

# Hepatotoxicity Induced from Exposure of Male Rats to Low Doses of Tributyltin Chloride

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**ABSTRACT:** Tributyltin (TBT) is a contaminant found all over the world. It is a member of the organotin class, which includes several widely disseminated, very hazardous compounds that have been linked to endocrine disruption. Consumption of TBT-contaminated seafood and drinking water are the most common routes of exposure among the susceptible population to man at low doses. The main goal of this study is to recognize the hepatotoxicity of low doses of TBT chloride (10-2000 µg/Kg body weight) in male rats for 45 days. TBT significantly induce hepatic impairment of antioxidant systems, oxidative stress and lipid peroxidation. In conclusion: the TBT at low doses could induce hepatotoxicity at low levels due to its lipophilic properties and the ability for accumulation in tissues.

**Keywords:** Tributyltin chloride; Molecular studies; Hepatotoxicity; Oxidative stress, Drug metabolizing enzymes, Antioxidants.



## 1. INTRODUCTION

Tributyltin is a contaminant found all over the world. It is a member of the organotin class, which includes several widely disseminated, very hazardous compounds that have been linked to endocrine disruption (1). TBT's widespread usage as a biocide in antifouling coatings and as a fungicide on food crops has polluted the environment and the sea (2). Consumption of TBT-contaminated seafood, drinking water, skin contact, and inhalation are the most common routes of exposure among the susceptible population. Because of its lipophilic properties, TBT and its organotin metabolites (such as dibutyltin (DBT) and inorganic tin) accumulate in the tissues of numerous experimental animals, causing hepatic, neurological, and immune system diseases (3).

Animal studies have shown that organotin can have a variety of adverse effects on humans include primary immunosuppression, endocrinopathy, neurotoxicity, metabolic impacts, and effects on enzyme activity. The cardiovascular, upper respiratory, gastrointestinal, and reproductive/developmental systems may not be the only ones negatively impacted by exposure to organotin compounds (OTC). Further, OTC exposure has the potential to cause bioaccumulation and carcinogenicity (4). TBT, an organotin compound, is toxic to aquatic ecosystems and humans. However, the mechanisms underlying TBT-induced CNS toxicity, hepatotoxicity, and reproductive toxicity remain unknown, particularly in mammals and freshwater aquatic vertebrates (5). TBT causes an increase in adiposity in mice livers, causing a disorder in the hepatic structures and an increase in lipid droplet accumulation (6).

Furthermore, TBT's deleterious effects have been studied, but the mechanisms by which TBT induces toxicity remain unclear (5). The liver pivotally affects the metabolism and detoxification of xenobiotics. Damage to the liver can occur because of oxidative stress, which occurs when many stressors throw off the liver's oxidative/antioxidative imbalance. The different chemical metabolic pathways all contribute to produce free radicals, and antioxidants remove them, where enzymes perform critical role in the defensive antioxidant system (7). The liver has many immune cells that can help removes damaged tissues when it is exposed to environmental toxins (8).

At low doses, tributyltin causes extensive cell death and apoptosis in a wide range of cultured cell types. Additionally, it causes thymocytes to undergo apoptosis *in vivo*. These effects of TBT are thought to be mediated through the binding of TBT to various external "death receptors," as well as by increasing the intracellular concentration of calcium and disrupting mitochondrial activity. It has been shown that TBT therapy causes a dramatic elevation of intracellular Ca<sup>2+</sup>, which is normally quite low in

quiescent cells (9). Tributyltin can stimulate the endoplasmic reticulum stress and apoptosis in hepatocytes according to (10), who studied the *in vitro* and *in vivo* toxicity of TBT chloride (TBTC) and dibutyltin dichloride (DBTC) in rat's liver and guinea pigs and the results showed an influence of TBTC and DBTC on mitochondrial respiration and the hepatocytes accumulated more fat than usual.

Previous studies have documented the toxicity of high doses of tributyltin (TBT) on different tissues, while there is not enough data on the effects of the micro-low doses of TBT on the rat liver. Therefore, the objective of this study is to explore the TBT hepatotoxicity at wide range of low doses which relevant to the human's exposure at different levels; molecular, biochemical, and histological changes.

