International Journal of Scholarly Research in
Life Sciences
Journal homepage: https://srrjournals.com/ijsrls/
ISSN: 2961-3264 (Online)

(RESEARCH ARTICLE)

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Effects of concurrent food-chain simulation of cadmium and crude petroleum oil pollutions on amylase activity in Wistar rats

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International Journal of Scholarly Research in Life Sciences, 2022, 01(01), 033–038

Publication history: Received on 23 June 2022; revised on 09 August 2022; accepted on 11 August 2022

Article DOI: https://doi.org/10.56781/ijsrls.2022.1.1.0024

Abstract

The bioavailability levels of cadmium (Cd) and (CPO) in water and polluted food has become worrisome over the years. The study was aimed at investigating the effects of concurrent food-chain simulation of cadmium and crude petroleum oil pollution on amylase activity in Wistar rats. Simulation of both Cd and CPO pollution was done in the water in which catfish were housed for 1 month at a daily dose of 4 and 0.8 ppm respectively. The fish were then used as source of protein in formulation of diet for feeding rats for 1 and 2 months respectively. The rats were granted access to drinking water at will, weighed at the end of the feeding periods and sacrificed followed by removal of the duodenal portion of the small intestine. Weight gain and intestinal/body weight ratio was measured and the activity of amylase assayed. The results showed a non-significant difference ($p > 0.05$) in the intestinal/body weight ratio and amylase activity after 1 month of simulated pollution. While, there was a significant decrease ($p < 0.05$) in weight gain and amylase activity after 2 months. Cd and CPO mixture therefore, has a deleterious effect on the environment and subsequently animals especially man as the final consumer in the food-chain.

Keywords: Cadmium; Food-chain; Simulation; Amylase; Concurrent

1. Introduction

Cadmium (Cd) is a heavy metal belonging to group 12 and period 5 of the periodic table of elements. It is both ubiquitous in the natural environment and extremely toxic. Cd pollution has been identified as a potential health threat to wildlife as its technological utilization has led to increased levels in the environment and also in the human body [1]. Animals normally absorb Cd into the body through inhalation or ingestion. Cd build-up in living organisms is a crucial ecological factor mostly due to its ability to build-up rapidly. It is known that exposure in diets is the major route for metal bioaccumulation in many marine and terrestrial animals [2, 3, 4, 5]. The effects of the toxicity are acute, when large amounts are ingested or the element is particularly toxic or chronic, with toxicity only being noticed after a long time [6, 7]. The latter may be the result of the bioaccumulation process across the food-chain [8, 9].

Oil exploration in the Niger- Delta of Nigeria over the years have led to serious pollution of the environment by these oil producing states and have taken a toll in the biological activity of the inhabitants. Recently, the Government of Nigeria has undertaken a clean-up process of the Ogoni land. While this is a welcomed development, the process is however slow in commencing and the impacting effects are still with the populace. The water-soluble fraction (WSF) of crude petroleum oil (CPO) is the solution of low molecular mass compounds naturally released from petroleum hydrocarbon mixtures in contact with water. Although generally regarded as hydrophobic, many petroleum hydrocarbons are water-soluble to a large degree. Low molecular mass compounds account for much of the toxic nature of hydrocarbon spills. Of great environmental concern particularly are benzene, toluene, ethyl benzene and the xylenes (BTEX) because they are readily available to organisms [10].

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Water is very essential for agriculture, industries and other human activities, and are normally obtained from two major natural sources which are surface water (water bodies) such as rivers, streams, fresh water lakes and ground water (geological water) such as borehole and well water [11]. Despite the numerous benefits of water, there is a very serious concern for the accessibility of this free and indispensable product due to the high rate of contamination of most water bodies. Among the various sources of pollution, the petroleum industry is considered the greatest source of water pollution in Nigeria. The increase in crude oil exportation and exploitation has resulted in remarkable increase in environmental degradation of terrestrial and aquatic ecosystems, and the degree of pollution may be significant where regular oil spillages occur [12]. Man-made activities that contribute to the pollution of water bodies may be intentional or accidental and these include gas glaring, oil spill discarding of used lubrication oils, tank cleaning, leakages from marine vessels and off-shore production, direct ocean dumping, coastal, municipal and industrial wastes, run-off from crude oil pollutes lands, seepage, refinery effluents etc. [13, 14, 15]. Among the hydrocarbons which are components of crude petroleum oil are total hydrocarbon (THC). Owing to the fact that pollutants in the environment often occur in mixtures, it is worthwhile to study the effects of Cd and CPO mixtures which occur in the natural environment by copying their transfer across the food-chain using a simulated medium in rats.

