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Experimental study on the impact of drinking water in plastic container with exposing to summer heat on animal health

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***Abstract*---**This study was conducted with the aim of evaluating the expected negative effects of using plastic containers in depicting hot weather on animal health by using some criteria for liver enzymes represented by Alinine transaminase ALT, gama glutamate tranaminase (GGT), as well as redox enzymes manuldehyde (MDA) and glutathione peroxidase (GPX) . The animal house in agriculture college university of Karbala was used for the purpose of housing the animals of this experiment and the experiment lasted from 4/6/2020 to 4/8/2020 . Thirty local male rabbits, average weight 1100-1400gm and age 4 months were divided into three groups equally : First group G1 served as controls negative (10 animals) were received water in metallic container at room temperature.Second group G2: Control positive (10 animals) were received water in plastic container at room temperature. Third group G3: also (10 animals) were received water in plastic container at 45°C temperature. Feeding and water offered ad-libtum. the result reviled that animals received water in plastic container with exposited to summer heat45°C, recorded increased significantly after 45 days in ALT,GGT and monoldihyd (MDA) , as well as glutathione peroxidase recoded decreased significantly was observed .An improvement was showed in group G1at the level of same parameters studied. as compared with second and third group.

***Keywords*---**plastic, health, drinking water.

Introduction

The major constituent of Earth's hydrosphere and all known living species' fluids is water, an inorganic, transparent, tasteless, odorless, and nearly colorless chemical substance (in which it works as a solvent) (Sharma., 2021). It is required for all known forms of life, despite the fact that it contains no calories or organic nutrients. It has the chemical formula H2O, which means that each of its molecules is made up of one oxygen and two hydrogen atoms connected by covalent bonds. Two hydrogen atoms are bonded to one oxygen atom at a 104.45° angle. (Saqlain, *et al* ., 2018).Plastics (or polymers) are made up of macromolecule chains that are formed by chemical reactions from monomer units. Polymer addition (continuous or stepwise) and condensation polymerization are two common processes for chain assembly (poly condensation) (He, *et al* ,2021). Bottled water is a consumer food product prepared from spring, filtered, mineral, effervescent, artesian, or well water that has undergone stringent processing to meet regulatory criteria (Daffi, *et al* , 2021). According to historical records, the sale of bottled water began in the 17th century in the United Kingdom (Holy Well bottling business), when water from mineral springs was thought to have medicinal and curative properties and was sold as a medicine for ailments (Walker, 2020). When Johann Jacob Schweppe discovered how to carbonate water in 1783, plain water's effervescent nature created a lot of rivalry for mineral water(Daffi, *et al* , 2021). Because of lower glass costs and novel bottling methods, bottled water and carbonated drinks became increasingly popular in the United States during the nineteenth century. Bottled water sales peaked in the early twentieth century, when chlorination was introduced to make tap water safer, resulting in a decline in bottled water sales. (Sawangjang, 2019).

Material and Methods

A total of 30 male rabbit divided into 3 groups G1 consider as control negative group,G2 used as control positive group(which take water with plastic container) and G3 is treated group (which take pre-treated water in plastic container at 45ºC temperature).blood sample taken direct from heart then isolated serum after centrifuge at 3000 ppm in 3 minute then Alanine aminotransferase ALT and Gamma-glutamyltransferase GGT calculate by using A serum sample was placed in the DC-40-Mindraydevice, and the measurement was done automatically. While Determination of Malondialdehyde (MDA)according to Buege and Aust 1978 method. And calculate Glutathione Peroxidase (GPx) (Pascual et al ., 1992).was measured using a special kit (Bio Assay Systems) by the quantitative colorimetric glutathione peroxidase determination

Statistical analysis

The statistical analysis of the data of the experiment was measured by using the SAS (Statistical Analysis System - version 9.1), Using two-way ANOVA for experiment two and Least significant differences (LSD) were performed to assess significant differences among means of the groups. The results were expressed as mean ± stander errors and $P < 0.05$ was considered statistically significant (SAS, 2010).

Result and Discussion

Table(1) Theeffect of drinking water exposed to a temperature (45 C0) in the plastic container on ALT(IU/L) in male rabbits.

The results represented in table (1) reveals the effect of oral supplementation of pre-treated Water in plastic container at 45°C temperature on male rabbits at libitum. There is no significant differences in G1 for all next periods. While G2 and G3 show there is a significant (P≤0.05) increase in all next periods. on the other hand at zero time there is no significant differences between all groups. But at 20 days G2 and G3 show there is a significant (P≤0.05) increase as compared with G1.

Table (1): The effect of drinking water exposed to a temperature (45 C^0) in the plastic container on ALT(IU/L) in male rabbits

Time / Group	Mean ± SE			L.S.D Values
	Zero time	after 20 days	After 45 days	
G1	38 ± 1.87 Aa	38.4± 1.77 Ba	39.4 ±1.16 Ca	
G2	39± 0.83 Ac	54.8± 2.57 Ab	66.8 ± 1.85 Ba	
G3	39.60±1.53 Ac	57.40± 1.19 Ab	73.8± 0.7 Aa	
ValuesLSD				5.1

Different capital letters denote a significant difference between groups vertically (p≤0.05), while small letters denote significant difference between periods for each group horizontally .Values represent mean ± SE (N=10).