## 2. MATERIAL AND METHODS

### 2.1. Chemicals

Tributyltin chloride ( $C_{12}H_{27}ClSn$ ), 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 adult male Wistar rats 8-10 weeks of age and weighing  $180 \pm 5$  g were 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 ( $25 \pm 5$  °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  $\mu$ g/kg BW (1/10000 of  $LD_{50}$ ), group 3 was treated with TBT at 50  $\mu$ g/kg BW (1/5000 of  $LD_{50}$ ), group 4 was treated with TBT at 100  $\mu$ g/kg BW (1/1000 of  $LD_{50}$ ), group 5 was given TBT at 250  $\mu$ g/kg BW (1/400 of  $LD_{50}$ ), group 6 was given TBT at 500  $\mu$ g/kg BW (1/200 of  $LD_{50}$ ), group 7 was given TBT at 1000  $\mu$ g/kg BW (1/100 of  $LD_{50}$ ), and group 8 was given TBT at 2000  $\mu$ g/kg BW (1/50 of  $LD_{50}$ ). Animals were orally treated with respective doses every day for 45 days. Body weights of rats were recorded at the 1<sup>st</sup> and 45<sup>th</sup> 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 45<sup>th</sup> 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. Biochemical parameters

The activities of plasma and liver aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma glutamyl transaminases (GGT) and total bilirubin were determined, total protein and protein content in liver was assayed according to (11). All parameters using their respective kits purchased from (Biosystems S.A. Costa Brava 30, Barcelona, Spain).

## 2.5. Drug metabolizing enzymes

### 2.5.1. Assay of cytochrome b<sub>5</sub> in liver

Total hepatic content of the cytochrome b<sub>5</sub> was estimated by the method of (12). The hepatic fraction (0.5 mL) was diluted with (4.5 mL) 0.1 mol/L phosphate buffer (pH 7.4), then few crystals of sodium dithionate was added to the sample, after mixing the binding spectrum was recorded spectrophotometrically over wave lengths 400 to 500 nm, with base line subtraction. The concentration of reduced cytochrome b<sub>5</sub> was calculated using extinction coefficient of 185 cm<sup>-1</sup>mmol<sup>-1</sup>.

### 2.5.2. Assay of cytochrome P<sub>450</sub> (CYP<sub>450</sub>) in liver

Total hepatic content of CYP<sub>450</sub> was determined according to the method of (12). The hepatic fraction (0.5 mL) was suspended in (4.5 mL) 0.1 mol/L phosphate buffer (pH 7.4) and reduced by adding few crystals sodium dithionate to both cuvettes, then carbon monoxide was subsequently bubbled in the sample cuvette with the rate of 20-30 bubbles for 1 minute. The reduced CYP<sub>450</sub>-CO complex was then recorded spectrophotometrically at wave lengths 450 nm. The concentration of hemoprotein-CO complex was determined using extinction coefficient of 91 Cm<sup>-1</sup>mmol<sup>-1</sup>.

### 2.5.3. Liver homogenate oxidative stress markers and antioxidants

The reduced glutathione content (GSH) according to the method described by (13) and the activities of glutathione peroxidase (GPx) according to the method described by (14), glutathione reductase (GR) according to the method described by (15), glutathione S-transferase (GST) according to the method described by (16) and superoxide dismutase (SOD) according to the method described by (17) in liver tissue homogenate was measured using colorimetric kits (Biodiagnostic, Egypt) according to the manufacturer instructions and using specific standard for each parameter.

Catalase was determined according to the method described by (18) with some modifications. The test principle is based on dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), with the formation of blue perchromic acid as an unstable intermediate. Hydrogen peroxide concentration is directly proportional to the concentration of chromic acetate that produced from the reaction. The chromic acetate produced can be measured calorimetrically at 570 nm against the reagent blank.

## 2.6. Statistical analysis

Mean and standard error values were determined for all the parameters and the results were expressed as mean ± 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 (19).

