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Preventive action of zinc against heavy metals toxicity in honeybee
Cevat Nisbet, Ahmet Güler, Neslihan Ormancı and Sena Cenesiz
Preventive action of zinc against heavy metals toxicity in honeybee

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A detrimental consequence of industrial growth has been the steady increase in heavy metal pollution which has major, negative impacts on living organisms. Heavy metals still pose a significant health threat, despite the implementation of many strategies to reduce pollution. The aim of this study was to investigate the possible effects of zinc on the accumulation of some heavy metals in honeybees. Groups totalling 15 hives of Apis mellifera were included in this study. Group A was fed sugar syrup, Group B was fed sugar syrup with Cu, Cd and Pb, and Group C was fed sugar syrup with Cu, Cd, Pb and Zn. Food stocks, number of mature worker bees, environment and colony management were standardised across the 15 hives. The data demonstrated that the co-administration of zinc to honeybees, exposed to heavy metals reduced Cu and Cd concentration in tissue samples, but had no effect on Pb concentration. Furthermore, Zn dietary supplementation was effective in reducing both the suppression of dopamine production and the negative effects of heavy metals on dopamine. The results of our study suggest that zinc supplementation in the diet of A. mellifera has a beneficial effect by reducing the cellular accumulation of some heavy metals.

Key word: Environment, honeybee, heavy metal, toxicity, zinc.

INTRODUCTION

Chronic, low-level exposure to toxic metals is increasing worldwide (Klassen et al., 2009). The sources of heavy metal pollution are anthropogenic emissions and natural sources like volcanic eruptions and erosion. Even at very low concentrations, these metals have toxic effects on living things (Hasiang and Diaz, 2011; Xu et al., 2009). These metals are absorbed from the environment along with food, during breathing or from dermal exposure. Following absorption, they bind to carrier proteins in the tissues and are transported through the cell membrane by non-specific and chemo-osmotic uptake systems or specific active transport mechanisms, and the toxic effects are seen as they accumulate in cells (Cicik et al., 2003; Hasiang and Diaz, 2011).
These toxic metals can compete with the target ions in an active transport mechanisms designed to allow the uptake of essential metals (Zn and Fe). Specifically, Cd interferes with the uptake of Zn and Ca, and Ni interferes with Fe uptake. The heavy metals cause toxic effects through interaction with physiologic ions. Lead has a higher electronegativity and ionic gradient than other metals, and causes toxic effects by substituting for polyvalent cations, such as Ca and Zn, which are involved in the fundamental molecular processes of living organisms (Godwin, 2001). Living organisms have developed mechanisms to inhibit uptake or accelerate elimination of such metals to prevent intracellular toxicity. This system maintains homeostasis for trace elements and allows for facilitates detoxification of toxic material (Choudhury and Srivastava, 2001; Onosaka et al., 2002). It has also been reported that some metals may alter the cellular distribution and proportions of other metals, by altering absorption and metabolism (Zatta et al., 2003). Bees are highly sensitive to pollution (Harano et al., 2005) but little is known about the potentional effects of metal pollutants (Gauthier et al., 2016).

Recent studies have reported an increased levels of heavy metal pollutants in honey bee hives and their products (Hladun et al., 2016; Gauthier et al., 2016; Polykretis et al., 2016). Recent, findings showed that some ingested metals alter the pathways of the honey bee that are involved in anti-oxidative responses, there by depressing the immune system and causing high bee mortality rate (Gauthier et al., 2016; Polykretis et al., 2016; Hladun et al., 2016). In addition, metal pollutants have harmful effects on honey bee behavior, development and survival (Hladun et al., 2013). To determine whether the harmful metabolic effects of heavy metals on honey bees could be reduced, the aims of this study were to investigate the effect of Zn on the metabolization of heavy metals and to examine the effect of Zn in combination with other heavy metals, on the concentration of the neurotransmitters, serotonin and dopamine.

MATERIALS AND METHODS

In this study, three study groups were formed with five bee colonies each, with a total of 15 colonies used. Firstly, the queen bees were changed. After the queen bee’s acceptance, the queen and worker bees were shaken off the empty hive medium.

After the shaking procedure, only the basic comb was given and no drug application was performed against pathogens and parasites. Thus, equivalence was established between colonies regarding food stocks, number of mature worker bees, nesting environment and beehive material.

Study groups

The three study groups (A, B and C) were subjected to the following treatments:

1. Group A comprised of the control group (5 colonies). Normal management was applied and only sucrose syrup at a ratio of 1:1.5 (water: sugar) was provided during the study period.
2. Group B comprised the heavy metal administration group. To each of the five colonies, 4 ppm Cu, 3 ppm Cd and 3 ppm Pb were added to 1:1.5 (water: sugar) mixture provided.
3. Group C comprised the heavy metal + Zn application group. To each of the 5 colonies, 4 ppm Cu, 3 ppm Cd, 3 ppm Pb, and 4 ppm Zn, were added to 1:1.5 (water: sugar) mixture provided. One and a half litres of the sugar syrup was provided at 3 day intervals to each colony, repeated 12 times.

