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DETERMINING THE ACUTE TOXIC EFFECTS OF POLY(VINYLFERROCENIUM) SUPPORTED PLATINUM NANOPARTICLE (PT/PVF+ NPS) ON APIS MELLIFERA

Yeşim DAĞLIOĞLU[1], Dilek KABAKCI[2], Gökhan AKDENİZ[2], Mutlu SÖNMEZ ÇELEBI[3]*

[1]Biology Department, Faculty of Arts and Sciences, Ordu University, 52200, Ordu, Turkey.
yozkan52@gmail.com

[2]Ministry of food, Agriculture and Livestock, Apiculture Research Station Department, Ordu, Turkey.
dilek.kabakci@tarim.gov.tr
gokhan.akdeniz@tarim.gov.tr

[3]Chemistry Department, Faculty of Arts and Sciences, Ordu University, 52200, Ordu, Turkey.
mustlu@yahoo.com

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*Corresponding author

Abstract

The use of engineering nanomaterials on a large scale along with their production, and their potential effects on the environment and on human health as well as their environmental emission have increased these concerns. For this reason, nanoparticles which are released into the environment are necessary determine the toxicity by using indicator organisms. With this study, it was aimed that the acute toxic effects of Polyvinylferrocene (PVF+)-supported platinum (Pt) nanoparticle (Pt/PVF+ NPs), Poly(vinylferrocenium) (PVF+) and K2PtCl4 be evaluted comparatively by using the honey bees (Apis Mellifera). LC50 values for 48 and 96 hours of these substances respectively; 713.290 ve 6.899 mg/l for K2PtCl4; 12458374.000 ve 178.262 mg/l for Pt/PVF+ NPs and 148.153 ve 0.344 mg/l for PVF+. When we look at this value, the toxic effect for all three substance had increased on a serious level, depending on the exposure time.
Keywords: Apis mellifera, plitanyum nanopartikül, nanotoksikoloji

APIS MELLIFERA ÜZERİNDE POLİVİNİLFERROSEN DESTEKLİ PLATİN (PT/PVF+) NANOPARTİKÜLLERİNİN AKUT TOKSİK ETKİLERİNİN BELİRLENMESİ

Özet

Mühendislik nanomalzemelerin büyük ölçüde üretimi ve kullanımı sonucu ve çevreye yayılımının artmasının yanı sıra çevre ve insan sağlığı üzerine potansiyel tehlikeleri üzerine kaygıları arttırmıştır. Bu nedenle, çevreye salınan nanopartiküllerin indikatör organizma kullanılmasıyla toksisitesinin tespit edilmeye ihtiyacı vardır. Bu çalışma ile polivinilferrosen (PVF+)- destekli platin (Pt) nanopartikülü (Pt/PVF+),polivinilferrosen (PVF+) ve K2PtCl4'ün akut toksisitesi bal arısı (Apis Mellifera) kullanımıyla mukayeseli olarak değerlendirildi. LC50 değerleri sırasıyla 48 ve 96 saatlerde K2PtCl4 için 713.290 ve 6.899 mg/l Pt/PVF+ nanopartikülü için 12458374.000 ve 178.262 mg/l ve PVF+ için 148.153 ve 0.344 mg/l olarak hesaplanmıştır. Bu değerlere baktığımızda, bu üç madde için maruz kalma süresine bağlı olarak toksik etki ciddi düzeyde artmıştır
Anahtar Kelimeler: Apis mellifera, plitanyum nanaopartikül, nanotoksikoloji

1 Introduction

Along with the rapidly developing nanotechnology, the environmental effects and potential dangers of nanoparticles (NPs) on health have created a great concern up till now [1-2]. More than 1000 products made of nanomaterials have been found in markets so far [3]. The revenue of nanotechnology-based products is assumed to reach up to 3.1 trillion American dollars across the globe by 2015 [4]. For this reason, the use of engineering nanomaterials on a large scale along with their production, and their potential effects on the environment and on human health as well as their environmental emission have increased these concerns [5]. Nanotechnology practices provide us with new materials through minimizing the sizes of conventional materials down to nano-scales and through changing the basic physical and chemical characteristics of materials, such as unique electrical, optical and mechanical features [6]. Since the potential toxicity and behaviours of nanoparticles during their analysis in different matrixes can be affected by the factors, such as the number of particles in nanoparticles as well as their load, size and dimensional distribution, and the structure and shape of the surface area, the identification of their concentration and consumption should not be restricted [6-8]. Thus, it is necessary to determine their toxic effects by using different living things around the environment. Today an increasing number of toxicological studies are being conducted as regards nanoparticles. Yet, as for the ecotoxicological studies conducted on terrestrial invertebrate animals as regards nanoparticles; there are