2. Material and methods

2.1 Chemicals/Reagents

All chemicals/reagents used in the study were of analytical grades.

2.2 Trophic Level 1: Simulation of Cd and CPO pollutions

Eighty (80) catfish were obtained from the fish farm in Fisheries Department of the Faculty of Agriculture, University of Benin, Edo state, Nigeria. The fish were sorted, divided into four (4) groups and left to get used to the new habitat for one (1) week before commencement of the First Feeding Level, which involved separate and combined exposures of the fish to Cd (in form of Cadmium chloride, $CdCl_2$) and water-soluble fraction (WSF) of CPO respectively.

The experimental design for the first trophic level of the study was:

- **Group A (Control) -** fish in this group were housed in 85 L plastic aquaria with fresh water for 4 weeks. This was marked as control group.
- **Group B (Cd) -** fish in this group were housed in 85 L plastic aquaria and exposed to Cd at a dose of 4 parts per million (4 ppm). The water was changed and re-contaminated every 24 hours for 4 weeks.
- **Group C (CPO) -** fish in this group were housed in 85 L plastic aquaria and exposed to CPO at a dose of 0.8 ppm as described by [16] for 4 weeks. The water was changed and re-polluted every 24 hours for 4 weeks.
- **Group D (Cd + CPO) -** fish in this group were housed in 85 L plastic aquaria and exposed to Cd + CPO polluted water. The water was changed and re-polluted every 24 hours for 4 weeks.
- All the fish received commercial fish feed daily for 4 weeks after which they were killed, dried in the oven, their bones removed and used as protein source (15% of total diet) for the diet of rats in the Second Feeding Level. This was done to copy the natural food-chain of fish to rat [17]. Other sources of nutrients in preparing the diet include: Corn starch (55%), Palm oil (10%), Groundnut shell (7%), Granulated refined sugar (8%) and Vitamins and minerals mix (5%).

2.3 Trophic Level 2: Feeding rats with prepared fish diets

Sixty (64) male Wistar albino rats, aged 8-12 weeks of weighing between 0.1 – 0.12 kg were procured from the Animal House, Department of Animal and Environmental Biology, University of Benin. The rats were divided into 4 groups (same as the fish in trophic level 1) with 16 rats in each group and housed in cages. Rats in all groups were fed 320 g of the formulated diet corresponding to the fish grouping daily and granted access to drinking water at will. Half the numbers of rats in all groups were sacrificed after 4 weeks of pollution for the half term study, while the other half was sacrificed after 8 weeks of pollution for the full-term study.

2.4 Crude Petroleum Oil (CPO) and its fractionation

CPO was obtained from Warri Refining and Petrochemical Company (WRPC), a subsidiary of NNPC in Delta State, Nigeria. The crude oil was fractionated by the method of [18] into Water-soluble fraction (WSF) and water insoluble fraction (WIF). For the fractionation, a 1:2 of 500 ml of crude oil was put in a 2 L conical flask covered with cotton wool and foil paper and constantly stirred with a table-top magnetic stirrer for 48 h. The WSF was then separated from the WIF in a sealed separating funnel and kept until required.

2.5 Collection of Intestine

After the 4- and 8-weeks pollution periods, the rats in all groups were sacrificed under mild anaesthesia. The duodenum was excised, washed in normal saline, homogenized (20% w/v) in ice-cold physiological saline and then centrifuged at 10,000 x *g* for 15 min. The supernatant gotten was then stored at 4°C prior to biochemical analysis.

