

In Vivo Evaluation of The Hepatotoxic Disorders of Tributyltin Low Doses in Male Rats

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ABSTRACT: Tributyltin (TBT) has been used widely for over 60 years as a pesticidal, preservation, rodent repellent, water-repellant coating, corrosion inhibitor, flame-resistant polyester, and household product. Previous research has demonstrated the toxicity of high doses of TBT, but there is insufficient data on the effects of relevant levels of TBT on at low doses.

The main goal of this study is to recognize the hepatotoxicity of Sublethal doses of TBT chloride (10-2000 µg/Kg body weight) in male rats for 45 days. The results TBT induced hepatic induction of oxidative stress as indicated by increasing levels of TBARS and 8-OHdG, induction of hepatic inflammation as indicated by the elevated NF-κB levels; inhibition of hepatic expression of PGC-1α and impairment of mitochondrial biogenesis, induction of SREBP-1c and induce hepatic lipogenesis and fatty liver, and distortion of hepatic structure at histological level in a dose-dependent manner significantly at doses higher than 250 µg/kg. In conclusion. the TBT at low doses can induce hepatotoxicity at different levels.

Keywords: Tributyltin chloride; Liver homogenate oxidative stress marker; Hepatotoxicity; oxidative stress, Liver homogenate, Antioxidants.



1. INTRODUCTION

Tributyltin (TBT) was detected in seawater, sediments, and aquatic organisms, as TBT levels are usually elevated at coastal waters, near harbors, marinas, and fishing ports (1). Tributyltin is one of the most dangerous man-made chemicals released into water systems, according to (5); it can enter benthic sediments directly through the particles and has an affinity for persistent, which could be a long-lasting problem. The bioaccumulation and ecological implications of TBT was poorly understood because of the little information available on how these compounds accumulate in fish and aquatic plants in freshwater environments, where, TBT bind to marine dead carp, shrimps and algae and consumed by living marine fauna were studied as toxins like TBT were found quite potent (38).

People are getting more worried about how tributyltin (TBT) might hurt their health; one way people get TBT is by drinking and eating contaminated water and food, especially seafood (4) reported that there were numerous routes of exposure to TBT, including ingestion, dermal, and respiratory exposure; particularly in countries where TBT use is not regulated (32), and substantial quantities of TBT had been found in the tissues of individuals all over the world (23).

demonstrated that the molecular routes of TBT-induced hepatotoxicity have been largely unexplored because most TBT research focused on cellular observations rather than gene or protein-level studies (42). Based on the results of the gene expression profiling, it was determined that TBT induced hepatotoxicity by altering the apoptotic pathway. Flow cytometry analysis shows an uptick in apoptotic cells after exposure to medium-and high-dose TBT, with more cells in the high-dose

TBT group undergoing late necrosis. During TBT-induced apoptosis, genes for heat shock proteins, tumor inflammatory factor receptors and kinases are all turned on. At the same time, tributyltin affects several of the hepatic cytochrome P450 enzymes involved in the oxidative metabolism and biotransformation of exogenous and endogenous medicines (25). As hepatic cytochrome P450 enzymes play a role in assessing the risks and advantages of xenobiotics in general and organotin chemicals in particular due to their involvement in detoxifying and activating foreign substances (3). TBT compounds are broken down into metabolites by cytochrome P450 enzymes, where mostly these metabolites are less dangerous than the chemicals they come from (28) (3).

Investigated how zebrafish growth, oxidative stress (40), and apoptosis were affected by dietary triphenyltin (TPT) and quercetin, and found that fish fed 100 mg/kg of quercetin outperformed fish subjected to 10 ng/L of TPT in terms of development performance after 56 day. MDA was lowered and antioxidant enzyme activity due quercetin's anti-oxidant capabilities. Both the central apoptotic gene and pro-inflammatory cytokine mRNA expressions were upregulated after TPT exposure, while, dietary quercetin inhibited TPT-induced increases in Bax, caspase3, and caspase9 transcript abundances. As well as decreasing inflammation, quercetin treatments impacted the NF- κ B signaling pathway. According to (34) in biochemical and histological examinations liver damage and tin accumulation in seahorses subjected to 50 and 500 ng/L TBT for 60 days, evaluating the impact of TBT on the antioxidant defenses and immunological responses of seahorse livers; malondialdehyde and SOD levels increased, whereas catalase activity decreased. According to transcriptomic research, many genes contributing to the antioxidant defense system were significantly activated to protect hepatocytes from oxidative injury.

Male rats were subjected to daily TBT doses of 0 mg/kg, 0.5 mg/kg, 1 mg/kg, and 2 mg/kg from postnatal day 56 to 86 (37). While TBT did not trigger apoptosis in H295R cells at 100 nM *in vitro*, it did cause them to create reactive oxygen species, and it did so *in vivo* and *in vitro* via increasing AMPK expression in the adrenal glands of rats. TBT inhibits glucocorticoid production in the rat adrenal cortex via reducing AKT1 phosphorylation and SIRT1/PGC-1 α levels, activating AMPK, and perhaps triggering ROS generation. (22) showed that TBT, TPT, and their combination decreased the DNA integrity in the liver, and the effect was dose dependent.

Most of the previous studies had focused on high and sublethal doses of TBT; while the effects of low doses of tributyltin at relevant levels of hepatotoxicity in humans are not well studied. As a result, the current study aims to investigate the molecular, biochemical, and histological changes that occur after male rats are exposed to low doses tributyltin, which causes hepatotoxicity.

2.MATERIAL AND METHODS

2.1. Chemicals

Tributyltin chloride (C₁₂H₂₇ClSn), in the form of clear liquid and light yellow, Product No: T50202-5G from Sigma–Aldrich Chemical Company, St. Louis, MO, USA, CAS- 1461-22-9, purity 96% and Molecular Weight: 325.51 g/mol, Reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), nicotinamide adenine dinucleotide phosphate (NADPH), thiobarbituric acid and other chemicals were also purchased from Sigma Chemical Company (Saint Louis, USA).