hardly any studies conducted on the honey bee (Apis mellifera) in particular, which has an economic value anywhere.

With this study, it was aimed that the acute toxic effects of Polyvinylferrocene (PVF+)-supported platinum (Pt) nanoparticle (Pt/PVF+), Poly(vinylferrocenium) (PVF+) and K2PtCl4 be evaluted comparatively by using the honey bees (Apis Mellifera), which are of great significance.

2 Methodology

2.1 The collection of bees and Test chemicals

Young worker bees are used from the same race (*Apis mellifera* Caucasica) the same age (5 days), living in the same hive etc. Young worker bees which grown in sterile conditions in order to work in the experiments were used. Also, there are no young bees in the bee honeycomb collected. Bees were collected on the morning of (It's 10 o'clock) experiments in August. Before starting the experiment, the collected bees are randomly placed in the experimental hive. Bees were fasted for 2 hours before starting the experiment. Before testing begins, unhealthy bees were taken and healthy bees were put in place.

Poly(vinylferrocenium) supported platinum nanoparticles (Pt/PVF+ NPs) were prepared according to the procedure described in the literature[9]. K2PtCl4 (>98.2%, Merck) and Poly(vinylferrocenium) was used as received.

2.2 The studies and the experimental setup of acute toxicity

In order to shelter the bees for 96 hours in vitro, 20x5 cm long and 8 cm wide self-covered plastic containers were utilized. Randomly selected 50 bees were put into each container. On one side of these containers, tiny holes were made to let the air in for the bees during the experiment, after which the sides of the cover were rubbed with emery in order not to let the cover open during the experiment. 2x1 cm hole was opened from the lower side of the plastic container to collect the bees that perished during the experiment, thanks to which the bees were easily collected. To feed the bees, on the other hand, 1 ml volume of droppers (pasteur pipettes) were used. The droppers were fixedly and vertically placed on the upper part of the containers, by means of which the bees were made to be fed easily from the droppers. The sucrose solution was put into the droppers with the help of a syringe. In this way, the groups exposed to the test were made to be fed with a test solution every four hours. On the other hand, the control group members were fed with the sucrose solution prepared only with deionized water. The test room was conducted at 25±2 ˚C temperature and in the dark. The relative humidity which was normally 50-70% was recorded throughout the test. No behavioural disorder or mortality was observed in the control groups all through the test. The perished bees in the test groups were counted at the 24th, 48th, 72nd and 96th hours. This study was carried out in 3 repetitions, independent of each other.

2.3 Statistical Analyses

All the experiments were repeated three times independently, and the data were recorded on average by means of standard deviation. The LC50 value was calculated through the probit statistical analysis of EPA. The other analyses were performed through ANOVA and TUKEY multiple comparison analysis.

3 Findings

3.1 Acute Toxicity/LC50 Study

In this study where the lethal concentration values of PVF+, K2PtCl4 and Pt/PVF+ NPs over Apis mellifera have been determined, the LC50 value for 48 and 96 hours were calculated through the probit analysis by taking the experiment results as the basis. These values have been shown below in the form of a tables. Throughout the experiment, no mortality was seen in the control group, nor were any behavioural abnormalities observed. To be able to provide the comparison, whether or not there was any difference in the mortality rate depending on time and concentration with respect to these three substances that were applied in the same concentrations was determined according to the statistical analyses.

