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Investigation of *in vivo* toxicity and biochemical perturbation of plasticizers identified in the Tunisian sweets and the fate in WWTP and adjacent coastal area

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ABSTRACT

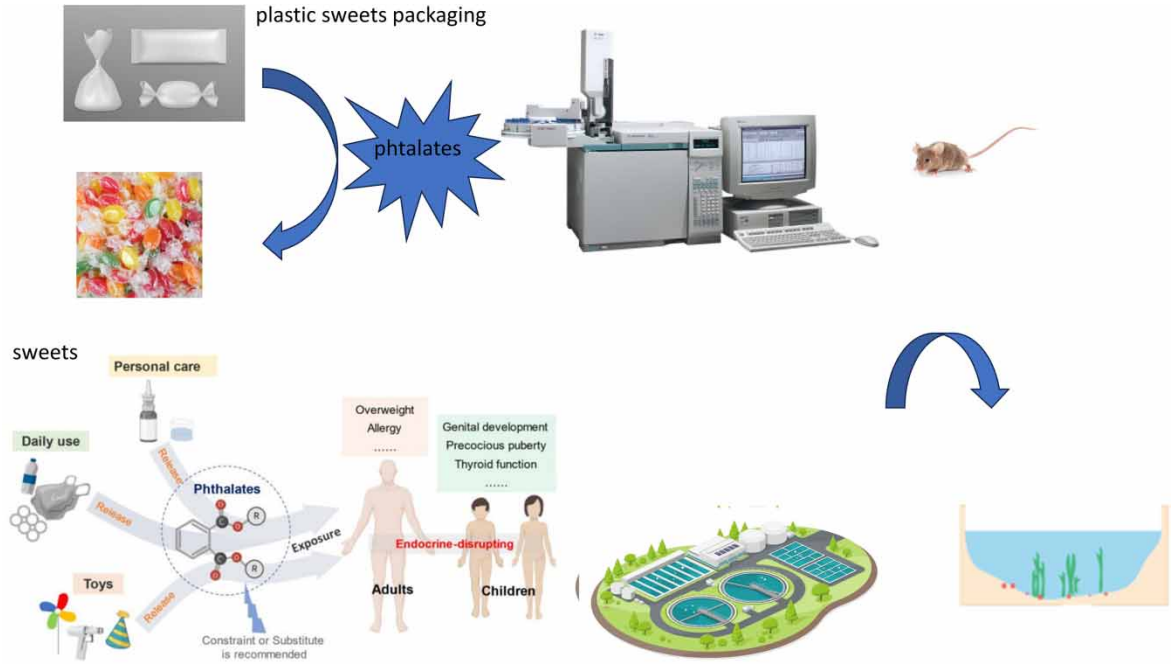
Plasticizers have been excessively used in food packaging and numerous studies show their migration into food and their fate in the environment after the release of plastic packaging into the receiving environment. Our results show the sweets products analyzed using GC-MS/MS indicated the presence of benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), bis(2-ethylhexyl) phthalate (DEHP), diisodecyl phthalate (DIDP), diisononyl phthalate (DINP), and 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINC). Slightly or not metabolized in the body, plasticizers can reach WWTPs and contaminated wastewater. Generally, the usual treatment procedures in WWTPs are not effective in removing these plasticizers, and therefore, they end up in the sea and concentrate in the sediments. This is what our study showed by detecting plasticizers at high concentrations such as DINB which reached 0.93 mg/L in the tested WWTP and 3.8 mg/kg in the sediment of the adjacent marine coast. These analyses generally have negative effects on human health. In fact, we have tested the toxicity of the main detected phthalates such as DINP, DEHP, and DBP. The results showed that the acute administration significantly induced liver and kidney injuries in male mice manifested by a rise in plasma uric acid, alanine aminotransferase, and glucose.

Key words: biochemical analysis, environmental fate, food contamination, plasticizers

HIGHLIGHTS

- GC-MS/MS analyses identified various phthalates in confectionery products widely consumed by children, highlighting the risk of plasticizer migration from packaging into food.
- The study found that these plasticizers are minimally metabolized in the body and eventually reach wastewater treatment plants (WWTP), where standard processes are generally ineffective at removing them.
- Significant concentrations of plasticizers, such as DINB, were detected in WWTP effluent (0.93 mg/L) and adjacent marine sediments (3.8 mg/kg), indicating environmental accumulation.
- Acute exposure to phthalates like DINP, DEHP, and DBP in mice resulted in liver and kidney damage, as evidenced by elevated plasma uric acid, alanine aminotransferase, and glucose levels.
- The study underscores the potential health risks posed by phthalate exposure, particularly for children, as well as the environmental impact due to the persistence of these chemicals in marine ecosystems.

