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Original Research Article

Amelioration of Cadmium-Induced Biochemical Alterations in Heart Tissues of *Wistar* Rats Using Bee Honey

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ABSTRACT

The hunt for potential remedies for cadmium (Cd) toxicity is an exciting topic of research that is now being investigated globally. This study investigated amelioration of cadmium-induced biochemical alterations in heart tissues of *Wistar* rats using bee honey. Six groups (1–6) of thirty adult *Wistar* rats were used for the study: control, sub-chronic Cd exposure, sub-chronic Cd exposure plus honey, acute Cd exposure, acute Cd exposure plus honey, and honey only. For the sub-chronic investigation, Cd chloride ($CdCl_2$) was injected intraperitoneally (IP) at a dose of 2 mg/kg body weight every two days for a period of four weeks, and for the acute trial, a dose of 4 mg/kg body weight was injected IP 12 hours prior to sacrifice. 1 ml/kg of body weight of honey was given orally once every day for four weeks. According to the study, groups 2 and 4 had substantially greater levels of lipid peroxidation and acid phosphatase (ACP) activity in the heart tissues compared to group 1. Catalase (CAT), superoxide dismutase (SOD), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) enzyme activity were also significantly decreased. This study shows that honey treatment significantly ameliorates Cd-induced toxicities in heart tissues by suppressing lipid peroxidation and normalizing antioxidant enzyme activity. Further work should be done to identify specific phytochemical antioxidants and its mode of action in ameliorating Cd-induced toxicities in heart tissues.

***Keywords*:** Cadmium, honey, toxicity, heart, acute exposure, chronic exposure.

Introduction

Environmental exposure to hazardous heavy metals is a widespread problem and a cause for serious concern because it can have a variety of negative health impacts on both humans and animals.[1,2] The soil, rock phosphate fertilizer, and tobacco plants are all sources of the hazardous element cadmium (Cd) in the environment.[3] Industrialization and mining have increased the amounts of Cd in soils, sediments, and water.[4,5]Cd is an extremely toxic metal with a very long biological half-life. Human exposure to Cd is unavoidable because it is not biodegradable; its levels in the environment are rising as a result of industrial activity. Chronic exposure to Cd frequently results in renal failure, anaemia, osteoporosis, and bone fractures. Acute Cd exposure causes toxicities in the lungs, liver, testes, and brain. Cd is a powerful carcinogen in a variety of rodent tissues and is categorized as a human carcinogen.[3] Oxidative stress is a significant factor in toxic effects of Cd in the tissues of the brain, kidneys, heart, liver, bone, testes, and ovaries. The production of reactive oxygen species (ROS) is stimulated by oxidative stress, which is among the several ways that lead to toxicity.

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This leads to RBCs and other tissues experiencing oxidative damage, which results in a loss of membrane integrity.[6,7] As a result, Cd can harm tissues through oxidative processes by accelerating the peroxidation of lipids in the membrane and disruption of cells' natural antioxidant mechanisms. Due to organelle interference from metal ions and cellular membrane peroxidation damage, cellular components may become disrupted.[1,8] In recent years, research on the amelioration of metal toxicity has switched to focus on natural antioxidant molecules rather than synthetic ones. Researchers have thus examined a number of natural products, such as honey, garlic, plant extracts etc, that have strong antioxidant benefits against the harmful effects of Cd and other metals.[1,9-11] Honey is frequently used in folk medicine to cure a variety of illnesses and to heal wounds. Its anti-inflammatory, antioxidant, anti-anaemic, and hepato-renal protective effects have all been demonstrated.[11-14]According to studies,[11,15,16] honey has a preventive effect against Cd toxicity. Honey is a rich source of both enzymatic and non-enzymatic antioxidants like flavonoids, catalase, and other polyphenols. Vitamins are also present in honey, which has been shown to be significantly effective against Cd, lead, nickel, arsenic, mercury, and chromium toxicities.[12,15] However, the use of honey in mitigating the effect of Cd-induced toxicity in the hearts tissues of animals has not been adequately addressed. Thus, this study investigated amelioration of cadmium-induced biochemical alterations in heart tissues of *Wistar* rats using honey after sub-chronic and acute exposures.