## 3. RESULTS AND DISCUSSION

### 3.1. Changes in body and liver weights

Table (1) show the changes in body weight, body weight gain, liver weight, and relative liver weight of male rats treated with different doses (10, 50, 100, 250, 500,1000, and 2000 µg/kg bw) of tributyltin chloride (TBTC) daily for 45 days. The result showed no significant changes in the final body weight in the groups treated with TBTC at doses of 10 and 50 µg /kg body, while the rats treated with the higher doses of TBT showed significant decline in the final body weights compared to the control group. Body weight gain showed no significant change in the rats treated with 10 µg /kg of TBTC while the rats treated with the higher doses of TBT showed dose-dependent decline in the body weight gain compared to control group.

Regarding the liver weight, only the rats treated with the highest dose of TBT (2000 µg /kg) have significantly higher value compared with the control rats, while the relative liver weights showed significantly higher values in the rats treated with 100 µg /kg of TBTC and higher doses in a dose-dependent manner compared to the control rats.

The liver is known as a major site of accumulation of organotins and decreases in liver weights and histopathological lesions had been observed in study done by (20) where rats were exposed to dietary TBT oxide (320 mg/kg food) or to TBT acetate by oral gavage (3 or 10 mg/kg/day) from post-natal day 2 until 29 (21). Decreases in liver weights also may point towards toxic effects of TBTC on the immune system (22).

The obtained results are contradictory to the results reported by (23) that showed significant increase in body weight gain in rats treated with TBTC. Other study reported that rats lose weight with high levels of TBTC treatments (20). Also, in agreement with our study, previous studies showed reduced body gain after exposure to high-dose of TBTC (about 10 mg/kg) in rodents (24). (25) observed a decrease in relative weight and simultaneous increase in cell density. At the same time, a rise in cell death in these organs suggests TBTC toxicity which may be the cause of tissue weight

loss and tissue damage. Moreover, increased cell density could be due to body’s natural response to replace the damaged cells.

Differences in body weight between genders following exposure to TBTC had been observed in mammalian studies, where reduced body weight in female rats (26), dose-relevant alterations in body weight of male mice, or no alterations at all (27) have been reported. Rat exposed to 0.05 mg TBTC/kg gained much more weight than those exposed to 0.01 mg.

**Table (1):** Changes in body and liver weights of male rats treated with tributyltin chloride (TBTC)

| Groups                    | Con              | TB            | TB            | TB            | TB             | TB             | TB             | TB             |               |
|---------------------------|------------------|---------------|---------------|---------------|----------------|----------------|----------------|----------------|---------------|
|                           | trol             | TC            | TC            | TC            | TC             | TC             | TC             | TC             |               |
|                           |                  | 10            | 50            | 100           | 250            | 500            | 100            | 200            |               |
|                           |                  | µg            | µg            | µg            | µg             | µg             | 0 µg           | 0 µg           |               |
| Body weight               | Initial (g)      | 174. ± 1.75 a | 173. ± 1.28 a | 174. ± 1.29 a | 173. ± 1.29 a  | 174. ± 0.51 a  | 174. ± 0.51 a  | 174. ± 1.39 a  | 173. ± 0.81 a |
|                           | Final (g)        | 245. ± 6.89 a | 243. ± 9.30 a | 236. ± 1.16 a | 208. ± 6.24 b  | 205. ± 7.42 bc | 200. ± 1.58bc  | 185. ± 4.47 bc | 180. ± 1.63 c |
| Gain (g)                  |                  | 76.5 ± 2.55 a | 72.0 ± 2.23 a | 63.0 ± 1.10 b | 50.8 ± 8.145 c | 38.4 ± 1.44 d  | 27.5 ± 1.02 e  | 18.5 ± 1.02 f  | 8.60 ± 0.17 g |
|                           | Liver weight (g) | 5.83 ± 0.24 a | 6.20 ± 0.27 b | 6.44 ± 0.08 b | 6.30 ± 0.29 b  | 6.52 ± 0.13 b  | 6.64 ± 0.49 b  | 7.06 ± 0.17 ab | 8.06 ± 0.44 a |
| Relative liver weight (%) |                  | 2.53 ± 0.16 b | 2.57 ± 0.13 c | 2.73 ± 0.03 c | 3.05 ± 0.21 bc | 3.19 ± 0.13 bc | 3.32 ± 0.24 bc | 3.83 ± 0.14 ab | 4.47 ± 0.26 a |

The results expressed as Mean ± SE, n=5 rats each group

(a,b,c,d,e) Mean values with in column not sharing common superscript letters were significant different, p ≤ 0.05

### 3.2. Changes in liver functions

in the liver of Syrian hamsters; this may be due to an increase in free radical production or a reduction in the effectiveness of the body's defense mechanisms.