Table 1. 96 and 48 hour LC/EC values calculated for particles K2PtCl4

Points	Exposure time	
	48 Hour	96 Hour
LC/EC 1.00	0.001	0.000
LC/EC 5.00	0.050	0.000
LC/EC 10.00	0.414	0.001
LC/EC 15.00	1.724	0.006
LC/EC 50.00	713.290	6.899
LC/EC 85.00	295191.094	7607.456
LC/EC 90.00	1228051.875	39906.375
LC/EC 95.00	10151345.000	465087.688
LC/EC 99.00	533349248.000	46540900.000

When we compared the lethal concentration values determined during the exposure for 48 and 96 hours in the acute toxicity study conducted in the same concentrations for the LC1,5 values of K2PtCl4 for 96 hours could not be calculated. The LC50 value was calculated as 713.290 mg/l for 48 hours and 6.899 mg/l for 96 hours. These values suggest that the toxic effect for K2PtCl4 had increased on a serious level, depending on the exposure time.

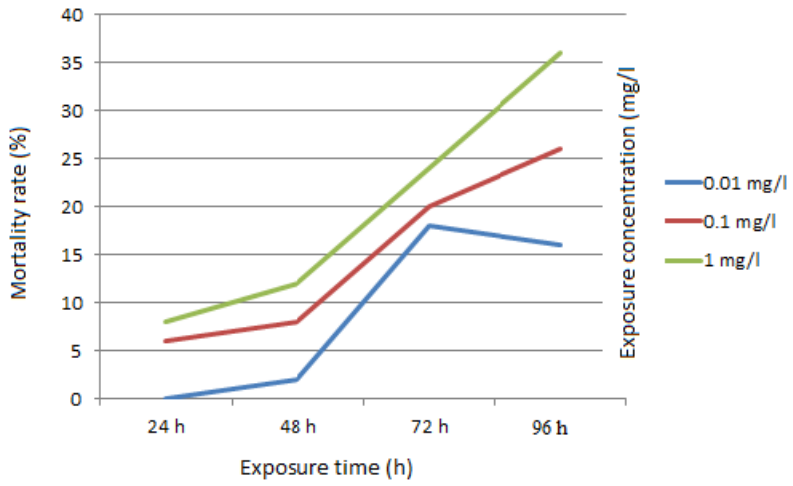

Figure 1. Mortality rates in Apis mellifera regarding K2PtCl4 according to time and concentration

When we take a look at the graphic, we see that the mortality rate continuously increased up to 96 hours at 0.1 mg/l and 1 mg/l concentrations. Yet, the mortality rate at the lowest concentration, 0.01 mg/l, decreases after 72 nd hour.

Table 2. The Difference K_2PtCl_4 concentrations groups according to ANOVA multiple comparison tests

	Con. (J)	Mean Difference (I-J)± Std.Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
0.01 mg/l	0* mg/l	0.12667*±0.03102	0.001	0.0439	0.2095
0.1 mg/l	0* mg/l	0.18000*±0.03102	0.000	0.0972	0.2628
1 mg/l	0.01 mg/l	0.10833*±0.03102	0.006	0.0255	0.1911
	0* mg/l	0.23500*±0.03102	0.000	0.1522	0.3178

0*: control group, con.: concentration, Sig.: significance

When we consider the ANOVA test results, significant differences were found according to the groups of K_2PtCl_4 concentration. These differences, as also seen in Table 2, were observed between 0.1 mgL^{-1} and 0* mgL^{-1}, 0.01mgL^{-1} and 0*mgL^{-1}, and 1 mgL^{-1} and 0.01 $mgL^{-1}$and 0* mgL^{-1} concentrations, which were found to be significant at P<0.05 level.

Table 3. Tukey test results regarding the differences in mortality rates among the different concentration groups of K_2PtCl_4

Concentration	N	Subset for alpha = 0.05		
		1	2	3
0* mg/l	12	0.0000[a]		
0.01 mg/l	12		0.1267[b]	
0.1 mg/l	12		0.1800[b,c]	0.1800[b,c]
1 mg/l	12			0.2350[c]
Sig.		1.000	0.326	0.300

N: The number of samples

As seen in Table 3, the concentration that poses the maximum difference among all the other concentrations of K2PtCl4 is the concentration, 1 mgL-1.