Materials and Methods

Chemicals and Reagents

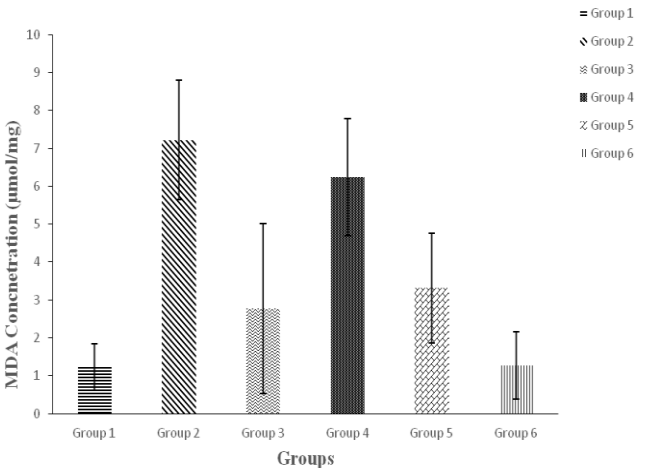
The source of the cadmium utilized in the experiment was Sigma Aldrich Co.'s cadmium salt ($CdCl_2$). The suppliers of all other analytically grade chemicals utilised were May & Baker, Dagenham, Sigma Aldrich Co., Titan Biotech Ltd, Bhiwadi-Rajasthan,

India, Loba Chemie Mumbai, India, British Drug House Chemicals, Poole, England; and England).

Honey
Natural bee honey purchased (August, 2023) from the Federal Ministry of Science and Technology's Black Ebony Resources Technology Incubation Centre (TIC), National Board for Technology Incubation (NBTI), Warri, Delta State was used for this study. Honey was diluted in distilled water at the ratio of 1:2 that is I mL of honey was diluted in 2ml of distilled water.

Animal
To conduct this investigation, thirty (30) adult *Wistar* rats with a mean body weight of 180±5.5 g were acquired from the College of Health Sciences' animal house at Delta State University in Abraka, Nigeria. According to the National Institute of Health (NIH)[17] handbook on the use and care for laboratory animals, the animals were fed, watered, and housed throughout the investigation in standard animal cages under controlled environmental conditions of a 12-hour dark-light cycle. They were given growers mash and water as needed over the week-long acclimatization process.

Ethical Clearance
The study was approved by the Faculty of Science research and ethics committee (REC) at Delta State University, Abraka with approval number REC/FOS/2024/01. All animal-related procedures followed the National Institute of Health (NIH)[17] handbook on the use and care for laboratory animals.



Experimental design
Atagana and Asagba's[15] experimental design was modified. Aqueous solution of 0.36mg/L and 0.72mg/L $CdCl_2$ for 2mg/kg and 4mg/kg respectively were prepared. The rats were separated into six groups, numbered 1-6, with five rats in each group. For a period of four (4) weeks, animals in group 1 (the control) were given feed mash and water every day. This was the control group. Animals in group 2 (sub-chronic Cd alone) received intraperitoneal injections of 2 mg/kg of Cd ($CdCl_2$) three (3) times a week for four (4) weeks. For a total of four weeks, group 3 animals (sub-chronic Cd + honey) received 1 ml/kg of honey diluted with water (in a ratio of 1:2) once daily and 2 mg/kg of Cd by intraperitoneal injection (IP), three times per week.
Twelve (12) hours before sacrifice, animals in Group 4 (acute Cd alone) received an intraperitoneal injection (IP) of 4 mg/kg body weight of Cd (in the form of Cd chloride ($CdCl_2$). Twelve (12) hours before sacrifice, animals in group 5 (acute Cd + honey) received intraperitoneal injections of Cd (in the form of Cd chloride ($CdCl_2$) at a dosage of 4 mg/kg body weight and oral administration of 1 ml/kg body weight of honey diluted with water (in a ratio of 1:2). Animals in Group 6 (honey only) received 1 ml/kg of honey diluted with water (at a ratio of 1:2) orally, once every day for four weeks. Animals in all the groups received growers mash and water as needed throughout the treatment.
The animals from each group were sacrificed under chloroform anaesthesia at the conclusion of the treatment period[15]. The animals' hearts were then removed after being dissected, and they were each individually blotted on ash-free filter paper, patted dry, weighed, and homogenized under cold conditions in normal saline. In order to get the supernatants, the homogenates were centrifuged at 5000 g for 10 minutes. The supernatants were then collected in standard bottles and kept in a refrigerator while waiting for biochemical assays.