2.2 Animals and experimental groups

Forty-five adults male Wistar rats 8-10 weeks of age and weighing 170 $\pm$ 185 g was used in this study. Animals were obtained from Faculty of Medicine, Alexandria University, Alexandria, Egypt. The research was carried out according to the Guide for the Care and Use of Laboratory animals (International Council for Laboratory Animal Science, ICLAS) and was approved by the local ethical guidelines of Institutional Animal Care & Use Committee (IACUC), Alexandria University, Egypt (AU14-211017-2-8A) and all the methods were performed according to the guidelines and regulations of the same Committee. Rats were housed 5 animals in each plastic cage (320 $\times$ 215 $\times$ 170 mm). Animals were kept on basal diet and tap water which were provided *ad libitum*. Rats were kept in normal atmospheric condition where room temperature (25 $\pm$ 5 $^{\circ}$ C) and humidity (50-60%) were maintained throughout the experiments. After two weeks of acclimation, animals were divided into 8 equal groups. Group 1 served as control, group 2 was treated with TBT at 10 μ g/kg BW (1/10000 of LD₅₀), group 3 was treated with TBT at 50 μ g/kg BW (1/5000 of LD₅₀), group 4 was treated with TBT at 100 μ g/kg BW (1/1000 of LD₅₀), group 5 was given TBT at 250 μ g/kg BW (1/400 of LD₅₀), group 6 was given TBT at 500 μ g/kg BW (1/200 of LD₅₀), group 7 was given TBT at 1000 μ g/kg BW (1/100 of LD₅₀), and group 8 was given TBT at 2000 μ g/kg BW (1/50 of LD₅₀). Animals were orally treated with respective doses every day for 45 days. Body weights of rats were recorded at the 1st and 45th days during the experimental period and body weight gain (g/45 days) was calculated.

2.3 Blood samples collection and tissue preparations

At the end of the 45th day of the experimental period, all animals of each group were sacrificed by using isoflurane 5% (2 mg/Kg BW or equal 2% inhaled in dictator). Blood samples were collected by cardiac puncture in test tubes containing heparin as an anticoagulant. The obtained blood was centrifuged at 860 Xg for 20 minutes for the separation of plasma. The plasma was kept at - 80 °C until analyses of the tested parameters. liver was immediately removed, washed using chilled saline solution (0.9%), and removed the adhering fat and connective tissues. liver was minced and homogenized (10%, w/v), separately, in ice-cold sucrose buffer (0.25 M) in a Potter–Elvehjem type homogenizer. The homogenates were centrifuged at 10,000 Xg for 20 min at 4 °C, to pellet the cell debris and the supernatant was harvested and stored at –80 °C for the determination of tested parameters.

2.4 Liver homogenate oxidative stress marker

Thiobarbituric acid reactive substances (TBARS) were measured as described by ,1 mL of homogenate was added to 2 mL of 7.5% trichloroacetic acid and mixed. The mixture was centrifuged at 1,000 xg for 10 min. 2 mL of supernatant added to 1 mL of 0.7 % 2- thiobarbituric acid. After boiling for 10 min, the reactants were cooled and TBARS were measured at 532 nm. An extinction coefficient of 156.000 mol⁻¹cm⁻¹ was used for calculation (35).

2.5 Oxidative DNA marker: 8-OH deoxyguanosine (8-OHdG)

Total DNA was isolated from liver tissues using DNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. 8-OH-dG was measured in DNA samples, using a commercial 8-OH-dG ELISA kit (Chongqing Biospes, China) following the manufacturer's protocol.

2.6 Determination of DNA breakages in liver homogenate

The DNA breakages, as an indicator of cell death, were assayed according to the method of (37).

2.7 Determination of nuclear factor kappa B (NF-κB)

Commercial ELISA kits (Chongqing Biospes, China) were used for determination of nuclear factor kappa B (NF-κB) in the liver tissue supernatants according to the manufacturer instructions.

2.8 Apoptotic marker: Caspase-3 activity in liver tissues

Caspase-3 has a specificity for cleavage at the terminal side of Asparatate residue of the amino acid sequence DEVD (Asp-Glu-Val-Asp). The caspase-3 enzymatic activity was assayed using Caspase-3 Assay Kit (Elabscainces, USA).

2.9 Quantitative real time-polymerase chain reaction (qRT-PCR) of gene expression of peroxisomes proliferator activated receptor γ co-activator -1 α (PGC-1 α), Sterol regulatory element binding protein-1c (SREBP-1c), and nuclear factor erythroid 2-related factor 2 (NRF2) in the liver tissues

Quantitative expression analysis of PGC-1 α and mtTFA in liver tissue was performed using for the relative quantitative determination of the gene expression of mtTFA (Piantadosi & Suliman, 2006) and PGC-1 α (Li et al., 2011) at mRNA level according to the manufacturer instructions. The primes sequences used: PGC-1 α ; F- GTGCAGCCAAGACTCTGTATGG3' ,and R- GTCCAGGTCATTCACATCAAGTTC3', SREBP-1c; F- 5-GACGACGGAGCCATGGATT3', and R- 5-CAAATCCCACCTTGAACACA3' , NRF2; F- CAAATCCCACCTTGAACACA3' and R- 5'-CGACTGACTAATGGCAGCAG3' and 18srRNA; F- 5-GTAACCCGTTGAACCCCAT3', and R- 5-CAAGCTTATGACCCGCACTT3'

Total RNAs were isolated from the hepatic liver tissues using RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer instructions. Reverse transcription was done using miScript II RT Kit (Qiagen,Germany) according to the manufacturer instructions.

2.10 Relative quantification of expression using PCR

Quantitative PCR was applied to determine the relative expression of cortical and hippocampal PGC-1 α , SREBP-1c, and NRF2 using the specific primer sets for each gene (Table 3.2). The relative quantification (RQ) using comparative threshold cycle (Ct) provides an accurate comparison between the initial levels of template in each sample. A normalizer or reference gene (18s rRNA) was used as internal control for experimental variability in this type of quantification. This method of relative quantification is called $\Delta\Delta C_t$ method or Livak method (20). Quantitative PCR assay was carried out using Rotor-Gene SYBR Green PCR Kit (Qiagen®, Germany).

2.11 Histological section preparation of liver

Histopathological examination was carried out according to (11). Livers were obtained from rats, and immediately fixed in 10% formalin, and then treated with a conventional grade of alcohol and xylol, embedded in paraffin and sectioned at 4-6 μ m thickness. The sections were stained with Haematoxylin and Eosin (H&E) stains and photographed on the PC screen using a light microscope with a digital color camera attachment and dial indicator for studying the histopathological changes.