The significant increase in the activity of numerous enzymes suggests that stress induces tissue damage in fish (34). (5) reported that the increased AST and ALT Activity suggested that fish with liver injury from TBT stress had their energy metabolism altered. Toxic stress and elevated transaminase activity may result from exposure of fish to TBT because stress generally stimulates this pathway. In this regard, the results demonstrated that male rats treated with a wide range of TBTC (from 10 µg/kg B.W.) had a dose-dependent increase in liver enzymes (AST, ALT, LDH, and ALP). Long-term exposure to TBT will reduce lysozyme activity since TBT and its breakdown products are known potent membrane toxicants. Tables (2) show the changes in aspartate aminotransferase, (AST) alanine aminotransferase (ALT), bilirubin, albumin, alkaline phosphates (ALP) and Gamma glutamyl transaminases (GGT) of male rats treated with different doses TBTC daily for 45 days. The results indicated no significant changes in the levels of bilirubin and albumin in all treated groups compared with the control. The activities of AST and ALT showed significant elevation only in the rats treated with the highest dose used (2000 µg/Kg) compared with the control. The activity of ALP showed significant elevation in the rats treated with TBTC at doses of 1000 and 2000 µg/Kg compared with the control rats. The activities of GGT showed significant elevation in the rats treated with TBTC at dose 100 µg/Kg or higher doses compared with the control rats.

These results indicate mild hepatotoxic effects of TBTC especially at the high doses used while the low doses showed no significant effects. In agreement to our study, different butyltin chloride compounds (TBTC, and dibutyltin dichloride; DBTC) have hepatotoxicity on both mice and guinea pigs with a considerable rise in the activity of the ALT and AST enzymes using higher doses than the used in our study (360 µmol/Kg of TBTC equivalent to about 117 mg/Kg). These findings were associated with mitochondria swollen and collapse which suggested that the effects of TBTC and DBTC on liver mitochondria are likely pivotal in the development of hepatic fatty liver disease (28).

Dose-dependent TBTC-induced hepatotoxicity was detected as elevations in serum ALT and GGT activities in male and female rats at 90 and 60 days old, respectively. Despite the lack of microscopic liver lesions at the doses used in this investigation, hepatic functional abnormalities were seen in all cases, suggesting that they may contribute to the possible hepatotoxicity of TBTC (29).

(30) reported significant drop in serum albumin levels indicating the existence of liver necrosis or inflammation caused by interference with the albumin-synthesizing process in the liver which associated with histological and antioxidant changes, as well as shifts in bilirubin level, characterize the toxicity caused by TBT in the hamster. (31) showed that histopathological study of liver tissue from TBT-treated hamsters confirmed that these animals exhibited higher tissue damage. The toxin probably exacerbated tissue damage by interfering with liver functions. Increased total bilirubin in TBT-treated mice may have resulted from decreased liver absorption, increased conjugation, or enhanced bilirubin synthesis because of hemolysis (32). Detoxification of bilirubin, epimerization of galactose to glucose as uridine-5-phosphate derivative products, and synthesis of protein fractions are all disrupted by oxidative damage to the liver produced by lipid peroxidation and other hepatotoxic substances (albumin and prothrombin) (33).

