyme CK plays an important role in energy metabolism by acting as phospho- kinases in vertebrates and invertebrates (Uda et al., 2006). It was determined that the enzyme CK activity in the hemolymph tissue of *G. mellonella* larvae which was fed with oxfendazole supplemented with artificial diet increased at a statistically significant rate at the highest concentration (1.5%). In a study in which Diaquabis (N, N-diethylnicotinamide-N1) bis (4-formylbenzoato-O) cobalt (II) complexes were injected into the larvae of *G. mellonella*, enzyme CK activity was found to increase significantly after injection (Sertçelik et al., 2018)

The role of GGT in biological systems is to metabolize extracellular reduction glutathione (GSH). Recently, oxidative stress has been used as a marker. In the case of oxidative stress, GGT may be increased to compensate for falling GSH (Whitfield 2001, Rasheed et al. 2010). In a study investigating the toxic effect of the oil from *Artemisia annua* on *Pseudococcus viburni* (Hemiptera: Pseudococcidae), it was found that allochemical statistically increased AST, ALT and GGT enzyme activity in insect larvae (Ramzia et al., 2017)

This study showed that oxfendazole as a new chemical which is alternative to toxic insecticides used intensively in agriculture fields. Understanding the physiological and biochemical mechanism of action of clinically important oxfendazole over model organism *G. mellonella* would allow the development of new chemical methods in management pest insects that are less harmful to the non-target organisms and less toxic to the environment.

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