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ALTERATIONS IN DISACCHARIDASES' ACTIVITIES FOLLOWING CADMIUM AND CRUDE PETROLEUM OIL CO-ADMINISTRATION IN RATS VIA FOOD-CHAIN

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ABSTRACT
It has long been established that cadmium (Cd) and crude petroleum oil (CPO) are deleterious to the ecosystem (soil, animals and plants). The link between these forms of pollution to man has been an area of interest. Contamination from metals and CPO components are mainly from the drinking water or food of a population. The study was aimed at assessing the alterations in disaccharidases activities following cadmium and crude petroleum oil co-administration in rats via food-chain. Cadmium and Total Hydrocarbon (THC) contents in control and test fish diets were analyzed and the activities of disaccharidases in the duodenum of control and test rats assayed. Sixty-four (64) male albino rats of Wistar strain were divided into four groups (A, B, C and D) of 16 rats in each group. Test groups (B, C and D) were fed formulated diet made from Cd (4 ppm), CPO (0.8 ppm) and Cd + CPO-exposed fish respectively. Control group (A) was fed diet not exposed to Cd and CPO contaminated fish. All animals were fed 320 g of diet daily and allowed access to drinking water *ad libitum*. Cd and THC contents were significantly increased ($p < 0.05$) in the test diets made from Cd and CPO-exposed fish (singly and combined) respectively, when compared to control. There was a significant reduction ($p < 0.05$) in the activities of disaccharidases in the rats after 8 weeks, while after 4 weeks there was no significant difference ($p > 0.05$) observed. Thus, Cd and CPO co-administration greatly alter digestion and absorption as a direct effect on toxicity.

KEYWORDS: Disaccharidases; Cadmium; Food-Chain; Co-Administration; Alterations.

INTRODUCTION
Feeding is very vital and is one of the characteristics of living organisms. The classes of food serve various functions; growth, repair of tissues, energy and defense against diseases. The food-chain is a linear feeding relationship where an organism serves as food for the next organism in the chain. In the natural habitat, the feeding order is from fish to man. Glucose, galactose, and fructose are the three monosaccharides that are commonly consumed and are readily absorbed. The digestive system is also able to break down the disaccharide sucrose (regular table sugar: glucose + fructose), lactose (milk sugar: glucose + galactose), and maltose (grain sugar: glucose + glucose), and the polysaccharides glycogen and starch (chains of monosaccharides). Our bodies do not produce enzymes that can break down most fibrous polysaccharides, such as cellulose. While indigestible polysaccharides do not provide any nutritional value, they do provide dietary fiber, which helps propel food through the alimentary canal. Three brush border enzymes hydrolyze sucrose, lactose, and maltose into monosaccharides. Sucrase splits sucrose into one molecule of fructose and one molecule of glucose; maltase breaks down maltose and maltotriose into two and three glucose molecules, respectively; and lactase breaks down lactose into one molecule of glucose and one molecule of galactose. Insufficient lactase can lead to lactose intolerance. All carbohydrates are absorbed in the form of monosaccharides. The small intestine is highly efficient at this, absorbing monosaccharides at an estimated rate of 120 grams per hour. All normally digested dietary carbohydrates are absorbed; indigestible fibres are eliminated in the faeces. The monosaccharides glucose and galactose are transported into the epithelial cells by common protein carriers via secondary active transport (that is, co-transport with sodium ions). The monosaccharides leave these cells via facilitated diffusion and enter the capillaries through intercellular clefts. The monosaccharide fructose (which is in fruit) is absorbed and transported by facilitated diffusion alone. The monosaccharides combine with the transport proteins immediately after the disaccharides are broken down.[1]

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades.[2,3] It appears that problem of heavy metals accumulation in aquatic organisms including fish needs continuous monitoring and surveillance owing to

biomagnifying potential of toxic metals in human food-chain.[4,5,6,7,8,9]

Food-chain contamination is part of the common routes for entry of metals into the animal system[10] and therefore, monitoring the bioavailability pools of metals in contaminated feed is of uttermost concern. Cadmium can be transported, dispersed to and accumulated in plants, to animals and passed across the food-chain to humans.[11]

A wide range of contaminants are continuously introduced into the aquatic environment mainly due to increased industrialization, technological development, growing human population, oil exploration and exploitation, agricultural and domestic wastes run-off, and may contribute greatly, to the poor quality of river water.[12,13,14] Also, many aquatic animals such as fishes and shellfishes either die or become polluted with trace metals and bio-contaminants often associated with petroleum and municipal wastes.[15,16,17] Among the various sources of pollution, the petroleum industry is considered the greatest source of water pollution in Nigeria.

