

Effect of chemical contaminants in local bottled drinking water on Blood glucose of male and female albino mice

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ABSTRACT

Eight local commercial bottled water brands (T1, T2, T3, T4, T5, T6, T7, and T8) were subjected to an investigation regarding chemical contamination. The investigation involved at sunlight, shad, refrigerator temperature 4°C, freezing -18°C, room temperature, at different time intervals (days). Samples to various storage conditions which were; direct sun light at (45–47°C), shade, room temperatures, cold storage (4°C), freezing (at 0°C and -18°C) all for different periods. The investigated chemical contaminants were acetaldehyde, formaldehyde, ethylene glycol, diethyl hexyl phthalate, dimethyl phthalate, diethyl phthalate, butyl benzyl phthalate, dibutyl phthalate, bisphenol A, and antimony. The maximum concentration for each chemical was measured amounting to 1275.50, 256.09, 6599.09, 82.61, 4.75, 172.69, 99.00, 458.83, 104.95, 1704.3 ppb, respectively. A combination of chemical contaminants was employed to investigate their impact on the blood sugar levels of lab mice in addition to 2 other concentrations (less and greater). Significantly different blood glucose levels (of male and female mice) were determined at ($P \leq 0.01$ and $P \leq 0.05$), respectively. An increase in both male and female mice blood sugar levels was noted correlated with the increase in contaminants concentrations. These levels were greater in male mice than female mice in comparison to the control group, which amounted to (230.00 - 150.33 mg/dL and 171.49 - 149.49 mg/dL), respectively.

Keywords: Phthalate esters, Carbonyl compounds, Water, Blood glucose, Mice.

Introduction

Polyethylene terephthalate (PET) is known as polyester, belongs to the family of long-chain polymers. PET exhibits significant economic value due to its chemical and physical characteristics. Manufacturing efficiency, resistance to chemical effects, high flexibility, transparency, and thermal stability are some of these properties. For the production of PET, Ethylene glycol and terephthalic acid or dimethyl terephthalate are subjected to a polymerization process, in addition to the use of catalysts such as titanium, germanium, or antimony (Sb) according to (Wu et al., 2024; Bach et al., 2012). Occasionally, the presence of high amounts of certain chemical substances (as by product of the polymerization process) such as acetaldehyde, in

associated to the manufacture of PET bottles (Mutsuga et al., 2006). On the other hand, according to (Nawrocki et al., 2002, Mutsuga et al., 2006), formaldehyde has also been detected in certain cases.

Antimony (Sb), which is a known as a byproduct catalyst, due to the exposure to various conditions such as temperature, storage, and sunlight, also been detected in PET bottles (Filella, 2020; Keresztes et al., 2009; Elamri et al., 2017).

Blood glucose is the main energy source of the human body; therefore, glucose levels should be within the range of 4 to 7 mmol/L in healthy humans during feeding and fasting times (Saltiel and Kahn, 2001). Insulin resistance and diabetes both were linked to the level of exposure to certain chemical substances

such as phthalates as reported by various studies (Gao et al., 2021; Mariana and Cairrao, 2023). Former research papers by (Peng et al., 2023; Amin et al., 2018) stated that urinary phthalate metabolites concentrations are correlated with escalated waist circumference or insulin resistance (IR) in adult American males and females. Furthermore, Different recent studies reported that exposure of animals to phthalates during pregnancy or maturity exhibited elevated blood glucose, lower serum insulin, reduced glucose tolerance, and declined insulin construction (Sun et al., 2014; Amin et al., 2018). However, opposite outcomes were reported in different research papers (Feige et al., 2010; Nakane et al., 2012).

It was reported that high doses of phthalates may have low effect on blood glucose and glucose tolerance of female Albino mice especially during initial phases of exposure (Zhou et al., 2015). However, after few weeks, this effect faded. According to Parsanathan et al., (2014), insulin signal transduction is altered by DEHP-induced ROS and lipid peroxidation in adipose tissue and glucose intolerance was promoted. The study aimed to identify potential chemical pollutants in locally produced water bottles in Baghdad and northern Iraq and to study their effect on sugar levels in male and female mice.

Materials and Methods

Analytical grade chemicals, phthalate esters, Sb and reagents were provided. Spin react kits (Spain) were used for the determination of lipid profile parameters. Eight-bottled drinking water brands (500 ml) of (T1, T2, T3, T4, T5, T6, T7, and T8) were provided from drinking water factories at Baghdad city (at zero-time storage). All samples were stored under different conditions, which included temperatures of (-18, 0, 4, and 25 °C), and (-18, 0, 4, and 25 °C). By using rotary vacuum evaporator, bottled water samples were prepared by reducing the concentration from one liter to 200 ml and were stored in screw glass bottle in refrigerator at 4°C.