Also, (30) found that serum bilirubin levels increased in 50, 100, and 150 mg/Kg TBT-treated hamsters, possibly indicating bile regurgitation because of liver damage. This research demonstrates that liver function was altered in hamsters after oral administration of TBT for 65 days and stated that TBT exposure causes free radical-mediated toxicity

**Table (2):** Changes in liver function of male rats treated with different doses TBTC for 45 days

| Groups               | Con<br>trol           | TB<br>TC<br>10<br>µg  | TB<br>TC<br>50<br>µg  | TB<br>TC<br>100<br>µg | TB<br>TC<br>250<br>µg  | TB<br>TC<br>500<br>µg | TB<br>TC<br>100<br>0 µg | TB<br>TC<br>200<br>0 µg |
|----------------------|-----------------------|-----------------------|-----------------------|-----------------------|------------------------|-----------------------|-------------------------|-------------------------|
| AST (U/L)            | 135.4<br>±5.7<br>4 b  | 134.2<br>±<br>8.66 b  | 136.0<br>±<br>7.53 b  | 145.0<br>±9.6<br>1 ab | 160.6<br>±11.<br>94 ab | 176.8<br>±6.2<br>1 ab | 171.6<br>±24.<br>06 ab  | 198.<br>±17.<br>01 a    |
| ALT (U/L)            | 49.80<br>±<br>3.40 b  | 56.40<br>±6.2<br>7 ab | 60.20<br>±8.0<br>6 ab | 62.60<br>±8.3<br>9 ab | 65.0<br>±4.2<br>1 ab   | 65.0<br>±4.2<br>1 ab  | 78.80<br>±0.3<br>4 ab   | 87.4<br>±6.3<br>3 a     |
| Bilirubin<br>(mg/dL) | 0.314<br>±0.0<br>26 a | 0.334<br>±0.0<br>09 a | 0.326<br>±0.0<br>19 a | 0.326<br>±0.0<br>28 a | 0.330<br>±0.0<br>20 a  | 0.326<br>±0.0<br>22 a | 0.322<br>±0.0<br>25 a   | 0.35<br>±0.0<br>25 a    |
| Albumin (g/dL)       | 4.13<br>±0.1<br>5 a   | 4.20<br>±0.3<br>1 a   | 4.06<br>±0.2<br>1 a   | 3.97<br>±0.1<br>4 a   | 3.92<br>±0.1<br>7 a    | 3.87<br>±0.1<br>2 a   | 3.85<br>±0.1<br>3 a     | 3.76<br>±0.1<br>8 a     |
| GGT (U/L)            | 135.4<br>±<br>5.74 b  | 134.2<br>±<br>8.66 b  | 136.0<br>±<br>7.53 b  | 145.0<br>±9.6<br>1 ab | 160.6<br>±11.<br>94 ab | 176.8<br>±6.2<br>1 ab | 171.6<br>±24.<br>06 ab  | 198.<br>±17.<br>01 a    |
| ALP (U/L)            | 49.80<br>±<br>3.40 b  | 56.40<br>±6.2<br>7 ab | 60.20<br>±8.0<br>6 ab | 62.60<br>±8.3<br>9 ab | 65.0<br>±4.2<br>1 ab   | 65.0<br>±4.2<br>1 ab  | 78.80<br>±10.<br>34 ab  | 87.4<br>±<br>6.33 a     |

The results expressed as Mean ± SE, n=5 rats each group

(a,b,c,d,e) Mean values with in column not sharing common superscript letters were significant different, p ≤ 0.05

### 3.3. Changes in cytochrome b<sub>5</sub> and cytochrome P<sub>450</sub> in rat's liver

Table (3) showed the changes in cytochrome b<sub>5</sub> and cytochrome P<sub>450</sub> in the liver of male rats treated with different doses of TBTC for 45 days. The results showed that cytochrome b<sub>5</sub> activity is significantly increased in the rat groups treated with TBTC at doses of 500 µg /kg body weight or higher while the rats treated with lower doses showed no significantly change in the activity of cytochrome b<sub>5</sub>. The hepatic activity of cytochrome P<sub>450</sub> showed no significant changes in the rats in all groups treated.

The liver is an intricate organ that serves as a hub for the body's metabolic processes in mammals. Furthermore, xenobiotics like TBT are mostly metabolized and stored in the liver (35). TBT appears to be transformed into inorganic

tin by hepatic cytochrome P<sub>450</sub>, and elevated serum tin levels indicate the presence of TBT in the body (36). In mammals, cytochrome P<sub>450</sub> enzyme systems dealkylate TBT chemicals into less toxic metabolites, the metabolites being generally less hazardous than the parent compounds (37).