The effect of drinking water exposed to a temperature (45 C^0) in the plastic container on GGT(IU/L) in male rabbits.

The results represented in table (2) reveals the effect of oral supplementation of pre-treated Water in plastic container at 45°C temperature on male rabbits at libitum. There is no significant differences in G1 in all periods .In G2 there no significant differences from zero to 20 days but there is a significant (P≤0.05) elevated at 45days. While there is a significant (P≤0.05) increase for all next periods.On the other hand there is no significant differences between groups at zero timeWhile after 20 and 45 days there is a significant (P≤0.05) increase in G2 and G3 compared with G1.

Table (2) The effect of drinking water exposed to a temperature (45 C^0) in the plastic container on GGT(IU/L) in male rabbits

Time / Group	Mean ± SE			L.S.D Values
	Zero time	after 20 days	After 45 days	
G1	6.6 ± 0.67	6.4± 0.24	5.6 ±0.40	

	Aa	Ba	Ba	
G2	6.4± 0.67 Ab	7.6 ± 0.40 Ab	8.8 ± 0.31 Aa	
G3	6.5 ±0.58 Ac	8.44± 0.44 Ab	9.7 ± 0.20 Aa	
ValuesLSD				1.2

Different capital letters denote a significant difference between groups vertically (p≤0.05),while small letters denote significant difference between periods for each group horizontally .Values represent mean ± SE (N=10).

Discussion of table 1 and 2 (ALT and GGT)

The increase of ALT and GGT in G2 and G3 due to the effect of plastic and reaction with water especially G3 when the temperature increase the reaction between plastic material and water .while the plastic contain 8 ppm in normal plastic container and in case of subjected to 45c contain 36.7 ppm of BPA which consider toxic effect induced several pathological processes in different animals. And it is known that ALT and GGT are frequently used in damage diagnosis in various tissues such as liver, muscle and kidney caused by pollutants (Coppo, Mussart, &Fioranelli, 2016). The reactive oxygen species (ROS) produced during BPA metabolism in the rabbit liver, can lead to an increase in the permeability of liver cells that results in leakage of GGT and ALT, enzymes into the plasma (Abdel-Rahman,et al, 2018). Therefore, the monitoring of liver enzymes into the blood has been a useful tool in liver toxicological studies (El-Sayed, Monier, et al., 2017). Significant increases in the activities of AST, ALT, and ALP were recorded due to BPA toxicity in the present study compared to the values from the control fish. Similarly, elevated of serum GGT and ALT levels in BPA-exposed animals compared to a control group were observed in rats (Ahmed, Moselhy, & Nabil, 2015) and fish (Hamed& Abdel-Tawwab, 2017). Additionally, similar results were reported in activities of liver enzymes of African catfish (Sayed&Hamed, 2017). In another hand the increase in G3more than G2 due to the effect of temperature on reaction between water and plastics that increase of precipitate of plastics materials (Keogh, et al,2015). The increase with time in the level of GGT and ALT in G3and G2 due to increase damage of liver cell by more free radical production (Jovanovic, et al. (2018).

Table(3)The effect of drinking water exposed to a temperature (45 C^0) in the plastic container on GPX (IU/L) in male rabbits

The results represented in table (3) reveals the effect of oral supplementation of pre-treated Water in plastic container at 45°C temperature on male rabbits at libitum. There is no significant differencesthroughout periods from zero to 20 days and 45 days of G1. While In G2 and G3 there is a significant (P≤0.05) decrease in all next periods .On the other hand there is no significant differencesbetween groups at zero time. While after 20-4 days there is a significant (P≤0.05) decrease in G2 and G3 compared with G1.

Table(3)The effect of drinking water exposed to a temperature (45 C^0) in the plastic container on GPX (IU/L) in male rabbits

Time / Group	Mean ± SE			L.S.D Values
	Zero time	after 20 days	After 45 days	
G1	190 ± 2.7 Aa	192.50± 1.54 Aa	194.50 ±2.04 Aa	
G2	188.7**52.36**± Aa	**5.85±175** Bb	**2.82±159.32** Bc	
G3	**189**.33 ±1.58 Aa	160.16± 2.66 Cb	136.32 ± 2.53 Cc	
ValuesLSD				8.56

Different capital letters denote a significant difference between groups vertically ($p \leq 0.05$),while small letters denote significant difference between periods for each group horizontally .Values represent mean ± SE (N=10).