Feeding was performed for 40 days. The starting doses of the metals were determined from a pilot study as published in reports (Zhelyazkova et al., 2001; Roman, 2010). The compounds used to expose the bees to heavy metals were cadmium chloride (CdCl₂), copper sulphate (CuSO₄), lead chloride (Pb(II)Cl₂) and zinc acetate (C₆H₅O₂Zn) from Sigma-Aldrich Ltd.

Laboratory analysis

After the administration of the treatments, 100 bees were randomly collected from each colony. That meant that from each group 100 bees x 5 hives = 500 individuals were collected. The collected bees were euthanased in a laboratory by freezing at about -20°C, and then dried at 45°C for 24 h.

One hundred bee bodies (body without head) from each group were ground and thoroughly homogenised. A portion of about 1g was taken from each sample and the Pb, Cd, Cu, and Zn concentrations in both the bee bodies and honey samples (honey and beeswax) were determined with an atomic absorption spectrophotometer (Shimadzu, AA 6701F) (Medici et al., 2005). The brains were removed from the frozen bees’ heads under and dopamine levels (Harano et al., 2005).

Statistical analysis

Samples were analysed with the one-way multivariate general linear model (completely randomised) analysis of variance (package program SPSS 2004). Tukey’s multiple comparison tests were employed for the comparison of means at P < 0.05.

RESULTS

Cu, Zn, Pb and Cd concentrations in bee tissue samples

The Cu concentration in group A, B and C, respectively was 0.57±0.04, 0.62±0.13 and 0.47±0.07 ppm. Analysis of tissue samples from worker bees revealed that Zn significantly reduced the increase in Cu concentration (P<0.05).

The Cd concentration in group B was 1.83±0.33 ppm and in group C it was 1.12±0.08 ppm. There was a statistically significant difference (P<0.05) between Cd concentrations in the groups of bees exposed to Cd, with or without Zn. The mean Pb level of those administered heavy metals (group B) was 0.87±0.25 ppm, whereas in the group receiving the heavy metal–Zn combination (group C), this value was 1.27±0.29 ppm. However, there was no significant difference between these groups (P>0.05) (Table 1).
Table 1. Means and standard errors (\( \bar{X} \pm S_{\bar{X}} \)) of Cu, Zn, Cd and Pb in bee tissue samples (ppm).

<table>
<thead>
<tr>
<th>Bee tissue</th>
<th>Cu (ppm)</th>
<th>Zn (ppm)</th>
<th>Pb (ppm)</th>
<th>Cd (ppm)</th>
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<tbody>
<tr>
<td>Group A</td>
<td>0.57±0.04(^{a})</td>
<td>3.01±0.41(^{a})</td>
<td>0.10±0.02(^{a})</td>
<td>0.01±0.007(^{a})</td>
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<td>Group B</td>
<td>0.62±0.13(^{b})</td>
<td>1.71±0.67(^{b})</td>
<td>0.87±0.25(^{b})</td>
<td>1.83±0.33(^{b})</td>
</tr>
<tr>
<td>Group C</td>
<td>0.47±0.07(^{c})</td>
<td>1.79±0.20(^{b})</td>
<td>1.27±0.29(^{b})</td>
<td>1.12±0.08(^{c})</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate a significant difference between means (P<0.05).

Table 2. Means and standard errors for Cu, Zn, Pb and Cd levels in honey samples (mean± SE) (ppm).

<table>
<thead>
<tr>
<th>Honey comb</th>
<th>Cu (ppm)</th>
<th>Zn (ppm)</th>
<th>Pb (ppm)</th>
<th>Cd (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>0.26±0.08(^{a})</td>
<td>1.22±0.62(^{a})</td>
<td>0.68±0.09(^{a})</td>
<td>0.98±0.12(^{a})</td>
</tr>
<tr>
<td>Group B</td>
<td>0.40±0.09(^{b})</td>
<td>0.78±0.19(^{a})</td>
<td>3.46±0.73(^{b})</td>
<td>2.214±0.26(^{b})</td>
</tr>
<tr>
<td>Group C</td>
<td>0.19±0.03(^{a,b})</td>
<td>4.37±1.00(^{b})</td>
<td>3.56±0.85(^{b})</td>
<td>2.336±0.33(^{b})</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate a significant difference between means (P<0.05).

Table 3. Mean values for biogenic amine values (Pg/brain).