It was documented that; TBT causes a decrease in liver cytochrome P<sub>450</sub> content at concentrations of 20 µg/Kg or above. Since cytochrome P<sub>450</sub> is a major component of the mixed function oxygenase system, TBT's effects on fish hepatic cytochrome P<sub>450</sub> concentration may result in a delay in the oxidative process of other organic contaminants (38). Also, (39) found that exposing olive flounder to TBT decreased the hepatic cytochrome P<sub>450</sub> content and suggested that TBT could be an antagonist in promoting mixed-function oxygenase in fish.

Cytochrome P<sub>450</sub>s are participate in the metabolism and activation of xenobiotics changes in their expression that have important toxicological effects (40). Some previous research had described the presence and xenobiotic stimulation of CYP<sub>450</sub>s in the liver of fish, Although research on xenobiotic induced CYP<sub>450</sub>s in other organs is limited; Experiments showed that TBT may inhibit ethoxyresorufin-O-deethylase (EROD) activity in freshwater fish by interacting with microsomal CYP<sub>450</sub> both *in vitro* and *in vivo* (41). Absolute values of EROD activity in the liver, gills, and muscles of common carp showed that the liver has the greatest levels. Because of its enormous metabolic capacity, the liver is thought to play a crucial role in the body's detoxification of xenobiotics and the processing of metabolic products before their eventual destruction (42).

**Table (3):** Changes in cytochrome b<sub>5</sub> and cytochrome P<sub>450</sub> in the liver of male rats treated with tributyltin chloride

| Groups  | Co                        | TB                        |                           | TB                         |                            | TB                         |                           | TB                        |    |
|---|---------------------------|---------------------------|---------------------------|----------------------------|----------------------------|----------------------------|---------------------------|---------------------------|----|
|   | ntrol                     | TC                        | TC                        | TC                         | TC                         | TC                         | TC                        | TC                        | TC |
|   |                           | 10                        | 50                        | 100                        | 250                        | 500                        | 100                       | 200                       |    |
|   |                           | µg                        | µg                        | µg                         | µg                         | µg                         | 0 µg                      | 0 µg                      |    |
| Cytochrom b <sub>5</sub><br>(n mole cytochrome /<br>mg protein)   | 1.95<br>±0.0<br>6<br>72 b | 1.96<br>±0.1<br>6<br>03 b | 1.93<br>±0.1<br>0<br>06 b | 2.12<br>±0.0<br>4<br>62 ab | 2.32<br>±0.1<br>8<br>27 ab | 2.35<br>±0.0<br>8<br>83 ab | 2.44<br>±0.1<br>6<br>00 a | 2.47<br>±0.1<br>0<br>15 a |    |
|   |                           |                           |                           |                            |                            |                            |                           |                           |    |
| Cytochrom p <sub>450</sub><br>(n mole cytochrome /<br>mg protein) | 2.81<br>±0.2<br>4<br>65 a | 2.41<br>±0.0<br>2<br>84 a | 2.45<br>±0.1<br>2<br>61 a | 2.43<br>±0.1<br>4<br>39 a  | 2.61<br>±0.1<br>4<br>13 a  | 2.68<br>±0.0<br>8<br>49 a  | 2.29<br>±0.1<br>4<br>29 a | 2.25<br>±0.1<br>6<br>48 a |    |
|   |                           |                           |                           |                            |                            |                            |                           |                           |    |

The results expressed as Mean ± SE, n=5

tributyltin chloride (TBTC) administrated successive doses of 10, 50 and 100 µg/kg bw

(a,b,c,d,e) Mean values with in column not sharing common superscript letters were significant different, p ≤ 0.05