Previous studies of the effect of cadmium and/or crude petroleum oil in animal models focus primarily on direct exposure of these toxicants, whereas the food-chain is the major route of entry of these chemicals. The effects of a direct CPO contamination in combination with cadmium (Cd) have not been exhaustibly studied. As relevant data is scarce in literature, studies are therefore needed to assess the alterations in disaccharidase activities following cadmium and crude petroleum oil co-administration in rats via food-chain.

MATERIALS AND METHODS

Chemicals/Reagents

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

Feeding Level 1: Simulation of Cd and CPO pollution

Eighty (80) jumbo 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 CPO respectively.

The experimental design for the First Feeding Level of 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 a concentration of 0.4 mg Cd/100 ml water (4 parts per million). 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 (LD_{50} toxicity in catfish, 823.3µl/L, 0.8 ppm) as described by[18] 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.[19, 20] Other sources of nutrients in preparing the diet include:

Carbohydrate: Corn starch was as the source of carbohydrate and made up 55% of the total diet.

Fat and oil: Palm oil, a rich supply of carotene was the source of fat and oil and made up 10% of the total diet.

Fibre: Groundnut shell was the source of fibre and made up 7% of the total diet.

Sugar: Granulated refined sugar was the source of sugar and made up 8% of the total diet.

Vitamins and Minerals: Vitamins and minerals mix made up 5% of the total diet. The feed formulation was made so that the rats received a balanced diet.

Feeding Level 2: Feeding rats with prepared fish diets

Sixty (64) male albino rats, aged 2-3 months of Wistar strain weighing between 100 – 120 g 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 feeding 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 avenue to drinking water at will. Half the numbers of rats in all groups were sacrificed after 4 weeks exposure for the half term study, while the other half was sacrificed after 8 weeks exposure for the full-term study. All experiments involving the rats was 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.

Crude Petroleum Oil (CPO)

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[21] 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.

Collection of Organ

After the 4- and 8-weeks administration 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.

Cadmium and Total Hydrocarbon (THC) Analysis

Cadmium concentration in Forcados water, WSF-CPO and the formulated rat feed 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.

Biochemical analysis

The duodenal supernatant was used to assess biochemical disaccharidase activities.

Assay of Disaccharidases' activities

Method

Maltase (EC 3.2.1.20), Lactase (EC 3.2.1.23) and Sucrase (EC 3.2.1.48) activities were assayed according to the method of.[22]

Procedure

In a test tube, 0.1 ml (maltase, lactase or sucrase assay respectively) of homogenate were incubated with 0.1 ml of 56 mM maltose, lactose or sucrose solutions in 100 mM maleate buffer, pH 6.0. The reactions were stopped after 1 h by adding 1.5 ml of assay reagent (Sigma GAGO20) dissolved in 0.5 M tris-HCl (pH 7.0). The reaction mixture was then incubated at 37°C for 1 h to let colour develop, and its absorbance was read at 450 nm. Maltase, Lactase and Sucrase activities were determined with a glucose standard curve and expressed in U (1µmol glucose liberated per minute) per gram wet weight of gut tissue.

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.

RESULTS AND DISCUSSION

Results

Analysis of water from the Forcados end of Warri River for Cd and Total Hydrocarbon Content (THC) showed that Cd level was 0.004 mg/L and THC <0.001 mg/L. WSF used for the study was also analyzed and Cd was not detected but the THC was <0.001 mg/L. Results of the Cd and THC of the formulated rat feed were given in Table 1.

Table 1: Concentration of Cd and THC in formulated feed given to rats.