<table>
<thead>
<tr>
<th>Brain tissue</th>
<th>Serotonin (Pg/brain)</th>
<th>Dopamine (Pg/brain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>5.28±0.53(^{a})</td>
<td>23.8±3.81(^{a})</td>
</tr>
<tr>
<td>Group B</td>
<td>4.77±0.92(^{a})</td>
<td>9.0±3.2(^{b})</td>
</tr>
<tr>
<td>Group C</td>
<td>6.77±0.50(^{a})</td>
<td>31.2±11(^{a})</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate a significant difference between means (P<0.05).

**Cu, Zn, Pb, and Cd concentrations in honey samples from honeycomb**

In honey samples the data obtained as the result of metal–Zn interaction significant differences were seen between the control group and other groups (P<0.05), whereas there was no statistically significant difference between groups B and C for all three metals (P>0.05). (Table 2).

**Serotonin and dopamine concentrations in bee brain tissue**

The serotonin levels in groups A, B and C, respectively was 5.28±0.53, 4.77±0.92 and 6.77±0.50 (Pg/brain). There were no significant differences in serotonin levels between the groups (P>0.05). On the other hand, there were significant differences in dopamine concentration between groups A and B (23.82±3.81 and 9.05±3.2), also between groups B and C (9.05±3.2 and 31.2±11) (P<0.05) (Table 3).

**DISCUSSION**

The data obtained in this study demonstrated that Zn supplementation in honeybees exposed to heavy metals reduced Cu and Cd accumulation in tissue samples. Analysis of tissue samples from worker bees revealed that Zn significantly reduced the increase in Cu concentration (P<0.05). This finding concurs with the results of previous studies (Onosaka et al., 2002; Cicik et al., 2003) that suggest that, routes of absorption and entry into the circulatory system are similar for Cu and Zn.

Zn finger transcription play an important role in the regulation of transcription of metallothionein (MT), which plays a major role in Cu detoxification (Kimura, 2010). Both zinc and copper induce MT synthesis but the effect of Zn was reported to be greater than that of Cu (Liu et al., 1991; Park et al., 2001). Other researchers have suggested that Cu and Zn homeostasis is maintained by the control of Zn–Cu metabolism via binding with metal-binding proteins and peptides in orgazima (Cicik et al., 2003).
Park et al. (2001) injected rats with small doses of Zn and Cu, and reported that, the same dosage of Zn and Cu increased synthesis of MT by 2.4 fold and 1.4 fold, respectively. This suggests that MT has a higher affinity for Zn than for Cu. Generally, MT is saturated with Zn (Kimura, 2010). Iarto and Albergoni (2005) reported that the administration of Zn after dietary Cu administration are more than doubled intestinal MT concentration, which reduced the Cu concentration to the level of the control values. Medici et al. (2005) reported that Zn-induced MT production plays a role in cellular protection against damage caused by excessive Cu accumulation in bee tissues.

The results of the present study suggest that Zn administration augments MT synthesis in honeybees. MT binds to Cu, reducing the intracellular concentration and protecting the cell against its toxic effects. The data from our study suggest that Zn also inhibits the absorption and metabolism of Cd. There was a statistically significant difference (P<0.05) between Cd concentrations in the groups of bees exposed to Cd, with or without Zn; in group B, the Cd concentration was 1.83±0.33 ppm and in group C it was 1.12±0.08 ppm. These results from the present study with honeybees approximate those from other studies that used a variety of animals (Onosaka et al., 2002; Seebaugh and Wallace 2004).

Zinc has been reported to have a substantial ability to reduce the toxicity of orally ingested Cd (Barata et al., 2002). Due to their chemical similarity, Zn and Cd ions compete for binding proteins. Zn pretreatment increased the endogenous concentration of MT in the intestine 25-fold. Following intraluminal administration, 93% of Cd in the intestinal cytosol of Zn-treated rats was bound to MT, whereas only 40% of the cytosolic Cd was bound to MT in saline-treated (control) rats (Goon and Klaassen, 1998). In another case, Zn–MT synthesis increased after the administration of Zn (Hao et al., 2012). Binding Cd into an MT–Cd complex prevents cell toxicity in the tissues (Seebaugh and Wallace 2004).

Intracellular MT also plays an important role in ameliorating Cd toxicity following prolonged exposure, particularly chronic Cd-induced nephrotoxicity, osteotoxicity, and toxicity in the lungs, liver and immune system (Klaassen et al., 2009). Individuals with a low MT level are at high risk from Cd toxicity (Nordberg, 2004). Another study showed that increased Zn absorption reduces the renal toxicity of Cd (Mudgal et al., 2010). In the light of these data, it is suggested that Zn induces MT synthesis, thus increasing the binding of MT to Cd in the tissues and contributing to the formation of non-toxic MT–Cd complexes, thereby preventing toxicity resulting from the accumulation of Cd (Hua et al., 2011).