GRAPHICAL ABSTRACT



1. INTRODUCTION

The diet of the world population in general and in Tunisia in particular is essentially based on ready-to-eat industrialized products that are sold directly in stores and markets (Beltifa *et al.* 2017, 2018; Jebara *et al.* 2021). The most widely consumed products are kept in plastic packaging and are most of the time packaged and then kept in inadequate conditions, which favor the migration of contaminants from the container to the contents. Among these contaminants, there are plasticizers which constitute additives to plastics and play a very important role in its elasticity. Phthalates (diesters of ortho-phthalic acid) are a group of organic lipophilic chemicals with a wide range of user applications (González-Castro *et al.* 2011; Sakhi *et al.* 2014; Carnevali *et al.* 2019; Yang *et al.* 2019; Giuliani *et al.* 2020; Gkrillas *et al.* 2021; Krithivasan *et al.* 2023; Mérida *et al.* 2023). They are esters of phthalic acid and are mainly used as plasticizers in numerous consumer products, especially food products. When the occurrence of plastic components is not properly controlled, it can affect the organoleptic food properties and produce endocrine disrupting effects even if the levels are lower than the legislated or toxicological values (Nishihara *et al.* 2000; Cao *et al.* 2014; Beltifa *et al.* 2017, 2018; Jebara *et al.* 2021). These additives are not covalently bonded to the polymer matrix and are therefore likely to leach from the materials, particularly if exposed to UV rays, heat (Net *et al.* 2015; Paluselli *et al.* 2019), or prokaryotes, although direct additive inputs from manufacturing processes or wastewater treatment plants (WWTPs) could be the primary means of entry into the environment (Sanchez-Avila *et al.* 2012). Over the past decades, the presence of phthalates in the environment has been reported in numerous studies (Paluselli *et al.* 2018a, 2018b). Many studies have demonstrated the contamination of different compartments of aquatic ecosystems such as sediment, *Posidonia*, fish with various types of phthalates, and the main source of this contamination is the discharge of urban effluent from WWTPs (González-Castro *et al.* 2011; Sakhi *et al.* 2014; Carnevali *et al.* 2019; Yang *et al.* 2019; Giuliani *et al.* 2020; Gkrillas *et al.* 2021; Jebara *et al.* 2021; Krithivasan *et al.* 2023; Mérida *et al.* 2023).

In this study, we will focus mainly on a food product widely consumed in Tunisia, especially by children, namely sweets. This type of food is packaged in plastic packaging and is constantly exposed to heat and UV rays, and according to the bibliography, these two parameters promote the migration of phthalates. Our first objective was therefore to highlight the migration of these molecules in food and their fate in the environment after the release of plastic packaging into the receiving environment by performing gas chromatography–mass spectrometry (GC-MS)/MS analyses to detect these plasticizers in some sweets from the Tunisian weekly market and also in the Jebeniana wastewater treatment plants (Tunisia) and the sediments of the adjacent sea. Our second objective was the *in vivo* investigation of the toxicity of the three main plasticizers detected in

sweets, the WWTP and marine sediments on male mice and the determination of certain biochemical parameters indicating their toxic effect.

2. MATERIAL AND METHODS

2.1. Sweets sampling

Sampling was carried out according to the method described by the Food and Agriculture Organization of the United Nations and detailed by Beltifa *et al.* (2018) which consists of the random sampling of three categories of candies (C1, C2, and C3) from the weekly market of Jebeniana City, Tunisia. Sampling was executed from February to December 2019. The sweets selection was based on the criteria of basic candies commonly consumed in a typical Tunisian children's diet.

2.2. Chemical analysis of sweets samples

Sweets samples were analyzed for phthalates and aliphatic ester by GC-MS according to the method described by Beltifa *et al.* (2018) and modified according to our study.