2.12 Statistical analysis

Mean and standard error values were determined for all the parameters and the results were expressed as mean $\pm$ standard error. The data were analyzed using a one-way analysis of variance (ANOVA) followed by Duncan multiple comparison. $P < 0.05$ was statically significant according to (27).

3. RESULTS AND DISCUSSION

3.1 Changes in oxidative stress markers in the liver of male rats

The result shows the changes in thiobarbituric acid reactive substances (TBARS) as a lipid peroxidation index and 8-OH-2'-deoxyguanosine (8-OH-dG) as an index of oxidative DNA damage in the liver of male rats treated with different doses of TBTC daily for 45 days, Figure (3.1). The results of hepatic TBARS revealed significant increase in the rats treated with TBTC at doses ≥ 250 μ g/kg in a dose-dependent manner compared with the control group. Also, the hepatic content of 8-OHdG showed significant dose-dependent elevation in the rats treated with TBTC start from the dose of 500 μ g/kg and higher with the highest levels observed in the rats treated with 2000 μ g/kg.

All aerobic organisms generate ROS, which can be in either physiological quantities, necessary for normal cell activity or excessive amounts; a condition known as oxidative stress (27). The methods through which TBT regulates ROS that led to induction of damage to lipids, proteins, and DNA were identified by (31). After three and seven days of exposure, TBT significantly increased hepatic superoxide anion production and increased reactive oxygen generation in a dose-dependent manner in the livers of rats (19).

Tiiphenyltin (TPT) induced toxicity is initiated and progresses in part due to oxidative stress (39). Free radicals have a crucial function in mediating organismal damage (16). Other studies on liver tissue from goldfish contains natural antioxidant enzymes treated with TPT demonstrating a reduction in the antioxidant enzymes activities, demonstrating that the antioxidant system is hindered by TPT exposure, which could lead to oxidative damage (8).

Results of present study agrees with the previous findings of (19), They indicated that oxidative damage was seen in TBT-treated rat liver tissues due to an increase in reactive oxygen species formation, (43) who found that the structural changes and cell death caused by Tributyltin were accompanied by an increase in an oxidative stress marker; this is significant because oxidative stress is linked to a broad range of liver diseases.

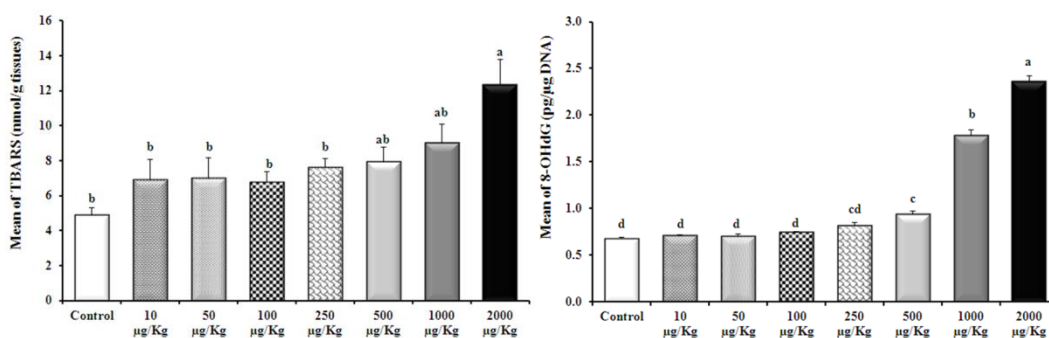


Figure (1): Changes in oxidative stress markers in the liver of male rats treated with tributyltin chloride
 The results expressed as Mean $\pm$ SE, n=5 tributyltin chloride (TBTC) administrated successive doses of 10, 50, 100, 250, 500, 1000, and 2000 $\mu\text{g/kg}$ bw. Mean values with in one measurement rats sharing common subscript letters were significant different, $p < 0.05$

3.2 Changes in the inflammatory and apoptotic markers in the liver of male rats treated with tributyltin chloride

Showed the changes in the Nuclear Factor Kappa B (NF- κ B) level, Caspase-3 activity and DNA breakages in the liver of male rats treated with different doses of TBTC daily for 45 days, Figure (4.6). The results showed no significant changes in the hepatic content of NF- κ B in groups treated with TBTC at low doses (10, 50 and 100 $\mu\text{g/kg}$ body weight), while the rats treated with the higher doses (≥ 250 $\mu\text{g/kg}$) showed dose-dependent increase in NF- κ B compared with the control rats. The hepatic activity of caspase-3 showed similar pattern of change as the NF- κ B. The percentage of DNA breakages showed dose-dependent increase which become significant only in the rats treated with the highest doses of TBTC (1000 and 2000 $\mu\text{g/kg}$).

From these results it is clear that; TBT causes cell death by impeding oxidative phosphorylation, a process that occurs predominantly in the mitochondria (7). Also, lipid peroxidation could disrupt cellular signaling pathways resulting in cell death enhanced and inflammation (15). TBTC induce hepatic inflammation as manifested as elevated pro-inflammatory marker; NF- κ B which may explain the increased liver weight and relative weights especially in the rats subjected to the high doses of TBTC. This inflammation may be caused by induced oxidative stress which subsequently may induce hepatic cell death as indicated by elevated activity of the apoptotic marker; caspase-3 and the necrotic cell marker; the DNA breakage.

In agreement with our findings, there was an increase in dose-dependent percentage of injured cells seen in the peripheral blood of TBT-treated rats; According to the literature, tropical freshwater fish exposed to TBT have degenerative nuclear changes such as aberrant nuclear shapes, chromatin condensation, the existence of intra-nuclear lipid bodies, and a lipid body buildup (30). Damage to DNA bases or strand breakage can occur when ROS attack the phosphate groups of DNA's deoxyribose sugars (36). Reactive intermediates formed when oxygen radicals oxidize lipids or proteins can create adducts with DNA bases (12), (21) DNA strands can be directly oxidized as a byproduct of lipid peroxidation (10). So, the DNA damage and inflammation have been connected to oxidative stress (6).