Zn concentration was increased by MT induction and MT-bound Zn significantly reduced the toxicity of the metals Cd and Cu (Kheradmand et al., 2013). MTs are chelators of harmful metals, like Cu and Cd. Under normal physiological conditions, when the organism is not contaminated with Cu and Cd, MT is bound to Zn. Zinc allows MTs to obtain their correct orientation inside the cytoplasm. When bees are exposed to heavy metals, they start overexpressing MTs as a defence in which Zn is needed for the correct fold of such proteins; thus an addition of Zn to their diet can benefit that process (Hua et al., 2011; Mudgal et al., 2010).

In the present study, in bee tissue samples the mean Pb level of those administered heavy metals (group B) was 0.87±0.25 ppm, whereas in the group receiving the heavy metal–Zn combination (group C), this value was 1.27±0.29 ppm. However, there was no significant difference between these groups (P>0.05). This suggests that Zn had no effect on Pb absorption and metabolism. This supports the finding of other researchers (Hanas, 1999; Castro et al., 2011), which showed a positive correlation between Pb and Ca concentrations, but found no correlation between Zn and Pb levels in dairy cows.

However, Verster (2011) reported that both Ca and Zn reduced Pb absorption in cows. In another study, Azooz et al. (2011) reported that a high dose combination of Zn and Pb produced a synergistic effect on the inhibition of plant growth parameters. Hanas et al. (1999) also reported that Pb inhibited the binding of Zn to proteins. Protein binding was reported to be higher for Pb than for Ca and Zn (Hasiang and Diaz, 2011; Godwin, 2001). Park et al. (2001) reported that MT did not provide protection against Pb and Fe. The differences in results between the studies may be the result of the different doses used and variations between animal species utilised, which covered a wide scale, including rats and cows (Hanas et al., 1999; Verster 2011).

In the present study of heavy metal-Zn interaction in bee tissues, significant differences were seen between the control group and the other groups (P<0.05), whereas there was no significant difference between groups B and C for all three metals (P>0.05). Table 1 shows that the concentration of Zn in the bees of the control group (Group A) which were not administered, three heavy metals was much higher than in Group C because excessive heavy metal concentration can affect zinc metabolism (Ghayour-Mobarhan et al., 2009). That may explain why in our study, excessive heavy metal administration decreased Zn absorption in group C while in the control group, Zn was absorbed through the intestine without interference.

In this study, the effect of the heavy metal–Zn combination on the concentrations of a number of neurotransmitters in honeybees was also investigated. There was no significant difference between the study groups in mean serotonin levels in brain tissue (P>0.05). This may be attributed to the low sensitivity of serotonin
to orally administered metals (Bhalla et al., 2007; Carlson et al., 2008). Carlson et al. (2008) reported that, the oral administration of Zn and Cu reduced the secretion of serotonin (5-HT) from intestinal epithelial cells. Cu was found to be more potent than Zn in this respect. Bhalla et al. (2007) reported that orally administered Zn did not cause a significant alteration in serotonin concentration. Lafuente et al. (2005) reported that rats exposed to Cd had no change in 5-HT concentration in the median eminence, although it increased in both the anterior and posterior pituitary.

The failure to demonstrate a significant difference in serotonin concentration between the groups in our study may be because, oral administration of metals has less effect on serotonin concentration. Dopamine is particularly sensitive to heavy metal toxicity and in this study, dopamine concentration was significantly different between the groups (P<0.05). These results support the theory that Zn supplementation can reduce the suppressive effect exerted by heavy metals on dopamine synthesis. Cd and Pb can cross the blood–brain barrier and reduce the concentrations of this amine (Lafuente et al., 2005; Romero et al., 2011).

In rats exposed to Cd, dopamine levels increased in the posterior pituitary but decreased in the median eminence (Lafuente et al., 2003). The effects of Pb and Cd on monoamine concentration varies with animal species and stage of development between different areas of the brain (Lafuente et al., 2003; Verstraeten et al., 2008). Bhalla et al. (2007) stated that when Zn was administered orally, dopamine concentration increased, with resultant positive effects on memory and cognitive behavior in rats. These results suggest that Zn supplementation may reduce the negative effects of Cd, Cu and Pb on dopamine levels.

Conclusions

Our results demonstrated that, the co-administration of zinc to honeybees exposed to heavy metals reduces Cu and Cd accumulation in tissue samples. These results support the theory that, Zn supplementation reduces the suppression of dopamine by Cu, Cd and Pb. This study complied with ethical standards.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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