2.2.1. Instrumentation

Plasticizers were analyzed using an Agilent 6890N (Agilent Technologies, Lake Forest, CA) gas chromatograph, equipped with a single quadrupole mass spectrometer running in the positive electron impact mode. The column was set at a constant flow rate of 0.5 mL/min using helium as carrier gas. Phthalates were separated on a capillary column from Agilent VF-ms 30 m × 0.2 mm ID × 0.3 μm df analytical column. The ion source and transfer line temperature were set at 250 °C. The injection volume was 2 μL in the splitless mode. A temperature gradient was run to analyze the phthalates within 20 min.

2.2.2. Extraction procedure

Three grams of homogenated sample sweets were transferred into a 50-mL-capped propylene tube (Falcon®) and spiked with 200 μL DEHP-d4 (1,000 mg/L) and 50 μL BPA-d16 (1.5 mg/L). After vortex mixing for 10 s, 10 mL of acetonitrile was added and again vigorously vortex mixed for 30 s. After centrifugation (4,000 rpm, 10 min, 10 °C), the supernatant was transferred to another Falcon® tube of 50 mL. For sufficient defatting, 1 mL Carrez I reagent (15 g potassium hexacyanoferrate (II) trihydrate in 100 mL of MilliQ water) and 1 mL of Carrez II reagent (30 g zinc sulfate heptahydrate in 100 mL of MilliQ water) were added. The sample was again vortexed, mixed for 10 s, and centrifuged at 4,000 rpm for 10 min (10 °C).

The supernatant was transferred to a new Falcon® tube of 15 mL containing 1.5 g of sodium sulfate to remove water residues from the extract. After a centrifugation step (4,000 rpm, 10 min, 10 °C), the sample was filtered through a 0.22-μm PVDF filter prior to GC-MS analysis. The following gradient elution method was used: 0–2 min: 95% A/5% B; 2–5 min: linear decrease to 5% A/95% B; 5–8 min: 5% A/95% B; 8.1 min: 95% A/5% B; 8.1–10 min: 95% A/5% B re-equilibrate to initial conditions. Since the matrices were of diverse origins, it was impossible to completely validate each matrix. In order to evaluate the extraction efficiency of each different matrix, each sample was analyzed in triplicate: spiked with the respective IS and at two QC levels. The method for phthalates was completely validated according to EU requirements including specificity, linearity, matrix effect, repeatability, reproducibility, detection limit, and quantification limit (Table 1).

Table 1 | Linearity, sensitivity, repeatability, and recovery of total phthalates esters

Compound	R^2	LOD (mg/kg)	LOQ (mg/kg)	RSD (%)	Recovery (%)		
					Sediment	Seagrass	Mussel
DEP	0.9911	0.012	0.040	3.25	108.6	110.4	101.3
DIBP	0.9933	0.021	0.065	4.22	98.4	95.4	94.4
DBP	0.9941	0.010	0.030	5.31	101.4	99.3	102.3
DEHP	0.9999	0.010	0.031	2.27	102.1	103.3	105.8
DEHT	0.9802	0.080	0.241	2.46	105.4	103.1	103.7

DEP, diethyl phthalate; DIBP, diisobutyl phthalate; DBP, dibutyl phthalate; DEHP, di-(2-ethylhexyl) phthalate; DEHT, di-2-ethylhexyl terephthalate; R^2, coefficient of determination; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation.

2.3. Chemical analysis of wastewater and sediment samples

2.3.1. Study area and sample collection of marine sediment

Jebeniana is a municipality in the governorate of Sfax (Tunisia) lying on the coast of Tunisia and facing the Mediterranean Sea. This coastal site is characterized by high marine biodiversity. Nevertheless, the environmental quality of this area is impacted by the huge volumes of wastewater discharged by a nearby sewage treatment plant and by industrial activities. Sampling was conducted during the wet January in 2019 (35°00′35.9″N; 10°59′39.2″E) according to the method described by Souaf *et al.* (2023). Surface sediment samples were gathered at a depth of 4 m using Niskin bottles with a Van Veen bucket with three different spots at the sampling point and immediately transferred to glass bottles. The collected sediments were freeze-dried and sieved with screen mesh (250 μm), and then, the meshed samples were stored at −20 °C until further processing.