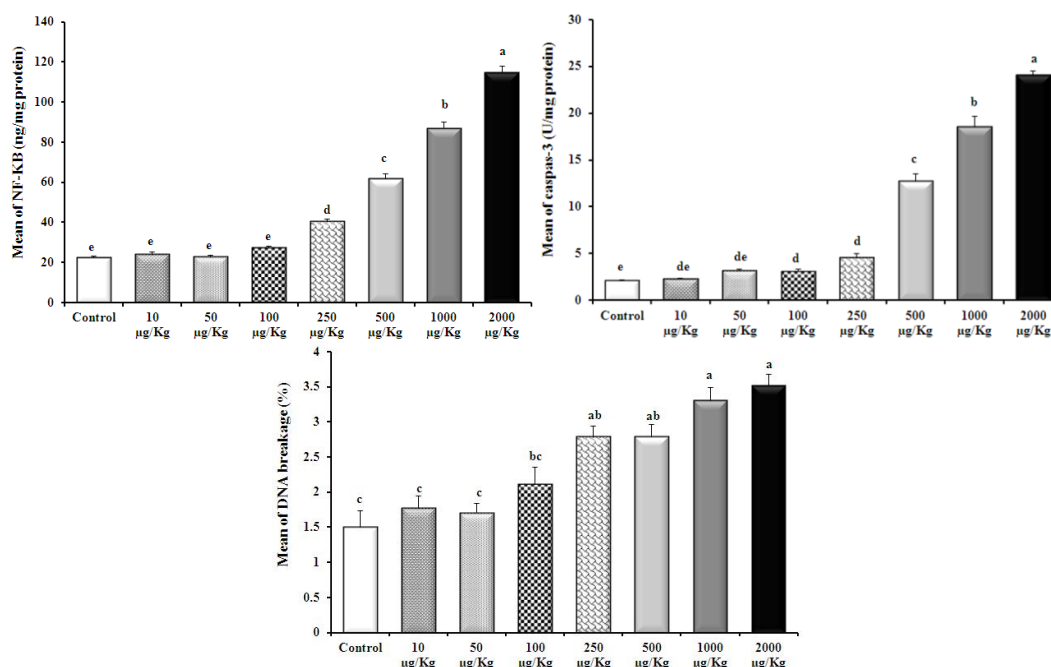


Figure (2): Changes in the inflammatory and apoptotic markers in the liver of male rats treated with tributyltin chloride

The results expressed as Mean $\pm$ SE, n=5

tributyltin chloride (TBTC) administrated successive doses of 10, 50, 100, 250, 500, 1000, and 2000 μ g/kg bw

Mean values with in one measurement rats sharing common subscript letters were significant different, $p < 0.05$

NF- κ B = Nuclear Factor Kappa B

3.3 Changes in the expression of PGC-1 α SREBP-1c in rat's liver

Show the changes in the expression of peroxisome proliferator Activated Receptor- γ Co-activator-1 α (PGC-1 α) and Sterol regulatory element binding protein-1c (SREBP-1c) in the liver of male rats treated with different doses of TBTC daily for 45 days, Figure (4.7). The results showed that PGC-1 α expression showed no significant changes in the rats treated with the low doses of TBTC (10-100 μ g/kg), while the groups treated with doses ≥ 250 μ g/kg the expression showed significant suppression compared with the control rats in a dose-dependent manner with the lowest expression was seen in rats treated with the highest dose (2000 μ g/kg). on the other hand, the hepatic expression of SREBP-1c showed dose-dependent upregulation in the hepatic tissues of rats treated with TBTC compared to control group.

PGC-1 α is a critical regulator of mitochondrial metabolism and antioxidant defense and a key regulator of function and mitochondrial biogenesis. Abnormalities in PGC-1 α expression have been linked to several chronic diseases. PGC-1 α enhances detoxification of ROS and cell survival and shields cells from the detrimental effects of oxidative stress, whereas PGC-1 α -deficient cells accumulate ROS and are more susceptible to oxidative stress-related cell death (33).

According to the current study, the reduced expression of PGC-1 α in the hepatic tissues of rats treated with TBTC could indicate a reduction in the mitochondrial biogenesis, replication, and transcription of mtDNA, which could contribute to malfunctioning in mitochondria. These findings shed light on the significant interaction between the TBT's hepatotoxicity and the gene expression of nuclear transcription factors that regulate cellular metabolism and mitochondrial biogenesis and function (PGC-1 α). Furthermore, TBTC increased ROS, lipid peroxidation and oxidative DNA damage while decreasing GSH levels, which indicates a defect in transfer of electron in the chain of mitochondrial respiratory as well as activity decreasing of the mitochondrial respiratory chain complexes.

showed that TBT can rise the intracellular lipid accumulation, and promote genes expression responsible in transport of lipid and lipogenesis which support our results regarding the dose-dependent induction of SREBP-1c (17); the main lipogenic protein. Found that there was a significant upregulation of genes involved in lipid storage, lipogenic enzymes, and lipogenic factors at 100 ng/L of TBT, suggesting that this concentration may be more likely to stimulate lipid synthesis and storage, and perhaps leading to ectopic lipids synthesis (41).

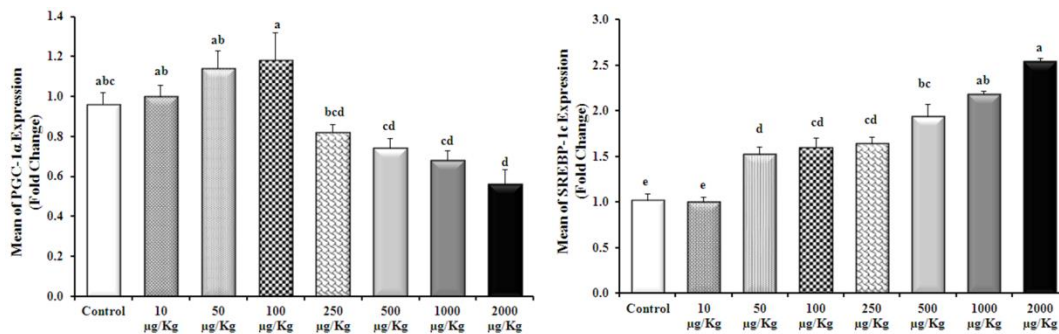


Figure (3): Changes in the expression of PGC1α and SREBP-1c in the liver of male rats treated with tributyltin chloride

The results expressed as Mean $\pm$ SE, n=5

tributyltin chloride (TBTC) administrated successive doses of 10, 50, 100, 250, 500, 1000, and 2000 µg/kg bw
Mean values with in one measurement rats sharing common subscript letters were significant different, $p < 0.05$