Parameter	Cd (mg/kg)	THC (mg/kg)
Group		
A (Control)	0.60 ± 0.34^{a}	1.38 ± 0.56^{a}
B (Cd)	1.80 ± 0.62^{b}	1.95 ± 0.07^{a}
C (CPO)	0.64 ± 0.32^{a}	2.33 ± 0.18^{b}
D (Cd+CPO)	1.60 ± 0.14^{b}	2.63 ± 0.26^{b}

Values are given as mean ± SEM. Values not sharing a common superscript letter in the same row differ significantly ($p < 0.05$.

The Cd and THC contents were significantly increased ($p < 0.05$) in the test feeds made from Cd and CPO-exposed fish (singly and combined) respectively, when compared to control group and the group that does not have either Cd or CPO-exposed fish as feed constituent respectively.

Table 2 showed the alterations in disaccharidase activities following cadmium and crude petroleum oil food-chain co-administration in the duodenum of rats.

Table 2: Alterations in disaccharidase activities following cadmium and crude petroleum oil food chain co-administration in rats.

Group	A (Control)	B (Cd)	C (CPO)	D (Cd + CPO)
Parameter				
LACTASE				
4 WEEKS	13.69 ± 3.06^{a}	13.79 ± 1.31^{a}	12.22 ± 1.62^{a}	11.39 ± 0.97^{a}
8 WEEKS	6.13 ± 1.93^{a}	3.82 ± 0.21^{b}	3.80 ± 0.51^{b}	6.76 ± 0.99^{a}
MALTASE				
4 WEEKS	12.91 ± 1.44^{a}	13.28 ± 1.35^{a}	12.63 ± 1.35^{a}	13.88 ± 1.40^{a}
8 WEEKS	5.83 ± 1.49^{a}	3.40 ± 0.10^{b}	3.80 ± 0.51^{b}	7.14 ± 1.48^{a}
SUCRASE				
4 WEEKS	24.23 ± 1.40^{a}	18.25 ± 2.53^{b}	13.41 ± 4.44^{b}	10.63 ± 3.58^{b}
8 WEEKS	17.85 ± 2.81^{a}	12.90 ± 0.26^{b}	13.23 ± 0.57^{b}	6.50 ± 1.34^{b}

Lactase, Maltase and Sucrase activities were expressed in U (1µmol glucose liberated/min/g weight of tissue). Values were given as mean ± SEM. Values not sharing a common superscript letter in the same column differ significantly ($p < 0.05$).

There were no significant differences ($p > 0.05$) in activities of amylase, lactase and maltase in the duodenum of rats in all test groups respectively compared to the control at the end of 4 weeks. Test groups when compared showed no significant difference. While at the end of 8 weeks, there were significant reductions ($p < 0.05$) in the enzyme activities of the rats in test groups in comparison with control. However, the lactase and maltase activities in the rats from Cd + CPO group showed no significant difference. There was a significant decrease ($p < 0.05$) in sucrase activity in the rats from test groups when compared to the control after 4 and 8 weeks respectively and test group comparisons showed no significant difference.