2.3.2. Sample preparation

Sediments were freeze-dried for 48 h (Martin Christ Alpha 1–2/LD Plus, Germany), homogenized, and sieved (2 mm). Then, 5 g of samples was spiked with 0.001 mg of DBP-d4 and 0.001 mg of DEHP-d4. Then, a centrifuge glass tube was prepared, containing the spiked sample with anhydrous sodium sulfate and 30 mL of *n*-hexane: acetone solution (1:1, v/v). The obtained mix was ultrasonically extracted for 10 min and then was centrifuged at 3,000 rpm for 10 min (Awel MF 20-R centrifuge, Awel SAS, France) to separate the organic supernatant from the bottom layer. The obtained supernatant was further extracted three times, according to the same procedure (Souaf *et al.* 2023). The final extract was completely dried using rotavapor (BUCHI Labortechnik AG, Switzerland) and re-suspended in 1 mL of hexane. The subsequent solid-phase extraction (SPE) was executed by a glass column (30 cm × 10 mm) packed with 5 g of Florisil (previously activated at 140 °C for 16 h) and 1 g of anhydrous sodium sulfate, which catches water molecules and prevents its passage to the extract. Eluate was processed with 60 mL of a diethyl ether:*n*-hexane solution (1:1, v/v) and evaporated to dryness (Souaf *et al.* 2023).

2.3.3. GC-MS analysis

Analysis was determined by a gas chromatography system (GC-2010, Shimadzu, Japan) equipped with an autosampler (HT300A, HTA, Italy) and combined to a single quadrupole mass spectrometer (QP-2010 Plus, Shimadzu, Japan). Chromatographic separations were carried out on an SPB5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, Supelco, USA). The oven temperature program was as follows: from 60 to 190 °C at 8 °C/min (5 min hold), from 190 to 240 °C at 8 °C/min (5 min hold), and from 240 to 315 °C at 8 °C/min. The injection port was at 260 °C and was provided with a narrow inlet liner (0.75 mm ID, Agilent Technologies). Sample injection occurred in splitless mode, with sampling time of 60 s, then split ratio 1:15. Injection volume was 1 μL. Carrier gas (He, 210.0 kPa, pressure control mode) was operated at a linear velocity of 30 cm/s. As for the MS setup, the temperature of the EI source was set at 200 °C, ionization energy and emission current were 70 eV and 250 μA, respectively, while interface temperature and electron multiplier voltage were, respectively, equal to 300 °C and 1.0 kV. Data acquisition was executed both in full scan (mass range: 40–400 m/z) and selected ion monitoring (SIM) by monitoring three characteristic mass fragments for every analyte. Data acquisition and processing occurred by GC-MS solution software. Identification of plasticizers were performed by comparison of their retention times and mass spectra with those of corresponding commercial standards. The quantitative analysis was realized in SIM mode, taking into account the relative base peak ions and exploiting the internal standard normalization (Souaf *et al.* 2023).

2.4. Acute animal treatment and toxicity investigation

Seven-week-old male mice (25 ± 3 g) were obtained from SPIT (Society of Pharmaceutical Industries of Tunisia). After a 15-h acclimatization period, the mice were divided into two control groups, A and B ($n = 6$/group), and three experimental groups, C ($n = 18$), D ($n = 18$), and E ($n = 12$). The mice in groups C, D, and E were housed in a large steel cage with unrestricted access to food from the Animal Nutrition Society (ANS; Sfax, Tunisia) and water. Each group was further divided into subgroups of six mice. All animals were maintained under laboratory conditions: temperature of 22 ± 2 °C, relative humidity of 70%, and a 12-h light/dark cycle. The treatment was administered once every 3 days for 10 days.

1. Animals in group A received intraperitoneal (IP) injections of corn oil (control group).
2. Animals in group B were not treated (control group).

3. Animals in group C were administered increasing doses of DEHP (three subgroups; $n = 6$/group) at 1,250, 2,500, and 5,000 μg/kg body weight (BW).
4. Animals in group D were administered increasing doses of DBP (three subgroups; $n = 6$/group) at 1,250, 2,500, and 5,000 μg/kg BW.
5. Animals in group E were administered increasing doses of DINP (three subgroups; $n = 6$/group) at 1,250, 2,500, and 5,000 μg/kg BW.
6. The tested concentrations of DEHP, DBP, and DINP were selected according to those described by Beltifa *et al.* (2017, 2018).