Tablo 4. 96 and 48 hour LC/EC values calculated for particles Pt/PVF+

Points	Exposure time	
	48 Hour	96 Hour
LC/EC 1.00	0.00	0.00
LC/EC 5.00	0.00	0.00
LC/EC 10.00	0.00	0.00
LC/EC 15.00	0.23	0.00
LC/EC 50.00	12458374.00	178.262
LC/EC 85.00	6540484.91	5071242240.00
LC/EC 90.00	4386387.82	294209388544.00
LC/EC 95.00	2230136.57	1206710.40
LC/EC 99.00	2655128.93	9603077.38

The $LC_{1,5}$ values of Pt/PVF+ NPs for 48 hours and $LC_{1,5,10,15}$ values for 96 hours could not be calculated. The LC_{50} values for 48 and 96 hours are 12458374.000 and 178.262, respectively. According to these results, when the exposure time for Pt/PVF+ is extended up to 96 hours, we see that the LC_{50} value decreases by almost 70 times, which indicates that the toxicity of Pt/PVF+ NPs increases as the exposure time goes up.

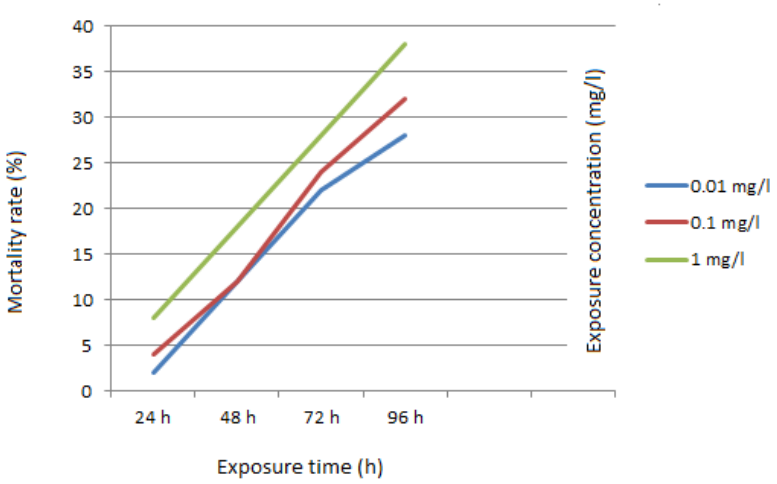

Figure 2. Mortality rates in *Apis mellifera* as regards Pt/PVF+ according to time and concentration.

When we take a look at the graphic above, quite a few mortality rates are observed at 0.01 mgL^{-1} concentration during 24-hour-exposure, whereas at other concentrations, this rate is almost twice as much. During 48-hour-exposure, on the other hand, the mortality rate at the highest concentration (1 mgL^{-1}) is much higher when compared with that in the others, while the mortality rates are equal in other concentrations. When considered in general, the more the exposure time extends, the more the toxicity of Pt/PVF+ nanoparticle increases.

Table 5. The Difference Pt/PVF+ NPs concentrations groups according to ANOVA multiple comparison tests.

con. (I)	Con. (J)	Mean Difference (I-J)*Std. Error	Sig.	95% ConfidenceInterval	
				LowerBound	UpperBound
0* mg/l	0.01 mg/l	0.15833*±0.04005	0.002	0.0514	0.2653
	0.1 mg/l	0.18167*±0.04005	0.000	0.0747	0.2886
	1 mg/l	0.22667*±0.04005	0.000	0.1197	0.3336

0*: control group, con.: concentration, Sig.: significance

According to the ANOVA-multiple comparison test given in Table 5, only between the control group (0* mgL^{-1}) and the other concentration groups was a significant difference at $P<0.05$ level recorded.

Table 6. Tukey test results regarding the differences in mortality rates among the different concentration groups of Pt/PVF^+ NPs

Concentration	N	Subsetforalpha = 0.05	
		1	2
0* mg/l	12	0.0000[a]	
0.01 mg/l	12		0.1583[b]
0.1 mg/l	12		0.1817[b]
1 mg/l	12		0.2267[b]
Sig.		1.000	0.333

As seen in Table 6, the concentration that poses the maximum difference among all the other concentrations of Pt/PVF^+ nanoparticle is the concentration, 1 mgL^{-1}.