2.6 Cadmium and Total Hydrocarbon (THC) Analysis

Cadmium concentration in the formulated rat diet was measured by atomic absorption spectrophotometry (Varian spectrAA-600). The test metal was dissolved in de-ionized water and used as standard. In all the determinations, blanks were prepared to determine the effect of reagent purity on the metal levels.

THC content was determined by Extraction-Infrared Absorption method using infrared spectrophotometer.

2.7 Assay of Amylase activity

The duodenal supernatant was used to assay the amylase activity according to the method of [19].

2.8 Procedure

Using starch as substrate, 1 ml of properly diluted enzyme solution was incubated for 3 min. with 1 ml of 1 % starch (1 g soluble starch and 0.0035 g NaCl in 100 ml of 0.002M Na_3PO_4, pH 6.9). The reaction was stopped by the addition of 2 ml of 3,5 dinitrosalicyclic acid reagent. The solution was then heated for 5 min. in boiling water, cooled and 20 µl distilled water added. The absorbance at 540 nm was read and a standard curve was established with maltose to convert readings into mg of maltose. Amylase activity was expressed as mg maltose liberated/min. g/tissue.

2.9 Data Analysis

Results obtained were expressed as mean ± standard error of mean (SEM). Data obtained were analyzed with one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS version 21.0) and group means compared with Duncan's Multiple Test. A probability of $p < 0.05$ was considered significant.

3. Results and discussion

The concentration of Cd and THC in the formulated diets made from the fish in the Cd and CPO groups was 1.80 ± 0.62 and 2.33 ± 0.18 mg/kg respectively while in the combined group it was 1.60 ± 0.14 and 2.63 ± 0.26 mg/kg respectively. This is significantly higher ($p < 0.05$) than the level in the diet made from fish in the control group. Table 1 shows the effects of concurrent food-chain simulation of cadmium and crude petroleum oil pollutions on weight gain and intestine/body weight ratio in Wistar rats.

Table 1 Effects of concurrent food-chain simulation of cadmium and crude petroleum oil pollutions on weight gain and intestine/body weight ratio in Wistar rats

Group	A (Control)	B (Cd)	C (CPO)	D (Cd + CPO)
Parameter Body weight gain (g)				
1 month	30.67 ± 1.32a	17.70 ± 3.46b	18.98 ± 4.96b	24.68 ± 6.32b
2 months	46.35 ± 3.88a	12.55 ± 8.06b	15.57 ± 3.88b	34.98 ± 3.47c
Intestine/body weight ratio (g)				
1 month	0.04 ± 0.01a	0.04 ± 0.01a	0.02 ± 0.01a	0.04 ± 0.01a
2 months	0.03 ± 0.01a	0.03 ± 0.01a	0.04 ± 0.00a	0.04 ± 0.00a

Amylase activity was expressed as mg maltose liberated/min g/tissue. Values were given as mean ± SEM. Values not sharing a common superscript letter in the same column differ significantly ($p < 0.05$).

The body weight gain of rats fed simulated Cd and CPO polluted fish diet concurrently was significantly decreased ($p < 0.05$) relative to control after 1 and 2 month periods. Test group comparison showed no significant difference ($p > 0.05$) after 1 month, while after 2 months the group fed concurrent Cd and CPO diets differed significantly from the respective

individual diet. There was no significant difference ($p > 0.05$) in the intestine/body weight ratio in the rats from test groups when compared to control and within groups respectively.

Table 2 Showed the effects of concurrent food-chain simulation of cadmium and crude petroleum oil pollutions on amylase activity in Wistar rats

Table 2 Effects of concurrent food-chain simulation of cadmium and crude petroleum oil pollutions on amylase activity in Wistar rats

Group	A (Control)	B (Cd)	C (CPO)	D (Cd + CPO)
Parameter Amylase				
1 month	27.39 ± 1.40[a]	30.85 ± 2.60[a]	43.16 ± 6.15[a]	21.16 ± 3.58[a]
2 months	21.75 ± 3.18[a]	13.96 ± 5.05[b]	17.73 ± 8.37[b]	12.28 ± 1.88[b]

Amylase activity was expressed as mg maltose liberated/min g/tissue. Values were given as mean ± SEM. Values not sharing a common superscript letter in the same column differ significantly ($p < 0.05$).