3.4 Histopathological changes of liver tissues

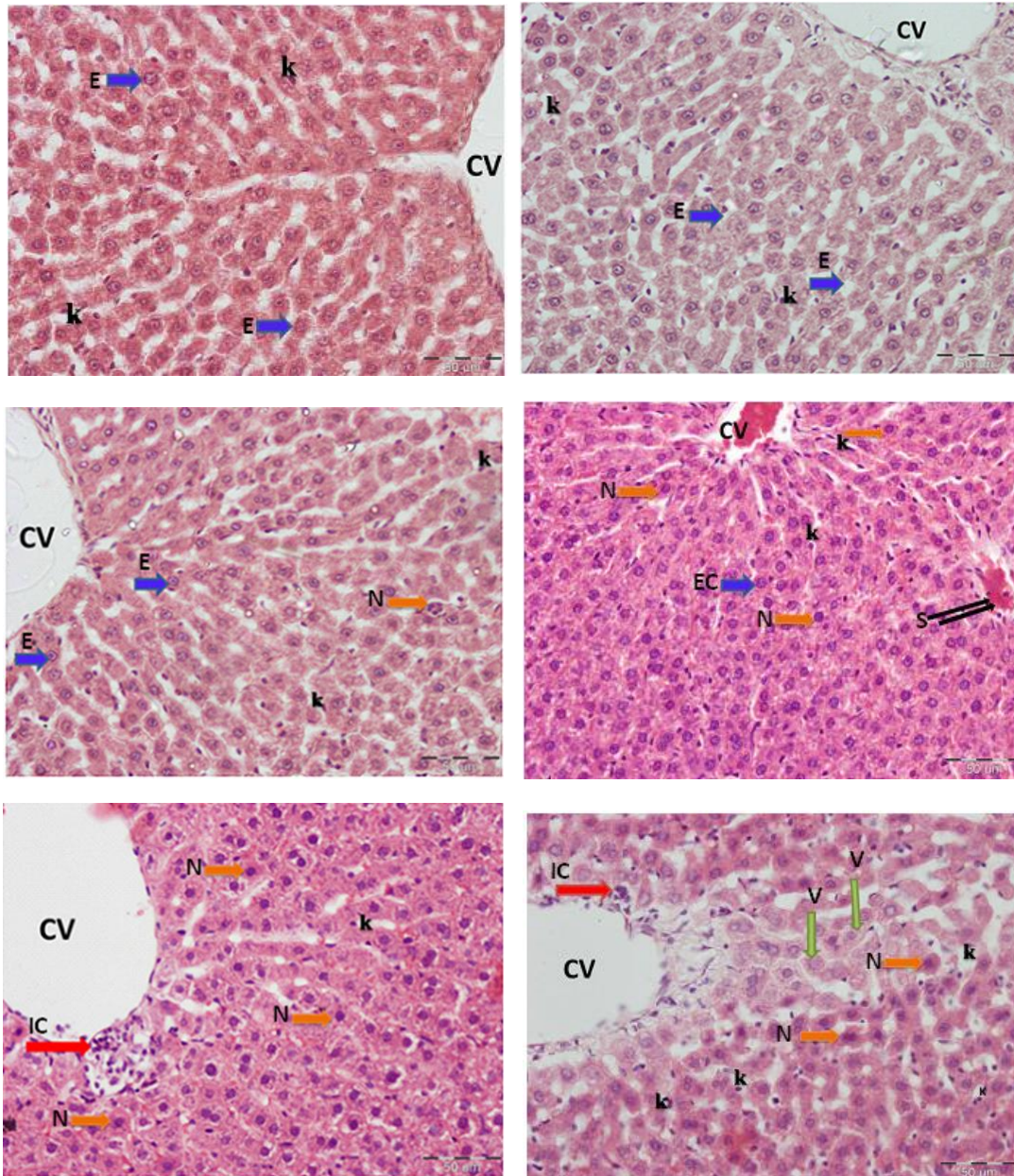
Sections from liver tissues of male rats stained with hematoxylin and eosin (H&E) were studied, for rats treated with tributyltin chloride (TBTC) in daily oral dosages of (10, 50, 100, 250, 500, 1000, and 2000 mg/kg B.W), and examined for different histopathological changes, where one representative image of each group of rats, at magnifications of X200 and bX400, was captured.

Microscopy of H&E stained sections of liver of control male rats indicated normal liver tissue architecture, where Kupffer cells seen within the sinusoids, and hepatocytes were seen with normal histological presence of polyhedral cells with granular eosinophilic cytoplasm (Figure 4.a). On other hand, liver tissue of rats treated with TBTC (10 µg/kg bw) revealed arrangement of hepatocytes in cords of one to two cells thick around hepatic central vein, mild appearance of Kupffer cells within the sinusoids; where hepatocytes seen in classical histological presence of polyhedral cells with granular eosinophilic cytoplasm (Figure 4. b). Where in liver tissue of rats treated with 50 µg/kg TBTC hepatocytes were arranged also in cords of one to two cells thick around hepatic central vein, mild appearance of Kupffer cells within the sinusoids, hepatocytes cells where seen in normal architecture of polyhedral cells with granular eosinophilic cytoplasm, mild number hepatocytes showed changes in nuclear shape (Figures 4. c). Meanwhile, liver tissue of the rats treated with 100 µg/kg TBTC showed congestion in the central vein, mild appearance of Kupffer cells within the sinusoids; where hepatocytes seen in classical histological presence of polyhedral cells with granular eosinophilic cytoplasm, mild presence of hepatocytes showing abnormal changes in nuclear shapes in the form of dark stained nucleus and hyper-eosinophilic cytoplasm (Figures 4.d). The liver section of rats treated with 250 µg/kg TBTC showed area of inflammatory cells appearing in between hepatocytes, moderate appearance Kupffer cells within the sinusoids; and hepatocytes observed mild presence of hepatocytes showing abnormal changes in nuclear shapes in the form of dark stained nucleus, and moderate presence of inflammatory cells seen around the central vein area (Figures 4.e). The liver section of the rats treated with 500 µg/kg TBTC showed distorted arrangement of hepatocytes around hepatic central veins, associated with moderate areas of inflammatory cells appearing in between hepatocytes, severe appearance Kupffer cells within the sinusoids and hepatocytes showed moderate nuclear changes in the form of dark stained nucleus and hyper-eosinophilic cytoplasm, mild cytoplasmic vacuolation in hepatocytes and moderate presence of inflammatory cells seen around the central vein area (Figures 4.f). Rats' liver section treated with TBTC at dose of 1000 µg/kg indicated distorted arrangement of hepatocytes around hepatic central veins, congestions were seen in the portal tracts, associated with moderate areas of inflammatory cells in portal tracts, hepatocytes show severe nuclear changes and severe increase in cytoplasmic vacuolation and dark stained nucleus; where, many exhibit a dark stained nucleus and hyper-eosinophilic cytoplasm, severe appearance of inflammatory cells observed in portal tract area (Figures 4.g). Rats' liver section treated with TBTC at dose of 2000 µg/kg indicted severe distortion in hepatocytes arrangements with severe congestions were seen in the portal tracts, associated with severe presence of inflammatory cells in portal tract area, hepatocytes showed severe nuclear changes and severe increase in cytoplasmic vacuolation and dark stained nucleus; to the extent, moderate cells appear empty and ballooned exhibiting dark stained nucleus and hyper-eosinophilic cytoplasm, severe presence of inflammatory cells seen in portal tract area (Figures 4.h).