2.4.1. Blood collection

All groups of mice were sacrificed by cervical decapitation to minimize handling stress during ether inhalation. After 10 days of treatment, blood samples were collected in tubes containing heparin to prevent coagulation and then centrifuged at 3,500 rpm for 5 min at 4 °C. The resulting plasma was collected in Eppendorf tubes and stored at −80 °C until biochemical analysis.

2.5. Statistical analysis

All experiments were independently repeated at least three times. Mean values were calculated; statistical analysis of the results was performed using SPSS standard version 13.0 software. Data were shown as means ± SD from at least three independent determinations. Values less than 0.05 are considered statistically significant (*) and those less than 0.01 are considered statistically highly significant (**).

3. RESULTS

3.1. Phthalate levels in sweets of the Tunisian weekly market

The concentrations of six different plasticizers in the three sweets items bought from the Tunisian market are shown in Table 2. Six plasticizers (DEHP, DBP, BBP, DINP, DIDP, and DINCH) were found in the three sweets sample categories at the concentrations ranging between the limits of detection (LOD) and the limits of quantification (LOQ) for each compound. The LOQ was at least 0.5 ng/g. Concentration of the analytes was calculated based on their peak areas in relation to the response for their respective internal standard (isotope dilution method). In this way, there is no need to establish a calibration curve when measuring sample series. Two of these compounds, DEHP and DIDP, were detected at high concentrations in sweets articles of candies C1 and C2. In fact, DEHP concentrations reached 3.339 and 0.053 mg/kg in C1 and C2, respectively. In addition, concentration of DIDP reaches 4.86 mg/kg in C1 but does not exceed LOQ in C2 and C3.

Table 2 | Chemical analysis of sweets samples using GC-MS/MS

	Concentration (mg/kg)					
Samples	**DBP**	**BBP**	**DEHP**	**DIDP**	**DINP**	**DINCH**
C1	0.46 ± 0.01**	<0.025[a]	3.339 ± 0.731**	4.86**	<0.75[a]	<0.5[a]
C2	<0.01[a]	<0.025[a]	0.053 ± 0.001*	<0.75[a]	<0.75[a]	<0.5[a]
C3	<0.01[a]	<0.025[a]	<0.0125[a]	<0.75[a]	<0.75[a]	<0.5[a]

C1, C2, and C3 were three types of candies; DBP, dibutyl phthalate; BBP, benzyl butyl phthalate; DEHP, bis(2-ethylhexyl) phthalate; DIDP, diisodecyl phthalate; DINP, diisononyl phthalate; DINCH, 1,2-cyclohexane dicarboxylic acid diisononyl ester.
[a]Identified, but not quantified (between LOD and LOQ).
*Significant difference: $p < 0.05$.
**High significant difference: $p < 0.01$.

3.2. Occurrence of plasticizers in wastewater and marine sediment

Of the 18 compounds investigated, six PAE congeners (namely, DEP, DPrP, DBP, DIBP, BBP, and DEHP) and two NPPs (namely, DEHA and DEHT) were reliably identified and quantified by the GC-MS method described above. Their concentrations and the related detection frequencies in every matrix are summarized in Table 3.

The overall contamination by all detected plasticizers in the sediment samples amounts to several milligrams per kilogram. Among these, DEHP is the most prevalent, with a concentration reaching 4.59 mg/kg. Additionally,

Table 3 | Descriptive statistics (range, mean, and median) of phthalates contents (mg/L and mg/kg) in the WWTP and sediment of the coast of Jebeniana (Tunisia)

Compounds	Range	Mean	Median	Detection rate (%)
Wastewater (mg/L)				
DEP	<MDL–0.0170	0.0226	0.0121	
DBP	<MDL–0.0305	0.0171	0.0161	
DINP	0.935–0.936	0.9355	0.0662	
DEHP	<MDL–0.168	0.0711	0.0452	
DEHT	0.645–0.930	0.634	0.782	
Sediment (mg/Kg, dw)				
DEP	0.0644–0.142	0.0950	0.0989	100
DPrP	0.0103–0.0202	0.0137	0.0124	100
DBP	0.0423–0.0824	0.0550	0.0490	100
DINP	3.801–3.394	3.819	0.171	100
BBP	<MDL–0.0425	0.0281	0.0284	92
DEHP	4.15–5.24	4.59	4.59	100
DEHA	<MDL–3.58	3.08	3.39	92
DEHT	1.70–2.86	2.42	2.73	100