Discussion of table 3 (GPX)

The decrease of GPX in G2 and G3 due to the effect of plastic and reaction with water especially G3 when the temperature increase the reaction between plastic material and water. Enzymatic radical scavengers, including CAT, SOD, GPx, and GST could be developed as biomarkers in aquatic animals (Geret, Serafim, &Bebianno, 2003; Hamed& Abdel-Tawwab, 2017). These enzymes may scavenge unwanted O2 − and H2O2, and ROOH produced by free radicals. For example, SOD catalyzes superoxide radical dismutation: $O2^- + O2^- + 2H^+ \rightarrow H2O2 + O2$. The resulting hydrogen peroxide in turn is decomposed by the enzymes GPx and CAT (Messarah ,et al,2010). Sharing this function with GPx. GPx is the family of enzymes that reduces hydroperoxides to hydroxy compounds using glutathione (GSH) as the substrate (Wilhelm Filho, 1996). The decrease in level of GPX in control positive group and treatment group related to reaction of GPX with free radical production by microplastic (bisphenol and other) and that agree with (Huang,et al ,2020). GPx-1 is one of the most abundant members of the GPx family of enzymes that include an epithelial-specific enzyme that is highly expressed in intestine (GPx-2); a secreted subtype (GPx-3); and GPx-4, which is widely expressed and differs in its substrate specificity compared to the other family members. Accordingly, GPx-1 is a crucial antioxidant enzyme involved in preventing the harmful accumulation of intracellular hydrogen peroxide. It is present in all cells; found in cytosolic, mitochondrial, and, in some cells, in peroxisomal compartments (EsworthyRS,et al ,1997, Li S.,et al,2000). Previous studies have shown that an inhibition in GPx may occur in response to elevated toxicity levels. Such inhibition was demonstrated in mussels (M. galloprovincialis) exposed to virgin and pyrene contaminated MP (Avio et al., 2015).

Table (4)The effect of drinking water exposed to a temperature (45 C⁰) in the plastic container on MDA(mmol /ml) in male rabbits

The results represented in table (4) reveals the effect of oral supplementation of pre-treated Water in plastic container at 45°C temperature on male rabbits at libitum. There is no significant differences in G1 throughout all next periods.While there is no significant differences throughout periods from zero to 20 days in G2 but there is significant (P≤0.05) increase in 45 days in G2.But In the G3 there is a significant (P≤0.05) increase in all next periods.There is no significant differencesbetween groups at zero time .While after 20 days there is a significant (P≤0.05) increase in G3 compared with G1 and G2. After 45 days there is a significant (P≤0.05) increase in G2 and G3 compared with G1.

Table (4):The effect of drinking water exposed to a temperature (45 C⁰) in the plastic container on MDA(mmol/l/ml) in male rabbits

Time / Group	Mean ± SE			L.S.D Values
	Zero time	after 20 days	After 45 days	
G1	2.03 ± 0.01 Aa	2.05± 0.01 Ba	2.08 ±0.01 Ca	
G2	2.15±0.04 Ab	**0.0±2.20**4 ABb	**2.5**3 **0.**±05 Ba	
G3	± **1.950.06** Ac	**0.07±2.47** Ab	**0.08±3.20** Aa	
ValuesLSD				0.3

Different capital letters denote a significant difference between groups vertically (p≤0.05), while small letters denote significant difference between periods for each group horizontally .Values represent mean ± SE (N=10).

Discussion of table 4 (MDA)

The present study showed a significant increase in renal MDA in G2 and G3 due to the effect of plastic and reaction with water especially G3 when the temperature increase the reaction between plastic material and water. This result suggests the induction of oxidative stress after micro plastic material(BPA) exposure (Bindhumol,et al , 2003). Thus, increased MDA levels could trigger the loss of membrane integrity causing increased cell permeability, enzyme inactivation, and structural damages of DNA (Dominguez-Rebolledo et al., 2010). It has been reported that levels of MDA increased as a result of oxidation of lipoproteins and lipids in membranes during oxidative stress (Moghaddam, Samarghandian, &Farkhondeh, 2015; Ozaydin et al., 2018). Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress and the antioxidant status(Gaweł, et al.2004) MDA in daphnids was significantly increased by ROX, indicating that the alteration of antioxidant enzymes in daphnids was unable to prevent and counteract the formation of ROS caused by ROX. It is similar to the literature that a high content of MDA was induced by

ROX in fish (Nie et al. 2017). MDA levels in daphnids were not significantly changed by PS alone, which has been demonstrated in previous studies with marine organisms. For instance, Paul-Pont et al. (2016) did not observe signs of LPO in mussels (Mytilus spp.) under oxidative stress from microplastic exposure. Microplastics at 1, 10, and 100 mg/L did not significantly change the MDA levels in the liver of O. niloticus in most cases after exposure for 14 days (Ding et al. 2018), whereas the co-exposure of PS and ROX decreased the MDA level induced by ROX alone to the control level. The results suggest that the addition of PS reduces the bioavailability of ROX in daphnids, which relieved the oxidative stress and consequently led to an inhibition of LPO(Nie.et al,2017).

Conclusion

supplementation drinking water with plastic container throw hot season may be negative impact on the progress in health .the present study has proven the summer heat that reach about 45C adversely affect liver functions and redox state of the body which appear clearly in the result of this study And therefore affect negatively on body functions.

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