Lipid peroxidation analysis: Following the procedure outlined by Varshney and Kale,[18] the production of malondialdehyde (MDA) was measured to assess lipid peroxidation.
Determination of alanine aminotransferase (ALT) activity: Using an assay kit (Randox) according to Reitman and Frankel[19] description, the activity of the enzyme alanine aminotransferase (ALT) was measured.
Determination of aspartate aminotransferase (AST) activity: AST activity was measured using an assay kit (Randox) in accordance with Reitman and Frankel.[19]
Determination of alkaline phosphatase (ALP) activity: In accordance with Roy,[20] an assay kit (Teco Diagnostics) was used to measure the alkaline phosphatase (ALP) activity.
Determination of acid phosphatase (ACP) activity: Teco Diagnostics' assay kit was used to perform the acid phosphatase (ACP) activity, which was done in accordance with Hillman.[21]
Determination of superoxide dismutase (SOD) activity: The procedure of Misra and Fridovich[22] was used to measure the activity of superoxide dismutase (SOD).
Determination of catalase (CAT) activity: The method reported by Kaplan et al.[23]was employed to assess the activity of catalase (CAT).

Statistical analysis
Data from the study were displayed using mean ± standard deviation. With the statistical programme SPSS version 21, one-way analysis of variance (ANOVA) was used to analyze the data of test samples, and Fisher's LSD test was used for significance testing ($P < 0.05$). Graphs were plotted with Microsoft excel.

Results and Discussion

This study investigated the amelioration of Cd-induced biochemical alterations in heart tissues of *Wistar* rats using bee honey. Cd causes biochemical changes in the heart that are indicative of oxidative damage and may lead to early cardiac failure and hypertension. Cd causes the most mitochondrial damage of all the toxicants.[24]
Figure 1 shows that at the end of the treatment period, the rate of lipid peroxidation (MDA levels) in the heart tissues of rats exposed to just Cd at sub-chronic and acute treatments was significantly higher ($P<0.05$) than the control. MDA levels were significantly lower ($P<0.05$) in honey-only-treated rats than in the control group. At acute and sub-chronic levels,

Figure 1: Effect of honey on cadmium-induced lipid peroxidation in the heart tissues
Values expressed as Mean ± Standard Deviation (SD). n = 5.
Values with different superscript are significantly different at ($P<0.05$). Group 1= Control, Group 2= Sub-chronic Cadmium, Group 3 = Sub-chronic Cadmium + Honey, Group 4 = Acute Cadmium, Group 5 = Acute Cadmium + Honey, Group 6 = Honey.

Cd-exposed rats treated with honey had MDA levels that were lower than those treated with Cd alone, but greater than those of the control group and the group treated with honey alone. This demonstrates how honey protects against lipid peroxidation in the heart tissues. These results demonstrated that, for both acute and sub-chronic doses, increased lipid peroxidation was a sign of Cd toxicity in the cardiac tissues. The impact of Cd on lipid peroxidation in the cardiac tissues was reduced in rats exposed to Cd when they were given honey.