Table 7. The Difference among the time groups of Pt/PVF^+ according to the Multiple Comparison Test

Exposure time (I)	Exposure time (J)	Mean Difference (I-J)*Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
24 h	72 h	0.15167*±0.04168	0.004	0.2629	0.0404
	96 h	0.21333*±0.04168	0.000	0.3246	0.1021
48 h	96 h	0.14500*±0.04168	0.006	0.2563	0.0337

Sig.: significance

As seen in Table 7, when we take a look at the ANOVA test results, there is a significant difference among the time groups, which are 24 hours and 72 hours and 96 hours as well as 48 hours and 96 hours at $P<0.05$ level.

Table 8. Tukey test results regarding the differences in mortality rates among the time groups of Pt/PVF^+ NPs

Exposure time (h)	N	Subset for alpha = 0.05		
		1	2	3
24 h	12	0.0333[a]		
48 h	12	0.1017[b]	0.1017[b]	
72 h	12		0.1850[b,c]	0.1850[b,c]
96 h	12			0.2467[c]
Sig.		0.368	0.204	0.458

As seen in Table 8, that which poses the maximum difference among all the other exposed time groups as regards Pt/PVF^+ nanoparticle is 96 hour-exposure.

Table 9. 96 and 48 hour LC/EC values calculated for particles PVF^+

Points	Exposure time	
	48 Hour	96 Hour
LC/EC 1.00	0.00	0.00
LC/EC 5.00	0.02	0.00
LC/EC 10.00	0.15	0.00
LC/EC 15.00	0.56	0.00
LC/EC 50.00	148.15	0.344
LC/EC 85.00	38771.10	34.13
LC/EC 90.00	144720.82	101.27
LC/EC 95.00	1018748.87	507.29
LC/EC 99.00	39599884.00	10416.57

The $LC_{1,5}$ values of PVF^{+} for 96 hours could not be calculated. The LC_{50} value was calculated as 148.153 mg/l for 48-hour-exposure, whereas this value was calculated as 0.344 mg/l for 96-hour-exposure. We see that when the exposure time is extended up to 96 hours, there is 430 times decrease in LC_{50} value. Hence, the toxicity of PVF^{+}, depending on the exposure time, increased at rather an excessive rate.

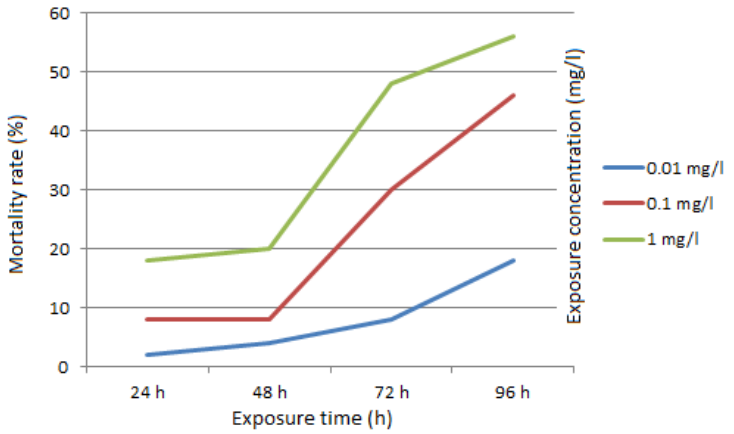

Figure 3. Mortality rates in *Apis mellifera* as regards PVF^{+} according to time and concentration.

In figure 3, With increasing time of exposure at all concentrations increased mortality. In the first 24 hours of exposure is the mortality rate low in all concentrations. When this duration is extended up to 72 hours, the mortality rate increased at a serious level at $1mgL^{-1}$ concentration in particular. In the lowest concentration, which is 0.01 mgL^{-1}, as the exposure time extends, the mortality rate drops down considerably when compared with other concentrations.

Table 10. The Difference PVF^{+} NPs concentrations groups according to ANOVA multiple comparison tests.