Accelerated weathering tester: An accelerated weathering tester was used to assess the stability of materials through speeding up the influence of different factors such as temperature, moisture, and sunlight (UV). For 20 days (480 hours), 500 ml of bottled drinking water were kept in the accelerated weathering testing equipment at 60°C and exposed to UV radiation with a capacity of (m/2 0.89W). According to the American Standard ASTM G154, 20 days is equal to 180 days.

Chemical contaminants extraction: The method described by (Ajaj et al., 2024; Jayaweera et al., 2020) was adopted to extract phthalate esters.

High performance liquid chromatography (HPLC): Phthalate esters in the bottled water samples were determined by using HPLC apparatus (Shimadzu LC-20A) equipped with (C18 MZ orbit 5 µm, 4.6 x 250 mm column) and (SPD 20-AD UV detector). Flow rate was set to 1 ml/min. the mobile phase consisted of Acetonitrile: Water (65:35 v/v) UV detection was set at 360 nm. Standards of phthalate esters (DBP, DEP, DMP, DEHP, and BBP) injection volume was 20 µl in addition to carbonyl compounds (acetaldehyde and formaldehyde), BPA and EG standards. The quantitative analysis was calculated according to the peak area (Abe et al., 2021).

Antimony determination: An atomic absorption spectrometer (Shimadzu, Kyoto, Japan) with an air and acetylene flame atomizer and a hollow cathode lamp was utilized to measure Sb. The wavelength was set to 217.6 nm. Experiment details: The experiment was carried out at Al-Nahrain University, Biotechnology Center. Three groups (10 mice/group) of male and female (25-28g for each) were subjected to oral administration (0.2ml/mouse) for 50 days, at controlled conditions. Three bottled water contaminant concentrations (the obtained in addition to less and above concentrations) and the fourth group was used as control (Table 1).

Blood collection and serum separation: One ml disposable syringe was used to withdraw a blood sample from the mouse heart after anesthetizing under the supervision of professional. The mouse blood sample was kept in Eppendorf tube. Eppendorf tubes were centrifuged for 10 minutes at 30°C and 3000 rpm. A Pasteur pipette was applied to collect the supernatant. Serum layer from each mouse was kept in a freezer for further analysis.

Blood glucose test: Prior to the extermination of male and female mice, blood glucose samples assay was performed by taking blood samples from cutting their tails. Accu-Chek Aviva, a glucose blood meter, was used to conduct the test.

Results and Discussion

Eight-brands of local bottled water (T1, T2, T3, T4, T5, T6, T7, and T8) were stored under different conditions and different after 10, 20, 30, 60, 90, 120, 150, 180 days of storage. The storage above one month was carried out by using accelerated weathering tester (60°C and UV) and time periods (Table 2).

Table (1): Chemical substances concentrations administrated to the male and female albino mice.

Chemical contaminant		0.2 m/mouse (25-28 g) weight	
	Obtained Conc.	Less	Above
DBP	0.49	DBP	0.49
DEP	0.18	DEP	0.18
DMP	0.005	DMP	0.005
DEHP	0.08	DEHP	0.08
BBP	0.10	BBP	0.10
BPA	0.11	BPA	0.11
EG	7.07	EG	7.07
Formaldehyde	0.27	Formaldehyde	0.27
Acetaldehyde	1.36	Acetaldehyde	1.36
Sb	1.82	Sb	1.82

Dibutyl phthalate (DBP), Diethyl Phthalate (DEP), Dimethyl Phosphate (DMP), Di(2-ethylhexyl) phthalate (DEHP), Benzyl butyl phthalate (BBP), Bisphenol A (BPA), Ethylene Glycol (EG).

Table (2): The average of chemical concentrations of eight brands under different conditions.

Chemical contaminants	Storage condition	Highest Conc. detected by (HPLC) in (ppb)
DBP	0°C	458.83
DEP	Shade (37°C)	172.69
DMP	Sunlight (48°C)	4.75
DEHP	0°C	82.16
BBP	-18°C	99.00
BPA	0°C	104.95
EG	Shade (37°C)	6599.09
Formaldehyde	Temp. room (25°C)	256.09
Acetaldehyde	Control	1275.50
Sb	-18°C	1704.3

Dibutyl phthalate (DBP), Diethyl Phthalate (DEP), Dimethyl Phosphate (DMP), Di(2-ethylhexyl) phthalate (DEHP), Benzyl butyl phthalate (BBP), Bisphenol A (BPA), Ethylene Glycol (EG).

Chemical contaminants effect on blood glucose levels:

The two primary sources of glucose (C₆H₁₂O₆) in the human body are diet (carbohydrates) and liver metabolic activities. Carbohydrate's digestion in the body provides glucose, which is the main energy source for cells. Glucose can be produced and stored in the liver to maintain stable blood sugar levels (Güemes et al., 2016). convulsions, unconsciousness, and even death are examples of negative impacts of Low blood glucose levels. On the other hand, elevated glucose levels can cause neuropathy, vascular disease, blindness, and renal failure. Therefore, blood glucose laval should be maintained within certain ranges (4–6 mM or 70–110 mg/dL), which is a process known as glucose homeostasis (Szablewski, 2011). Means were compared by the Duncan's Multiple Range test at 5% probability.