The MDA assessment helps to confirm the extent of biological membrane damage caused by xenobiotics.[25] The chain oxidation of polyunsaturated phospholipids by the super-oxide anion radical may be responsible for the considerable increase in LPO levels. This suppression of antioxidant activities resulted in oxidative tissue damage. Several tissues exposed to Cd were shown to have higher LPO levels, which is consistent with our findings.[26,27] Honey protects the rat heart from lipid peroxidation brought on by Cd. According to Al-Kafaween et al.[28] honey contains a variety of constituents in trace amounts, including minerals, free amino acids, proteins, vitamins, enzymes, organic acids, flavonoids, phenolic acids, and other organic acids. It also contains additional phytochemical compounds that act as antioxidants to scavenge free radicals. Atagana and Asagba,[15] Diakparomre et al.[16] and Shalaby and Saleh[29] have also previously reported on this decrease in lipid peroxidation in the presence of honey.

Figures 2 and 3 show the effect of honey on the superoxide dismutase (SOD) and catalase (CAT) activities in the heart tissues of rats exposed to Cd respectively. The activity of SOD and CAT was considerably reduced in rats exposed to Cd alone (Groups 2 and 4) compared to control (Group 1) at the end of both the sub-chronic and acute exposures.

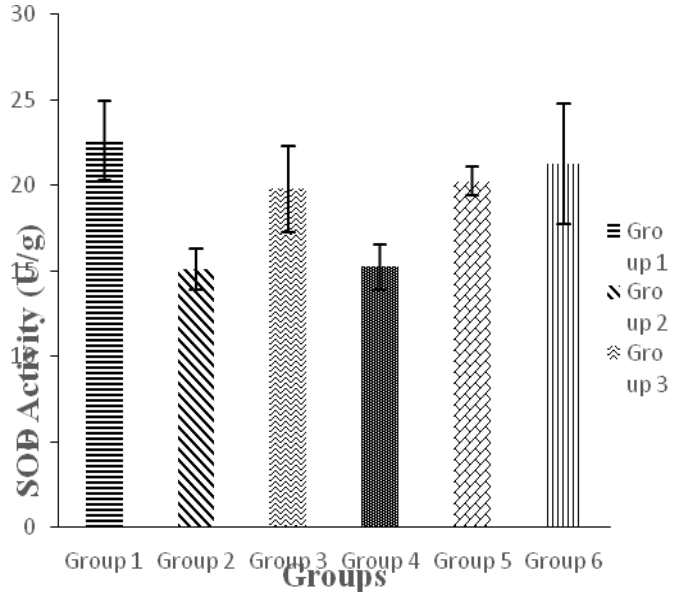


Figure 2: Effect of honey on SOD activity in the heart of rats exposed to cadmium.
Values expressed as Mean ± Standard Deviation (SD). n = 5.
Values with different superscript are significantly different at (P<0.05). Group 1= Control, Group 2= Sub-chronic Cadmium, Group 3 = Sub-chronic Cadmium + Honey, Group 4 = Acute Cadmium, Group 5 = Acute Cadmium + Honey, Group 6 = Honey.

In contrast, rats fed on honey alone (Group 6) did not substantially differ from control in terms of SOD and CAT activity at the conclusion of the exposure periods. When compared to rats exposed to Cd alone (Groups 2 and 4) following the treatment periods, rats treated with honey (Groups 3 and 5) showed significantly higher (P<0.05) SOD and CAT activity. This suggest that exposure to Cd diminished the activity of SOD and CAT for both treatment durations, whereas the treatment of Cd-exposed rats with honey led to a considerably increased SOD and CAT activity in the heart tissues.

Antioxidant enzymes like SOD and CAT guard cells against oxidative damage. The production of superoxide anions by toxicants initiates the activity of SOD, which eliminates dangerous radicals in cells, causing a fall in SOD activity whereas the decline in CAT could be attributed to the excess free radical generation's inhibition of CAT activity.