### 3.4. Changes in antioxidants in the liver tissues

Table (4) show the changes in the activities of glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), and the levels of reduced glutathione (GSH) and expression of nuclear factor erythroid 2-related factor 2 (NRF2) in the liver of male rats treated with different doses of TBTC daily for 45 days. The results showed that the GR is the most sensitive antioxidant enzyme for TBTC as it significantly inhibited at the dose ≥ 50 µg/Kg, followed by GPx which significantly inhibited by TBTC at dose ≥ 500 µg/Kg, then activity of SOD which significantly declined by TBTC at the highest doses used (1000 and 2000 µg/Kg) compared with the control rats. The activity of catalase showed no significant changes at all TBTC doses used. On the other hand, the level of GSH showed significant decline at the highest doses used compared with the control rats. The hepatic expression of NRF2 showed biphasic pattern of change as it was increased in the groups treated with lowest dose (10 µg/kg) of TBTC and remain unchanged in the rats treated with doses of 50, and 100 µg/kg, then its expression significantly declined in the rats treated with TBTC at doses ≥ 250 µg/kg compared with the control group.

TBT's toxicity occurs mainly through oxidative damage. There is evidence that pollutants like TBT can cause organ damage by generating reactive oxygen species (ROS) (43). Organotin chemicals may be directly interacting with sulfhydryl group (-SH) in proteins and glutathione leading to a decrease in the antioxidant capacity of the blood. Inhibition of certain activities of antioxidant enzyme may potentially contribute to the stimulation of lipid peroxidation and the disruption of the antioxidant defense system.

Antioxidant enzymes are recognized as the primary defense system against ROS production and oxidative damage to macromolecules. Systemic antioxidants, such as superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), and glutathione peroxidase (GPx) are synthesized by the liver (44). GPx is a component of the primary protective enzyme system, which efficiently eliminates free radicals and protects cells from oxidative damage. In line with the current study, the zebrafish treatment with high concentrations of TBT lead to a reduction in CAT, SOD, and GPx activities suggesting a lack of defense against cellular superoxide toxicity (45). Also, (46) reported that after exposure of fish to TBT (100mg/kg), the GSH level, and GPx, CAT, and SOD activities dropped.

(47) agrees to our study when he found a general decline in antioxidant enzyme levels in liver tissues. (48) reported the TBT's ability to react with cellular macromolecules like lipids, proteins, and DNA may produce mitochondrial damage, lowering antioxidant enzymes activity, which is concerning given that the Nrf2-antioxidant system has been identified as a primary molecular target against oxidative stress. Also, TBT has been found to inhibit Nrf2 expression in rats in a direct fashion.

(49) after treating abalone with TBT, the results showed decreasing in ACP and AKP activity in the liver of abalone. Studies for *Sebastiscus marmoratus* treated for 4 days with 9.6 and 19.3 or 193 µg TBT/kg, indicated a notable rise in hepatic CAT and SOD activities (50).

(51) found that the hamster treated with different doses of TBTC (50, 100, and 150 ppm/kg/day for 65 days showed oxidative stress-induced damage to liver and significant decline in the enzymatic antioxidants; CAT, SOD, GSH, GPx and the non-enzymatic antioxidants, vitamin C and vitamin E levels in a dose-dependent manner.

Nuclear factor erythroid 2-related factor-2 (NRF2) is the master transcription factor that regulate the transcription of the antioxidants. Under the oxidative stress Nrf2 translocate into the nucleus where it binds to antioxidant response elements (ARE), activates ARE-regulated gene expression of phase II detoxifying enzymes and antioxidant enzymes, and enhances cell resistance to oxidative stress and nucleophilic compounds (52). The observed induction of NRF2 in rats treated with TBTC at low doses (50-100 µg/kg) may indicate as adaptive mechanism that counteract the oxidative stress induced by low TBTC doses by maintaining the level of antioxidant system at normal level, while at the higher doses (≥250 µg/kg) TBTC significantly and markedly suppressed the expression of NRF2 that explain the decreased activities of the antioxidant enzymes (GR, GPx, and SOD) and decline in GSH in the rats subjected to high doses of TBTC.