Organotins tend to build up in the liver, where weight loss and changes in the liver's structure have been seen in agreement to our findings (18) found that when rats were exposed to dietary TBT oxide (320 mg/kg food) or to TBT acetate by oral gavage, observed inflammation and dilatation and cholestasis in the biliary tract (24). Microscopic liver lesions signify that TBTC procures passive effects on the functions of the liver before the appearance of morphological changes in aged animals treated with TBT (4). In agreement with our results, hepatocellular necrosis was evident at rats' livers cured

with 500 µg/kg TBT. These histological changes were identified as hepatocyte responses to toxic substances and were considered a helpful index for observing the biological influence of pollutants at rats (13).

Liver tissue from rats subjected to 500 mg/kg TBT for 45 days showed hepatocellular necrosis, which is a hepatocyte response to toxic substances and serves a good biomarker for tracking the biological impacts of pollutants in the animal population (13). Also, the histological and morphological examinations of TBT-exposed fish livers revealed a significant increase in lipid content and damage shown as vesicular steatosis in the 100 ng/L TBT-treated group. This finding was consistent with morphological and histological examinations of mammalian tissues (41). Mice Treatment with TBT at doses of 0.5, 5, and 50 mg/kg for 45 days has been shown to increase lipid droplets and hepatocyte degeneration (44). On other hand, (14) found that prenatal TBT exposure leads to increased adiposity in the livers of mice, characterized by a disruption of hepatic structure and an uptick in the accumulation of lipid droplets.



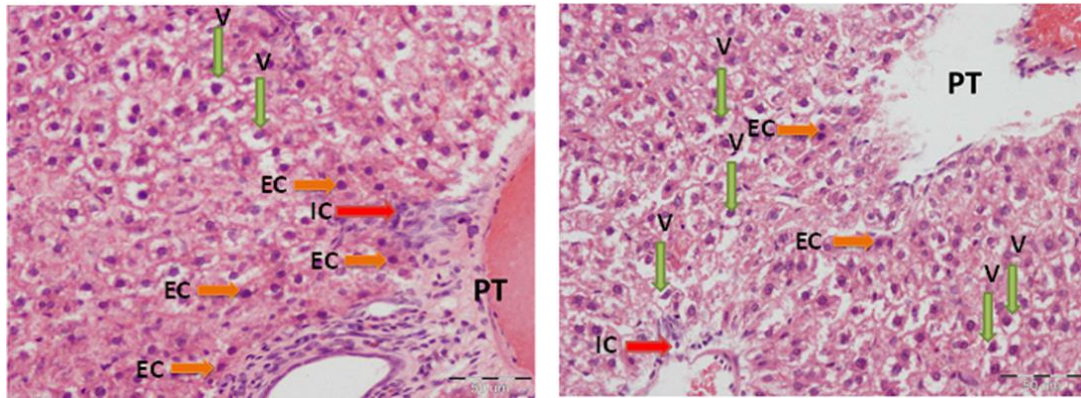


Figure (4.a, b,c,d,e,f,g,h):

Photomicrographs of liver section of male albino rats **(a)**: control group showing normal architecture of hepatic cells, where Kupffer cells (k) are seen within the sinusoids, hepatocytes are seen with normal histological appearance of polyhedral cells with granular eosinophilic cytoplasm (E). **(b)**: TBT 10 µg/kg bw treatments (dose1) illustrating, hepatocytes arranged in cords around hepatic central vein (CV) and Kupffer cells (k) seen within the sinusoids; Hepatocytes are observed with their classical histological appearance of polyhedral cells with granular eosinophilic cytoplasm (E). **(c)**: TBT 50 µg/kg bw treatments (dose2) illustrating, hepatocytes arranged in cords of one to two cells thick around hepatic central vein (CV), Kupffer cells (k) seen within the sinusoids; hepatocytes cells observed in normal architecture of polyhedral cells with granular eosinophilic cytoplasm (E), very few hepatocytes show change in nuclear shape (N). **(d)**: TBT 100 µg/kg bw treatments (dose3) illustrating, congestion in hepatic central vein (CV) Kupffer cells (k) seen within the sinusoids; hepatocytes cells observed in normal architecture of polyhedral cells with granular eosinophilic cytoplasm (EC), very few hepatocytes show change in nuclear shape in the form of dark stained nucleus and hyper-eosinophilic cytoplasm (N). **(e)**: TBT 250 µg/kg bw treatments (dose4) hepatic central vein (CV), Kupffer cells (k) seen within the sinusoids; show nuclear change in the form of dark stained nucleus and hyper-eosinophilic cytoplasm (N), and inflammatory cells seen around the CV area (IC). **(f)**: TBT 500 µg/kg bw treatments (dose5) illustrating, distorted general architecture of hepatic cells around the central vein (CV), Kupffer cells (k) are seen within the sinusoids in big numbers; hepatocytes show nuclear change in the form of dark stained nucleus and hyper-eosinophilic cytoplasm (N). Some hepatocytes start to show increased cytoplasmic vacuolation (V), also, inflammatory cells seen around the CV area (IC). **(g)**: TBT 1000 µg/kg bw treatments (dose6) distorted general architecture of hepatic cells, where, congestion seen in the portal tract (PT), presence of inflammatory cells (IC) in portal tract area, most hepatocytes show nuclear changes and increase in cytoplasmic vacuolation and dark stained nucleus (V); where, many exhibit a dark stained nucleus and hyper-eosinophilic cytoplasm (EC), inflammatory cells are present in abundant (IC) in portal tract area (PT). **(h)**: TBT 2000 µg/kg bw treatments (dose7) distorted general architecture of hepatic cells, congestion seen in the portal tract (PT), and presence of inflammatory cells (IC) in portal tract area, hepatocytes show nuclear changes and increase in cytoplasmic vacuolation and dark stained nucleus (V) to the extent that the cells appear empty and ballooned; where, many exhibit a dark stained nucleus and hyper-eosinophilic cytoplasm (EC), inflammatory cells are present in abundant (IC) in portal tract area (PT) (H&Ex400).