DEP, diethyl phthalate; DBP, dibutyl phthalate; DINP, diisononyl phthalate; DEHP, bis(2-ethylhexyl) phthalate; DEHT, di-(2-ethylhexyl) terephthalate; DPrP, dipropyl phthalate; BBP, benzyl butyl phthalate; DEHA, diethylhexyl adipate.

the two NPPs, DEHA and DEHT, are present at concentrations of 3.819 and 2.42 mg/kg, respectively. DEHA and BBP have a very high detection frequency of 92%, while DINP, DEHP, DBP, DEHT, BBP, and DEHT were found in all the samples studied, showing a 100% detection rate (Table 3). In the urban effluents collected from the WWTP of Jebeniana, significant quantities of DINP, DEHT, DEHP, and DBP were detected, with concentrations reaching 0.634, 0.9355, 0.0711, and 0.0171 mg/L, respectively (see Table 3).

3.3. DEHP, DIDP, and DBP effects on the glycemic homeostasis and biochemical markers

Figure 1 shows that DEHP, DBP, and DIDP injections induced diabetes in the mice, which was evidenced by a significant ($p < 0.01$) increase in their blood glucose level (hyperglycemia) as compared with the controls. We observed that the increase of plasma glucose (GLU) was inversely proportional to the dose of DBP. In fact, it reached the highest concentration of 11.2 mmol/L at the lowest tested dose of 1,250 μg/kg (BW) compared with the negative control ($p < 0.01$). However, the GLU concentration increases in a dose-dependent manner if the mice are treated with DEHP and reaches the highest concentration of 11.5 mmol/L at the higher tested dose of 5,000 μg/kg (BW) compared with the negative control ($p < 0.01$).

In the same way, we observed that the GLU concentration in the mice submitted to nourishment supplemented with 100 g/kg (BW) of candies (C1) is much higher ($p < 0.01$) compared with the negative control. Figure 1 shows also that ALT and uric acid recorded for the DEHP and DBP underwent a significant increase ($p < 0.01$) compared with the negative control. These parameters increase with dose-dependent manner of tested compounds. DEHP shows a more significant ($p < 0.01$) effect on the biochemical parameters previously cited compared with those observed when testing DBP.

4. DISCUSSION

In this study, chemical analysis was conducted using gas chromatography–tandem mass spectrometry (GC-MS/MS), which combines a GC with two mass spectrometers. This combination offers enhanced selectivity, sensitivity, and detailed molecular structure analysis. The analysis revealed that DEHP and DBP concentrations in the C1 sweets sample were up to 200 and 46 times higher, respectively, than the quantification limits. This sample had been repackaged in flexible plastic and adherent retail films, facilitating the migration of DINP from the packaging into the sweets. Our findings align with those of Cao *et al.* (2014) regarding DEHP concentrations in flexible plastic and retail films. However, Wormuth *et al.* (2006) reported lower concentrations of DBP and DEHP in sweets from European markets, with DBP ranging from 0 to 0.3 mg/kg and DEHP from 0.041 to