(I) con.	(J) con.	MeanDifference (I-J)* Std. Error	Sig.	95% ConfidenceInterval	
				Lower Bound	Upper Bound
0.1 mg/l	0.01 mg/l	$0.14667^{*}\pm0.05239$	0.037	0.0068	0.2865
	0* mg/l	$0.23000^{**}\pm0.05239$	0.000	0.0901	0.3699
1 mg/l	0.01 mg/l	$0.26333^{**}\pm0.05239$	0.001	0.1235	0.4032
	0* mg/l	$0.34667^{**}\pm0.05239$	0.000	0.2068	0.4865

0*: control group, con.: concentration, Sig.: significance

In table 10, according to ANOVA Multiple Comparison Test, significant differences were found according to $P<0.05$ level between concentrations of PVF^{+} 0.1 mgL^{-1} and 0.01 mgL^{-1}; 0.1 mgL^{-1} and 0* mgL^{-1} and 1 mgL^{-1} and 0.01 mgL^{-1}; 1 mgL^{-1} and 0* mgL^{-1}.

Table 11. Tukey test results regarding the differences in mortality rates among the concentration groups of PVF^{+}

Concentration	N	Subset for alpha = 0.05	
		1	2
0* mg/l	12	0.0000^{b}	
0.01 mg/l	12	0.0833^{b}	
0.1 mg/l	12		0.2300^{a}
1 mg/l	12		0.3467^{a}
Sig.		0.394	0.132

N: The number of samples

As seen in Table 11, that which posed the maximum difference among all the exposed time groups of PVF^+ is 96 hour exposure.

Table 12. The Difference PVF^+ NPs time groups according to ANOVA multiple comparison tests

(I) Hours	(J) Hours	Mean Difference (I-J)* Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
24 h	96 h	0.2316*±0.0650	0.005	0.4054	0.0579
48 h	96 h	0.2250*±0.0650	0.006	0.3987	0.0513

As seen in Table 12, when we take a look at ANOVA test results, there is a significant difference at $P<0.05$ level among the time groups, 96 and 24; 96 and 48 hours.

Table 13: Tukey test results regarding the differences in mortality rates among the time groups of PVF^+

Exposure time (h)	N	Subsetforalpha = 0.05	
		1	2
24 h	12	0.0683[a]	
48 h	12	0.0750[a]	
72 h	12	0.2167[b,c]	0.2167[b,c]
96 h	12		0.3000[c]
Sig.		0.118	0.580

N: The number of samples

As seen in Table 13, that which posed the maximum difference among all the exposed time groups of PVF^+ is 96 hour- exposure. On the other hand, when we compared these three substances among themselves, the most toxic substance was recorded as PVF^+ during both 48-hour and 96-hour-exposure, then came K_2PtCl_4, whereas the least toxic one was recorded as Pt/PVF^+ nanoparticle. Considering all these results, we can say that the toxic effect of PVF^+ was minimized by the Pt nanoparticle.

4 Result and Discussion

When compared with classic substances, nanoparticles gthat produce serious toxicity due to their large surface area and nano scales. The nano-sized surface areas of nanomaterials are directly associated with their several basic characteristics, such as surface properties, chemical reactivity and the capacity of physical absorption. All these factors strongly pose nanotoxicological behaviours in *in vivo* [10]. The toxicity of nanomaterials have been studied in the animals of various systematic groups. These comprise gene expression, sublethal and lethal concentrations, embryo toxicity, genotoxicity and cytotoxicity, cell proliferation effectiveness, chromosome aberrations, fertility (viviparity), life cycle, respiratory distress and oxidative stress [5, 11-13, 15,16,18]

Today the studies that evaluate the effect of nanoparticles on honey bees are rather few in number. One of them is the study conducted by [17] on the acute toxicity of nanoparticles referred to as TiO_2, $ZnO\text{-}TiO_2$ and $Ag\text{-}TiO_2$.. [19] exposed *Apis mellifera* to PVF^+ K_2PdCl_4 and Pt/PVF^+ nanoparticle for 48 and 96 hours. They stated that as the exposure time for each of the three substances according to LC50 value extended, the required amount to show the same toxic effect decreased, and its toxic effect showed rather a great deal of increase in time.

Also in this study, the toxic effect was seen to have increased when the exposure time for K_2PtCl_4, Pt/PVF^+ NPs and PVF^+ extended. The LC50 values are 713.290, 12458374.000, 148.15 for 48 hours, and 6.899, 178.262, 0.344 for 96 hours, respectively.

According to these results, the most toxic of all was recorded as PVF^+, both for 48 and for 96 hours.

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