4. CONCLUSION

Considering the obtained results, we can conclude that TBT can induce dose-dependent hepatotoxicity in rats, where doses higher than or equal to 250 µg/kg showed significant alterations in the studied parameters in a dose-dependent manner with the worst effects observed in the rats treated with the highest doses (1000 and 2000 µg/kg). The hepatotoxic effects of TBT may be mediated by induction of oxidative stress as indicated by increasing levels of TBARS and 8-OHdG, induction of hepatic inflammation as indicated by the elevated NF-κB levels; inhibition of hepatic expression of PGC-1α and impairment of mitochondrial biogenesis, induction of SREBP-1c and induce hepatic lipogenesis and fatty liver, and distortion of hepatic structure at histological level.

REFERENCES

- [1] Abreu F. E., da Silva, J. N. L., Castro, Í. B., & Fillmann, G. (2020). Are antifouling residues a matter of concern in the largest South American port? *Journal of Hazardous Materials*, 398, 122937.
- [2] Antizar-Ladislao, B. (2008). Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review. *Environment international*, 34(2), 292-30.
- [3] Appel, K. E. (2004). Organotin compounds: toxicokinetic aspects. *Drug metabolism reviews*, 36(3-4), 763-786.
- [4] Azenha, M., & Vasconcelos, M. T. (2002). Butyltin compounds in Portuguese wines. *Journal of agricultural and food chemistry*, 50(9), 2713-2716.
- [5] Bangedphol, S., Keenan, H. E., Davidson, C., Sakultantimetha, A., & Songsasen, A. (2009). The partition behavior of tributyltin and prediction of environmental fate, persistence and toxicity in aquatic environments. *Chemosphere*, 77(10), 1326-1332.
- [6] Barzilai, A., & Yamamoto, K. I. (2004). DNA damage responses to oxidative stress. *DNA repair*, 3(8-9), 1109-1115.
- [7] Brookes, P. S., Yoon, Y., Robotham, J. L., Anders, M. W., & Sheu, S. S. (2004). Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *American journal of physiology. Cell physiology*, 287(4), C817-C833.
- [8] Chang, X., Wang, X., Feng, J., Su, X., Liang, J., Li, H., & Zhang, J. (2020). Impact of chronic exposure to trichlorfon on intestinal barrier, oxidative stress, inflammatory response and intestinal microbiome in common carp (*Cyprinus carpio* L.). *Environmental Pollution*, 259, 113846.
- [9] Chien, L. C., Hung, T. C., Choang, K. Y., Yeh, C. Y., Meng, P. J., Shieh, M. J., & Han, B. C. (2002). Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. *Science of the total environment*, 285(1-3), 177-185.
- [10] Dedon, P. C., Plataras, J. P., Rouzer, C. A., & Marnett, L. J. (1998). Indirect mutagenesis by oxidative DNA damage: formation of the pyrimidopurine adduct of deoxyguanosine by base propenal. *Proceedings of the National Academy of Sciences*, 95(19), 11113-11116.
- [11] Drury, R. A., & Wallington, E. A. (1980). *Carleton's histological techniques* (p.p. 241-242). New York: Oxford University Press.
- [12] Esterbauer, H., Eckl, P., & Ortner, A. (1990). Possible mutagens derived from lipids and lipid precursors. *Mutation Research/Reviews in Genetic Toxicology*, 238(3), 223-233.
- [13] Feist, S.W., Lang, T., Stentiford G.D., & Köhler, A. (2004). Biological effects of contaminants: Use of liver pathology of the European flatfish dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.) for monitoring. *ICES Techniques in Marine Environmental Sciences*, 38, 42.
- [14] Grün, F., & Blumberg, B. (2006). Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. *Endocrinology*, 147(6), s50-s55.
- [15] Higdon, A., Diers, A. R., Oh, J. Y., Landar, A., & Darley-Usmar, V. M. (2012). Cell signalling by reactive lipid species: new concepts and molecular mechanisms. *Biochemical Journal*, 442(3), 453-464.
- [16] Hill, M. F., Palace, V. P., Kaur, K., Kumar, D., Khaper, N., & Singal, P. K. (2005). Reduction in oxidative stress and modulation of heart failure subsequent to myocardial infarction in rats. *Experimental & Clinical Cardiology*, 10(3), 146.
- [17] Jie, J., Ling, L., Yi, Y., Tao, L., Liao, X., Gao, P. & Weng, D. (2021). Tributyltin triggers lipogenesis in macrophages via modifying PPAR γ pathway. *Environmental Pollution*, 271, 116331.
- [18] Krajnc, E.I., Wester, P.W., Loeber, J.G., van Leeuwen, F.X., Vos, J.G., Vaessen, H.A., & van der Heijden, C.A. (1984). Toxicity of bis(tri-n-butyltin) oxide in the rat. I. Shortterm effects on general parameters and on the endocrine and lymphoid systems. *Toxicology and applied pharmacology*, 75(3), 363-386.
- [19] Liu, H. G., Wang, Y., Lian, L., & Xu, L. H. (2006). Tributyltin induces DNA damage as well as oxidative damage in rats. *Environmental Toxicology*, 21(2), 166-171.
- [20] Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods*, 25(4), 402-408.
- [21] Marnett, L. J. (2000). Oxyradicals and DNA damage. *Carcinogenesis*, 21(3), 361-370.
- [22] Martínez, R., Codina, A. E., Barata, C., Tauler, R., Piña, B., & Navarro-Martín, L. (2020). Transcriptomic effects of tributyltin (TBT) in zebrafish eleutheroembryos. A functional benchmark dose analysis. *Journal of Hazardous Materials*, 398, 122881.
- [23] Mitra, S., Srivastava, A., & Khandelwal, S. (2013). Tributyltin chloride induced testicular toxicity by JNK and p38 activation, redox imbalance and cell death in sertoli-germ cell co-culture. *Toxicology*, 314(1), 39-50.
- [24] Mushak, P., Krigman, M. R., & Mailman, R. B. (1982). Comparative organotin toxicity in the developing rat: somatic and morphological changes and relationship to accumulation of total tin. *Neurobehavioral toxicology and teratology*, 4(2), 209-215.
- [25] Nelson, D. R., Koymans, L., Kamataki, T., Stegeman, J. J., Feyereisen, R., Waxman, D. J.,... & Nebert, D. W. (1996). P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics*, 6(1), 1-42.
- [26] Nordberg, J., & Arnér, E. S. (2001). Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free radical biology and medicine*, 31(11), 1287-1312.