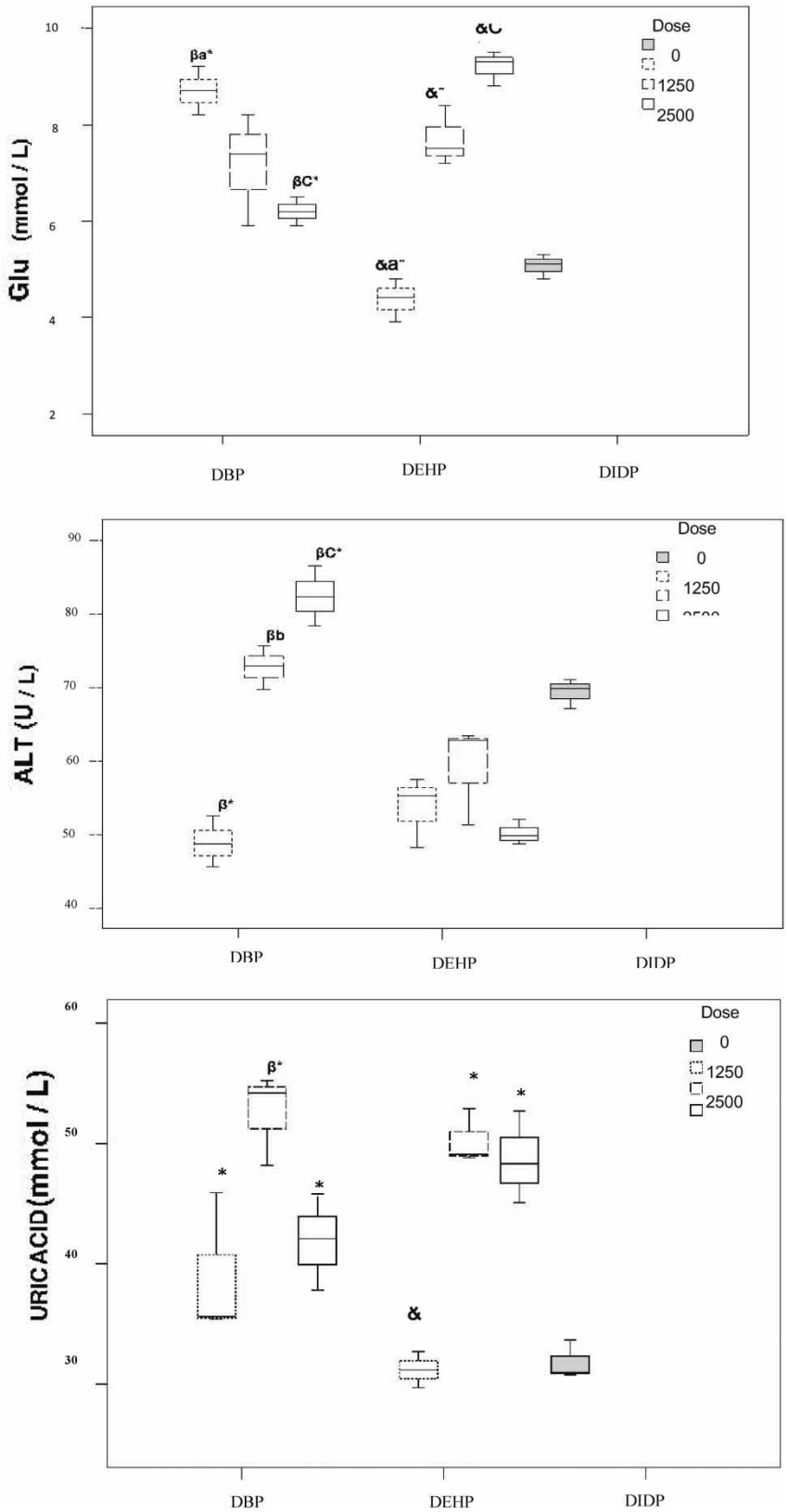


Figure 1 | Hepatic and renal functional markers in plasma of control, di-(2-ethylhexyl) phthalate (DEHP)- and dibutyl phthalate (DBP)-treated mice. The means ± SE are plotted. GLU, plasma glucose; ALT, alanine aminotransferase, uric acid, and urea. The significance of differences between mean values was estimated using the non-parametric Kruskal–Wallis test. *Significant differences ($\alpha = 0.01$) compared with the corresponding controls. a, b, and C are significant differences between DBP and DEHP at different tested concentrations; β and ampersand are significant differences between various doses of each tested compound; β for DBP and ampersand for DEHP.

1.23 mg/kg, which are both lower than the levels found in our C1 sweets sample. The DEHP concentration in C1 sweets is particularly concerning, given the EU's specific migration limits (SMLs) for individual contaminants or groups of contaminants under regulation 10/2011, which set limits at 0.3 mg/kg food simulant (fs) for DBP and 1.5 mg/kg fs for DEHP (Sakhi *et al.* 2014; Yang *et al.* 2019).