DISCUSSION

Heavy metals as well as crude oil pollution has been reported to produce reactive oxygen species and other free radicals which induce oxidative stress and peroxidation of lipids when plants and animals are exposed to them.[23] The present study was aimed to assess the possible chemical effect of separate and combined cadmium (Cd) and crude petroleum oil (CPO) on digestive and absorption enzymes in the serum and tissues of rats via the food-chain. The test fish were exposed to Cd at a concentration of 0.4 mg/100 ml and CPO 823.3 µl/L of water (separately and combined) daily to obtain a high level of the metal and hydrocarbon in the tissues for 1 month. This concentration of Cd was the same in the study of[24], who studied the effect of a controlled food-chain on oxidative enzymes in the tissues of rats exposed to Cadmium and Arsenic. Also,[25] used 0.4 mg/100 ml in the study of the effects of cadmium chloride on biochemical characteristics of fresh water fish, *Cyprinus carpio*.[26] Likewise studied the effects of Cadmium and Arsenic on Lipid profile in rats using the same concentration. The river in Warri is one of the most important rivers in Africa in terms of economic development. It lies within latitude 5^o 21'- 6^o 00'N and longitude 5^o 24'- 6^o 21'E. It runs from its source in Utagba-Uno in a direction southwest crossing Oviorie and Ovu-Inland and through Agbarho to Otokutu southwards. It then reaches Effurun to Ode-Itsekiri and drains into Forcados and Bight of Benin.[27] The World Health Organization, WHO has set a maximum permissible concentration of Cd in water of 0.005 mg/L.[28] The concentration of Cd in Forcados water in this study aligns with the WHO standard. Although[27] reported that the levels of Cd in Warri River were far above the WHO permissible limit between 1986 and 1991. Therefore, the use of water from the Warri River for drinking and ingestion of aquatic lives housed in this river is hoped to constitute a serious threat to the riverine inhabitants. Cd and total hydrocarbon (THC) content analyses of the formulated feed (Table 1) revealed a significant increase from control occasioned by the metal and THC accumulation in the fish used in formulating the diet as well as traces from the various feed components. This is believed to have caused the marked distortions in the digestive and absorption enzymes levels as the rats feed on the diets. Bioaccumulation of heavy metals (including Cd) by fishes has been reported in literature.[29]

The rising Cd and CPO contamination in Nigeria as a result of increased industrial activity calls for immediate attention. Animals are thought to be dormant in their digestive enzyme production in response to diet, because the metabolic expense of producing large amounts of digestive enzymes would be wasted by animals ingesting low levels of the substrates for those enzymes.[30,31] Digestive enzyme activity levels can serve as an indicator of nutrient retention ability, metabolic status and the degree of adaptation to the environment, thus it can be used as biomarkers to monitor the environment in which the organisms are placed.[32] Quantitative assessment of enzyme is a reliable indicator of stress imposed on the organism by environmental pollutants such as heavy metals.[33] Many physiological processes including activity of many lysosomal hydrolytic enzymes are inhibited by heavy metals even though these metals may also activate certain enzymes. Comparing digestive enzyme activities between different studies is difficult because of differences in methodology, including differences in assay substrates, assay temperatures, instruments used for analysis, units of reported activity, and species as well as in the quantity and composition of diet.[34,35,36]

Carbohydrate digestion was altered as a result of the co-administration of cadmium and crude petroleum oil through the food-chain. As nutrient digestion is paramount in other to be utilized by the cells of the body, the altered carbohydrate digestive enzyme activities result in maldigestion of substrates which will lead to malabsorption. The metabolic functions of the disaccharidases in the breakdown of disaccharides into their building blocks were altered. The absorbable products of carbohydrate digestion are the simple sugars. Thus, the organism is weak and there is growth impairment evidenced by the decrease in body weight gain.[20]

The non-significant difference in the activities of the disaccharidases in the duodenum of the intestine analyzed after 4 weeks shows that the contaminants were capable of disrupting enzymes activities for that duration of exposure. This is suggestive that the level of these contaminants was still at a lower accumulated state and also may be due to adaptive response of the immune system of the rats. However, the significantly decreased activities of the disaccharidases in the intestine of the rats

after 8 weeks of exposure could be linked to increased histopathological conditions of the intestine as the contaminants enter and build-up in body systems quicker than the elimination pathways of the rats can expel them. The decreased enzyme activities could be attributed to cadmium's affinity for sulfhydryl groups via covalent bond formation.[26]

There was a duration-dependent decrease in the activities of the disaccharidases in the control and test groups. In their study,[37] revealed that Cd decreases sucrase activity and D-galactose absorption in the jejunum tissue of rabbits. Also,[38] found out that Cd exposure had an inhibitory effect on the activities of disaccharidases and amylase.

CONCLUSION

The dangers arising from this co-contamination of pollutants in the environment cannot be over-emphasized. Therefore, people especially riverine inhabitants who consume water and fish from industrial and oil spill areas, should be cautioned as alterations in carbohydrate metabolism is often the aftermath of such exposures. Thus, Cd and CPO co-administration greatly alter digestion and absorption of carbohydrates and has a direct effect on toxicity.

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