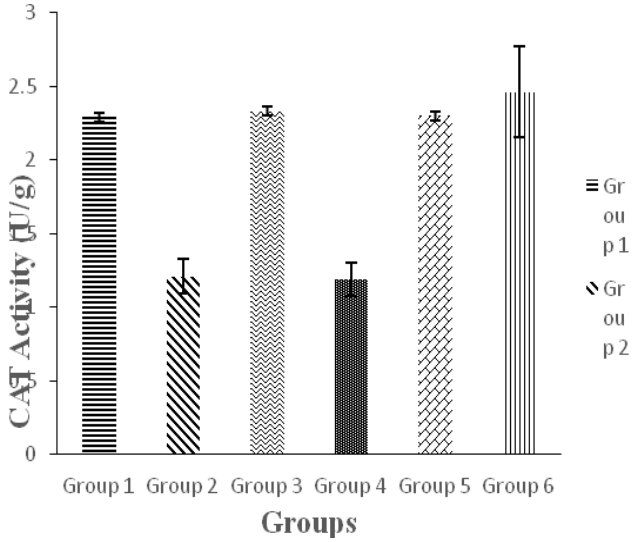


Figure 3: Effect of honey on CAT activity in the heart of rats exposed to cadmium.
Values expressed as Mean ± Standard Deviation (SD). n = 5.
Values with different superscript are significantly different at (P<0.05). Group 1= Control, Group 2= Sub-chronic Cadmium, Group 3 = Sub-chronic Cadmium + Honey, Group 4 = Acute Cadmium, Group 5 = Acute Cadmium + Honey, Group 6 = Honey.

This is in line with the study of Kadiri[30] and Wang et al.[31] that reported decrease in SOD and CAT activity in the tissues of animals exposed to Cd. Similar results reported by Ezedom et al.[32]showed that tissues from organisms exposed to Cd had higher MDA concentrations, along with concurrently declining SOD and CAT activity. In the present work, honey administration to Cd-exposed rats at sub-chronic and acute doses resulted in a notable rise in the antioxidant enzymes (CAT and SOD) in comparison to animals who received only Cd. According to Cheng et al.[34] phenolic compounds have hydroxyl groups joined to the aromatic ring that can serve as hydrogen donors in the scavenging of free radicals. Additionally, phenolic chemicals might lower the metal ions since they are electron donors. The phenolic content is therefore thought to be the key to the honey's antioxidant properties.[33,35,36]

Table 1 and 2 shows the impact of honey on ALT and AST activity in heart tissues of rat after exposure to Cd and the activities of ALP and ACP in the heart tissues of rat of the various groups respectively. Rats exposed to Cd at sub-chronic levels (Group 2) and acute levels (Group 4) had significantly lower ALT and AST activities in their heart tissues than the control group (Group 1) (P<0.05). However, no significant difference was observed in the activities of ALT and AST in the heart tissues of Cd-exposed rats treated with honey (Groups 3 and 4), those maintained on honey alone (Group 6) and the control group. The result therefore demonstrates that honey treatment increased ALT and AST activities while Cd exposure decreased their activity in the cardiac tissues of rat.

When compared to the control, rats exposed to Cd had significantly lower (P<0.05) levels of ALP activity in their tissues. This was seen with both sub-chronic (Group 2) and acute (Group 4) treatment types. On the other hand, honey treatment of Cd-exposed rats led to ALP activities that were higher than those in groups 2 and 4, but were comparable to rats exposed to honey alone (Group 6). According to the findings, treatment with honey restored ALP activity to that of the control while exposure to Cd reduced ALP activity in the cardiac tissues of rats.

When compared to the control group (Group 1), the activities of ACP in the heart tissues of Cd-exposed rats (Groups 2 and 4) were considerably higher (P<0.05), demonstrating that Cd greatly increased

Table 1: Effect of honey on ALT and AST activities in the heart of rats exposed to cadmium.