The amylase activity of rats fed simulated Cd and CPO polluted fish diet concurrently was not significantly different ($p > 0.05$) relative to control after 1 month and other test groups after 1 and 2 months. But, the activity decreased significantly ($p < 0.05$) from control after 2 months.

The levels of Cd and THC increased in the formulated test diets. This is probably due to their gradual accumulation in the fish up to toxic levels and transferred along the food-chain. [20] reported that as mineral levels may always bio-magnify from one feeding level to another, animals at the latter part of the food-chain may gather-up enough toxins than the composition is supposed to give.

As amylase is one of the carbohydrate digestive enzymes, the significantly decreased activity (Table 2) will affect carbohydrate digestion. Alpha amylase is the major form of amylase found in humans and other mammals [21]. It is also present in seeds containing starch as a food reserve, and is secreted by many fungi. Amylase hydrolyses polysaccharides such as starch to yield shorter chains such as maltose which can then be acted upon by maltase to give two glucose molecules which is the building block of carbohydrate, and the form in which it is utilized by the body. The affected amylase activity therefore, may lead to improper digestion and absorption. Cells thus, are starved of nutrients which can lead to growth retardation as seen in the decreased body weight gain of the rats (Table 1) and even death. The decreased amylase activity could be as a result of cadmium's affinity for sulfhydryl groups (-SH) via covalent bond formation [22]. Also, [23] found out that Cd exposure had an inhibitory effect on the activities of disaccharidases and amylase. The nutrient composition of the diet could be an important factor contributing to the Cd co-toxicity observed. The formulated diet in the study has a low protein and high carbohydrate content, typical of an African diet. The Cd ions will thus have less –SH groups to bind to and reduce its toxicity, resulting in numerous Cd ions which is transported to target organs. It has been reported that the metal-binding protein Metallothionein (MT) produced in the GIT can sequester Cd thereby reducing its toxicity [24]. MTs are a family of low molecular heavy weight metal binding proteins, which are unique in their high cysteine content [25]. The typical African diet is low in protein and high in carbohydrate. Increased accumulation of Cd^{+2} in the liver, kidney and bones occurs after exposure to Cd in the form of inorganic salts (e.g., $CdCl_2$) than the cadmium present in conjunction with metallothionein (Cd-MT). The Cd-MT complexes which are released from the liver into the blood is cleared by glomerular filtration in the kidney and taken up by the renal tubule cells where the MT is cleaved and Cd is released. The synthesis of MT in the kidney is lower and insufficient to bind all the free Cd, resulting in tubular damage or cell membrane destruction through activation of reactive oxygen species (ROS) which leads to increased excretion of sugars, amino acids and minerals [26].

4. Conclusion

The study showed that concurrent administration of Cd and CPO hindered the digestion of carbohydrate in the intestine as seen in the decreased enzyme activities. Cd and CPO simulated pollution in the fish led to the gradual accumulation of the toxicants from the little amounts given and bio- magnify to toxic levels which when fed to the rats resulted in disturbance of the normal digestive process. The concurrent effects of Cd and CPO cannot be evaluated from their respective individual effects as observed in the study.

Compliance with ethical standards

Acknowledgments

The authors are grateful to the staff in the Departments of Biochemistry, University of Benin, Benin City and Delta State University, Abraka, Nigeria.

Disclosure of conflict of interest

The authors state that there are none.

Statement of ethical approval

All experiments involving the rats were conducted in accordance with the Guide for the Care of Laboratory animals, as approved by the Ethics committee, Faculty of Pharmacy of the University of Benin with approval number: EC/FP/021/05.

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