- [27] Norušis, M. J. (2006). *SPSS 14.0 guide to data analysis*. Upper Saddle River, NJ: Prentice Hall.
- [28] Ohhira, S., Enomoto, M., & Matsui, H. (2006). *In vitro* metabolism of tributyltin and triphenyltin by human cytochrome P-450 isoforms. *Toxicology*, 228(2-3), 171-177.
- [29] Quintas, P. Y., Alvarez, M. B., Arias, A. H., Garrido, M., & Marcovecchio, J. E. (2019). Spatiotemporal distribution of organotin compounds in the coastal water of the Bahía Blanca estuary (Argentina). *Environmental Science and Pollution Research*, 26(8), 7601-7613.
- [30] Ribeiro, C. O., Schatzmann, M., De Assis, H. S., Silva, P. H., Pelletier, E. M. I. L. I. E. N., & Akaishi, F. M. (2002). Evaluation of tributyltin subchronic effects in tropical freshwater fish (*Astyanax bimaculatus*, Linnaeus, 1758). *Ecotoxicology and Environmental Safety*, 51(3), 161-167.
- [31] Rice, C. D., & Weeks, B. A. (1990). The influence of *in vivo* exposure to tributyltin on reactive oxygen formation in oyster toadfish macrophages. *Archives of environmental contamination and toxicology*, 19(6), 854-857.
- [32] Santos-Silva, A. P., Andrade, M. N., Pereira-Rodrigues, P., Paiva-Melo, F. D., Soares, P., Graceli, J. B., & Miranda-Alves, L. (2018). Frontiers in endocrine disruption: Impacts of organotin on the hypothalamus-pituitary-thyroid axis. *Molecular and cellular endocrinology*, 460, 246-257.
- [33] St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J. M., Rhee, J., Jäger, S. & Spiegelman, B. M. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell*, 127(2), 397-408.
- [34] Tang, L., Zhang, Y. H., Wang, X., Zhang, C. C., Qin, G., & Lin, Q. (2021). Effects of chronic exposure to environmental levels of tributyltin on the lined seahorse (*Hippocampus erectus*) liver: Analysis of bioaccumulation, antioxidant defense, and immune gene expression. *Science of The Total Environment*, 801, 149646.
- [35] Tappel, A. L., & Zalkin, H. (1959). Inhibition of lipid peroxidation in mitochondria by vitamin E. *Archives of Biochemistry and Biophysics*, 80(2), 333-336.
- [36] Ward, J. F., Limoli, C. L., Calabro-Jones, P., & Evans, J. W. (1987). Radiation vs chemical damage to DNA. In P. A. Cerutti, O. F. Nygaard & M. G. Simic (Eds.), *Anticarcinogenesis and radiation protection* (pp. 321-327). Boston, MA: Springer.
- [37] Wu, K., Li, Y., Liu, J., Mo, J., Li, X., & Ge, R. S. (2020). Long-term triphenyltin exposure disrupts adrenal function in adult male rats. *Chemosphere*, 243, 125149.
- [38] Xiao, X., Zhu, S., Zou, X., He, G., Jiang, J., & Sheng, G. D. (2020). Relation of tributyltin and triphenyltin equilibrium sorption and kinetic accumulation in carp and *Ceratophyllum demersum*. *Ecotoxicology and Environmental Safety*, 192, 110289.
- [39] Zhang, C. N., Zhang, J. L., Ren, H. T., Zhou, B. H., Wu, Q. J., & Sun, P. (2017). Effect of tributyltin on antioxidant ability and immune responses of zebrafish (*Danio rerio*). *Ecotoxicology and environmental safety*, 138, 1-8.
- [40] Zhang, C., Jiang, D., Wang, J., & Qi, Q. (2021). The effects of TPT and dietary quercetin on growth, hepatic oxidative damage and apoptosis in zebrafish. *Ecotoxicology and Environmental Safety*, 224, 112697.
- [41] Zhang, J., Sun, P., Kong, T., Yang, F., & Guan, W. (2016). Tributyltin promoted hepatic steatosis in zebrafish (*Danio rerio*) and the molecular pathogenesis involved. *Aquatic toxicology*, 170, 208-215.
- [42] Zhou, M., Feng, M., Fu, L. L., Ji, L. D., Zhao, J. S., & Xu, J. (2016). Toxicogenomic analysis identifies the apoptotic pathway as the main cause of hepatotoxicity induced by tributyltin. *Food and Chemical Toxicology*, 97, 316-326.
- [43] Zhu, R., Wang, Y., Zhang, L., & Guo, Q. (2012). Oxidative stress and liver disease. *Hepatology Research*, 42(8), 741-749.
- [44] Zuo, Z., Chen, S., Wu, T., Zhang, J., Su, Y., Chen, Y., & Wang, C. (2011). Tributyltin causes obesity and hepatic steatosis in male mice. *Environmental toxicology*, 26(1), 79-85.
- [45] Wu, B., Ootani, A., Iwakiri, R., Sakata, Y., Fujise, T., Amemori, S., & Fujimoto, K. (2006). T cell deficiency leads to liver carcinogenesis in Azoxymethane-treated rats. *Experimental Biology and Medicine*, 231(1), 91-98.