The results of the statistical analysis show distinct and highly significant differences between blood differences were noted between blood sugar levels

and above chemical contaminants introduced to male and female mice in comparison to the control sample amounting to (230.00 and 124.67 mg/dl) for male mice and (171.67 and 122.33 mg/dl) for female mice. No significant differences were recorded between sugar levels and chemical concentrations for both obtained and less concentrations in comparison with control amounting to (149.67, 146.00, and 122.33 mg/dl), respectively as demonstrated in Figure (1). Moreover, according to the results, a clear correlation was observed between blood glucose levels and chemical contaminants concentrations in both male and female mice compared to the control. Likewise, chemical contaminants negatively impacted blood glucose levels in both male and female mice, which resulted on elevated glucose levels in comparison to the control. These results can be attributed to the insulin hormone resistance, which in turn regulates blood glucose levels. To be more precise, body insulin can regulate glucose levels, which allows the body

cells to prevent the use of excess glucose as energy source, hence, sustaining different bodily processes (Estela, 2011).

The majority of Sb compounds present in the environment are mostly found as Sb trioxide, a byproduct of the production of polyethylene terephthalate (PET) (WHO, 2011). Therefore, there is a remote possibility of poisoning incidents because of its limited absorption in the digestive system. On the other hand, long-term animal studies revealed the occurrence of blood alterations and liver damage due to the exposure and consumption of Sb (WHO, 2011). Also, according to Kanuchova et al. (2020), the continuous exposure to high levels of Sb can lead to lower blood sugar levels and higher blood cholesterol. No clinical toxicity symptoms were observed in male

and female mice after exposing to Sb trioxide for 21 days, except for a slight drop in body-weight growth in high dosages (Kirkland et al., 2007). In case of inhaling Sb for extended period of time, stomach discomfort, diarrhea, vomiting, heart and lung issues, and stomach ulcers can be noticed due to the irritating behavior of Sb (Cooper and Harrison, 2009). Lung cancer and infertility were reported to be exhibited as reported by (ATSDR, 1992) as a result of inhalation of elevated levels of Sb. According to the inadequate evidence of Sb trioxide's carcinogenicity in humans and extensive evidence of its carcinogenicity in experimental animals, Sb was classified as probably carcinogenic to humans within 2B group (IARC, 1989).

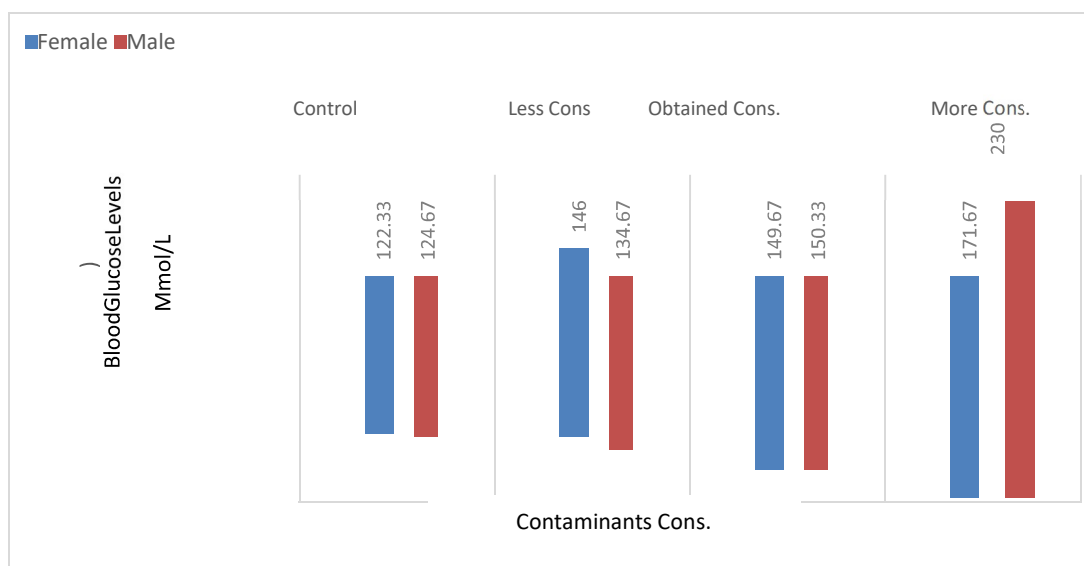


Figure (1): The effect of chemical contaminants on blood glucose levels in male and female mice after 50 days of introducing different concentration (less, obtained, and above).

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