**Table (4):** Changes in antioxidants in the liver of male rats treated with tributyltin chloride

| Groups  | Con                   | TB                    | TB                     | TB                     | TB                    | TB                    | TB                    | TB                   | TB   |
|---|-----------------------|-----------------------|------------------------|------------------------|-----------------------|-----------------------|-----------------------|----------------------|------|
|   | trol                  | TC                    | TC                     | TC                     | TC                    | TC                    | TC                    | TC                   | TC   |
|   |                       | 10                    | 50                     | 100                    | 250                   | 500                   | 100                   | 200                  |      |
|   |                       | µg                    | µg                     | µg                     | µg                    | µg                    | 0 µg                  | 0 µg                 |      |
| <b>GR</b><br>(U/mg protein)                       | 15.38<br>±1.5<br>0 a  | 11.28<br>±1.4<br>7 ab | 8.62<br>±0.8<br>8 b    | 7.62<br>±0.6<br>1 b    | 7.92<br>±1.0<br>5 b   | 7.86<br>±0.5<br>9 b   | 8.30<br>±1.0<br>6 b   | 6.80<br>±0.8<br>2 b  | 18.5 |
| <b>GPx</b><br>(U/mg protein)                      | 29.80<br>±1.7<br>3 ab | 34.74<br>±4.3<br>4 a  | 23.40<br>±4.8<br>5 ab  | 21.14<br>±3.6<br>8 ab  | 19.56<br>±2.0<br>1 b  | 19.14<br>±2.5<br>1 b  | 17.14<br>±2.2<br>8 b  | 17.14<br>±2.2<br>0 b | ±3.6 |
| <b>CAT</b><br>(U/mg protein)                      | 32.34<br>±2.9<br>4 a  | 29.30<br>±0.8<br>1 a  | 29.02<br>±1.2<br>2 a   | 28.80<br>±0.5<br>4 a   | 28.54<br>±2.1<br>4 a  | 28.06<br>±2.0<br>4 a  | 25.34<br>±1.3<br>4 a  | 25.34<br>±1.3<br>7 a | ±1.7 |
| <b>SOD</b><br>(U/mg protein)                      | 185.2<br>±15.<br>01 a | 162.3<br>±8.3<br>2 ab | 167.4<br>±5.1<br>5 ab  | 153.5<br>±6.8<br>4 ab  | 155.5<br>±8.2<br>3 ab | 151.2<br>±5.6<br>6 ab | 138.1<br>±5.2<br>9 b  | 138.1<br>±5.2<br>b   | 8.36 |
| <b>GSH</b><br>(mg/g tissues)                      | 17.06<br>±0.8<br>3 a  | 16.0<br>±1.1<br>5 a   | 15.36<br>±2.2<br>7 a   | 14.77<br>±1.1<br>0 a   | 14.08<br>±1.4<br>0 a  | 13.39<br>±1.4<br>0 a  | 12.59<br>±1.4<br>2 a  | 12.59<br>±1.4<br>4 a | ±1.1 |
| <b>NRF2</b><br><b>Expression</b><br>(Fold Change) | 1.020<br>±0.0<br>86 b | 1.420<br>±0.1<br>24 a | 1.300<br>±0.0<br>55 ab | 1.280<br>±0.0<br>37 ab | 0.680<br>±0.0<br>58 c | 0.620<br>±0.0<br>66 c | 0.520<br>±0.0<br>20 c | 0.520<br>±0.0<br>c   | ±0.0 |

The results expressed as Mean ± SE, n=5

tributyltin chloride (TBTC) administrated successive doses of 10, 50 and 100 µg/kg bw

(a,b,c,d,e) Mean values with in column not sharing common superscript letters were significant different, p ≤ 0.05

GSH = Glutathione, GR = Glutathione Reductase, GPx = Glutathione peroxidase, SOD = Superoxide dismutase, CAT= Catalase, NRF2= Nuclear factor erythroid 2-related factor-2

## CONCLUSION

Considering the obtained results, we can conclude that TBT can induce hepatotoxicity in a dose-dependent manner, hepatic impairment of antioxidant systems, oxidative stress, lipid peroxidation. In conclusion: the TBT at low doses, and could induce hepatotoxicity at low levels due to its lipophilic properties and the ability for accumulation in tissues.

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