Groups	Enzyme Activity in Heart Tissues (U/L)	
	ALT	AST
Group 1: Control	22.61 ± 2.32[a]	42.40 ± 2.72[a]
Group 2: Sub-chronic Cd Only	15.10 ± 1.20[b]	17.40 ± 2.51[b]
Group 3: Sub-chronic Cd + Honey	19.80 ± 2.50[a]	41.27 ± 3.52[a]
Group 4: Acute Cd Only	15.22 ± 1.32[b]	16.12 ± 1.12[b]
Group 5: Acute Cd + Honey	20.23 ± 0.83[a]	39.32 ± 1.92[a]
Group 6: Honey Only	21.26 ± 3.54[a]	41.22 ± 4.12[a]

Values are expressed in Mean ± Standard Deviation (SD). n = 5.
Values on the same column with different superscript are significantly different at (P<0.05).

Table 2: Effect of honey on ALP and ACP activities in the heart tissues of rats exposed to cadmium.

Groups	Enzyme Activity in Heart Tissues (U/L)	
	ALP	ACP
Group 1: Control	139.84 ± 16.32[a]	8.33 ± 2.44[a]
Group 2: Sub-chronic Cd Only	73.60 ± 12.20[b]	17.40 ± 2.51[b]
Group 3: Sub-chronic Cd + Honey	125.91 ± 24.32[a]	10.66 ± 2.52[a]
Group 4: Acute Cd Only	81.63 ± 9.65[b]	16.94 ± 5.44[b]
Group 5: Acute Cd + Honey	127.23 ± 14.51[a]	11.79 ± 1.77[a]
Group 6: Honey Only	134.26 ± 21.32[a]	10.94 ± 1.33[a]

Values are expressed in Mean ± Standard Deviation (SD). n = 5.
Values on the same column with different superscript are significantly different at (P<0.05).

the enzyme's activity in the heart tissues after acute and sub-chronic exposure. On the other hand, rats exposed to Cd and given honey showed dramatically reduced ACP activity, which was comparable to that of control rats (Group 1) and rats given honey alone (Group 6). The results show that treatment with honey reduced the activity of the enzyme to a normal level while exposure to Cd increased ACP activity in the rat heart tissues.

While some authors reported an increase in enzyme (ALT, AST, and ALP) levels,[37-39] others[40-42] observed a drop. The competitiveness between harmful metals and divalent ions, such as Mg, Co, and Mn; enzyme activators; and Zn, an enzyme component, can be used to explain the observed decline. The activity of these enzymes (ALT, AST, and ALP) were restored to normal by administration of honey, indicating less harm had been done to the heart's cells and tissues. This finding is consistent with other researches[15,16,43] where they asserted that the activities of ALT, AST, and ALP seen in tissues of rats exposed to Cd were restored to normal by honey. The rise in tissue acidity brought on by oxidative stress may be the cause of increased ACP activity. According to Ciriolo et al.[44] the initiation of lipid peroxidation brought on by oxidative stress has been associated with a decrease in intracellular pH. Oladele et al.[45] and Onwuka et al.[46] have also previously demonstrated an increase in ACP activity caused by Cd-induced oxidative stress. Diakparomre et al.[16] previously reported an increase in ACP activity in the brain tissues of Cd induced *Wistar* rat which was ameliorated by administration of honey.

Conclusion

Cd caused oxidative stress and changed the activity of a few key enzyme systems in the heart tissues. However, honey treatment showed considerable amelioration against Cd-induced toxicities in the heart tissues, which was caused by oxidative stress, through the suppression of lipid peroxidation and the normalization of antioxidant enzymes (SOD and CAT) activity and significant enzymes (ALP, AST, ALP, and ACP) in the heart tissues. Honey plays a protective role in the body and therefore, may be considered a possible therapeutic and ameliorative choice in the management of cellular and tissue damage resulting from Cd-induced oxidative stress in the heart tissues.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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