Excessive consumption of food products, particularly sweets, by children has environmental consequences. A significant portion of plasticizers enters wastewater and reaches treatment plants. This accounts for our detection of plasticizers, especially DEHP, DBP, and DINP, in WWTPs. Wastewater is typically discharged into coastal areas, such as Jebeniana. The presence of these residues in sediments indicates their impact on marine environments, as confirmed by *in vivo* toxicity studies. The estimated acceptable level of exposure and daily intake (DI) are based on a considerable degree of certainty, often derived from animal studies by competent authorities. Our study shows that the outcome of risk assessments is highly dependent on the chosen methodology. It has been reported that DBP and DEHP, when combined, pose the greatest risk to humans among phthalates (González-Castro *et al.* 2011; Carnevali *et al.* 2019; Giuliani *et al.* 2020; Gkrillas *et al.* 2021; Krithivasan *et al.* 2023; Mérida *et al.* 2023). Additionally, DEHP is the most widely used plasticizer globally, accounting for 37.1% of the market and is still extensively used in medical devices. It is now classified as a carcinogenic, mutagenic, or toxic for reproduction (CMR1B) substance. Similar to DEHP, alternative plasticizers such as DINP and DINCH are not covalently bound to the polymer matrix. Consequently, they can migrate from medical devices to their contents, potentially coming into direct contact with patients and exposing them to toxic risks (Genay *et al.* 2017). To investigate this, we studied the acute toxicity of DEHP, DIDP, and DBP in male mice, comparing the results to two control groups. Our findings indicate that the consumption of contaminated food has health consequences, as evidenced by a significant increase in ALT activity. The variation in these parameters can be attributed to the presence of DEHP and DBP in the diet. Tests on these compounds individually confirmed their role in causing significant liver and kidney damage (Beltifa *et al.* 2017). Several studies have shown that, compared with the normal control group, DEHP- and DBP-treated groups exhibited disrupted liver tissue architecture, disordered hepatocyte cords, vacuolar degeneration, and slight cytoplasmic staining. Specifically, the DEHP-treated group showed central necrosis of the liver lobule (Miura *et al.* 2007; Ge *et al.* 2015; Venturelli *et al.* 2015; Beltifa *et al.* 2017). Many studies attribute the various damages caused by phthalates to their ability to generate reactive oxygen species (Kasahara *et al.* 2002; Miura *et al.* 2007; Pérez-Albaladejo *et al.* 2017, 2020). Inoue *et al.* (1986), Jebara *et al.* (2021), and Beltifa *et al.* (2018) reported that mild oxidative stress from a nephrotoxic agent induces free radical generation, accompanied by an increase in hepatorenal glutathione levels in rats. It was also noted that DEHP and DBP administration significantly increased glutathione concentration in both the liver and kidneys but decreased it in the testis (Kasahara *et al.* 2002; Beltifa *et al.* 2018; Souaf *et al.* 2023).

To limit the use of plasticizers and mitigate their harmful effects, a structured methodological approach is necessary. First, it is essential to prioritize research and development of alternative materials that can replace traditional plasticizers, such as biodegradable plastics and bio-based polymers. Subsequently, scientists must conduct toxicological studies to ensure the safety of these substitutes. Legislation and policies must align with this approach by implementing strict regulations and encouraging the adoption of safer materials. Indeed, stricter regulations on the use of harmful plasticizers like phthalates are necessary, along with financial incentives for companies that adopt alternative materials and sustainable manufacturing processes. Additionally, reducing plasticizers at the source is essential. This involves designing sustainable products that require little to no plasticizers and improving manufacturing processes to minimize the need for their use. These efforts should be complemented by the development of more efficient recycling technologies and effective waste management systems. Finally, ongoing monitoring and evaluation are essential to accurately assess environmental quality and its impact on human health. A study like ours aligns well with this approach, as it provides insights into the current situation, enabling the adjustment of strategies to significantly reduce the use of plasticizers and their harmful effects on human health and the environment.

5. CONCLUSION

In this work, an in-depth study was conducted to detect and trace the source and fate of certain phthalates (DEP, DBP, DINP, and DEHP) and an NPP (DEHT) in sweets and the environment, specifically in wastewater and marine sediment samples from the coast of Jebeniana in the governorate of Sfax (Tunisia). These plasticizers are classified as carcinogenic, mutagenic, or toxic to reproduction. A close relationship between the level and

type of contamination by these plasticizers was demonstrated, and their toxicity was confirmed. Indeed, the presence of these various plasticizers in murine plasma disrupted essential biochemical parameters in mice, indicating a potential for similar disruptions in humans, posing a serious health risk. This study must be supplemented by further investigations to explore potential areas for source reduction of these toxic substances, thereby reducing treatment costs and limiting their use in product packaging.

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AUTHORS' CONTRIBUTION

All authors contributed to this work: A. Belaid: Data analysis + investigation + writing the original draft. K.B.: Investigation. A. Beltifa: Statistical analysis. H.B.: Conceptualization + supervision + validation.

ETHICAL APPROVAL

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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