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*Original Research Article*

# Comparative Effect of the Toxicity of 4-*Tert*-Octylphenol on Male and Female Wistar Rats

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**Abstract**

**4-tert-Octylphenol (4-tOP), an endocrine disruptor used in industrial and domestic products, has been detected in environmental matrices and is linked to the rising incidence of non-communicable diseases and aquatic ecosystem impairments. This study investigated the gender-specific effects of 4-tOP toxicity in Wistar rats. Forty Wistar rats (20 male, 20 female) were divided into four groups, including a control group. They were administered intraperitoneal doses of 0.62, 0.41, and 0.2 mg 4-tOP/kg body weight, dissolved in DMSO. The treatment was repeated after 36 hours, and after 72 hours, urine, blood, kidney, and liver samples were collected for biochemical and histological analyses. Enzymatic activities and oxidative stress markers, including trehalase, creatinine, AST, ALT, SOD, GSH, and MDA, were assessed using spectrophotometry. Trehalase and creatinine levels in urine significantly increased in the higher-dose groups compared to controls. Serum AST and ALT activities, along with quinine oxidase, were also elevated in treated groups. Conversely, renal and hepatic SOD activity and GSH levels were significantly reduced, while MDA levels increased, indicating oxidative stress. Females exhibited heightened sensitivity to renal and hepatic toxicity compared to males. Female rats were more susceptible to 4-tOP-induced renal and hepatotoxicity, likely driven by oxidative stress. This highlights the urgent need for stricter regulations on 4-tOP usage and the promotion of safer alternatives to mitigate its health and environmental impact.**

**Keywords:** 4-tert-Octylphenol, Endocrine disruptor, Gender differences, Hepatotoxicity, Oxidative stress, Renal toxicity

## INTRODUCTION

4-tert-octylphenol (4-tOP) is a component of non-ionic surfactants alkyl phenol polyethoxylates present in personal care products. Reports of detectable levels of 4-tert-octylphenol in the environmental matrix and in biologic fluids such as blood are in legion (Jang and Jeong 2024; Olaniyan *et al.,* 2020). The compound is an environmental toxicant following its demonstrated endocrine disruption (Jang and Jeong 2024). Data are accumulating linking environmental factors via endocrine disrupting molecules to the increase in metabolic disease (Heindel et al., 2017). Cells such as β-cells which express oestrogen receptors have been affected by 4-tOP (Maciuszek et al., 2020) probably confirming its oestrogenic or possibly anti-oestrogenic activity. Study suggested that exposure of mice to low doses (0.01-1µg/kg/day) of 4-tOP for 7 days interferes with nucleic acid and amino acid metabolism in the liver, resulting in hepatotoxicity (Zhou et al, 2018). According to the report of Sukuroglu *et al.* (2022), the increased urinary concentration of creatinine in the studied human population exposed to 4-tOP could be linked to renal imbalance. However, to the best of our knowledge none of the reported toxicities have been related to gender. The present work has therefore decided to fill this void.

## MATERIALS AND METHODS

### Experimental Animals

**A**dult male and female Wistar rats weighing 196-200g were used for this study which was procured from Ladoke Akintola University of Technology animal house. The animals' acclimatization took two weeks before the experiments.

### Test substance

4-*tert*-Octylphenol {4-(1, 1, 3, 3- tetramethylbutyl) phenol} CAS No. 140-66-9 was a product from Sigma-Aldrich. Other reagents are of analytical grade which were supplied by local suppliers.

### Dosing and Treatment

There were three dose levels that were exposed to the rats intraperitoneally as shown in Table 1. These doses represent 10% of the in vitro doses that were applied to the rat insulinoma cells (Olaniyan *et al.,* 2018). Dimethylsuphoxide (DMSO) was the vehicle of administration in a total volume of 1 ml.

### Experimental Design

Each dose was given singly and was repeated once after 36 hours of first administration.

### Animal Sacrifice and Sample Collections

Following the administration of the last treatment dose, rats were fasted overnight and urine samples were collected individually from the rats in dedicated metabolic cages. Rats sacrifice took place by cervical dislocation. Blood samples were collected via cardiac puncture into sample plain bottles for serum preparation. The serum was separated from the clot by low-speed centrifugation in cooling conditions. Kidneys and liver harvested for biochemical assays and some portions were stored in 10% formalin for histopathological analysis. The organs were washed in ice cold 1.15% KCl and were homogenized with ice-cold 5ml 0.25 M sucrose solution in Tris.HCl buffer (40mM Tris.HCl, pH 7.4) using teflon-lined homogenizer. The homogenates were centrifuged at 2500 rpm for 10 minutes to remove nuclear fraction and cell debris. The supernatant obtained was centrifuged at 10000 rpm for 10 minutes in other to obtain the mitochondrial fraction. Mitochondrial pellets obtained after supernatant had been discarded were re-suspended in 5ml sucrose Tris buffer.

### Biochemical Assay

The activity of urine trehalase was evaluated to assess renal functions following the method of Sasai-Takedatsu et al., (1995), concentration of creatinine (Tietz 1995) and urea was evaluated to assess liver and kidney functions. The activities of quinine oxidase (Balazs *et al.,* 1961), aspartate aminotransferase (AST), alanine aminotransferase (ALT) were evaluated to assess kidney and liver functions. Commercially available kits (Randox) were used according to the manufacturer's instructions.

### Estimation of Redox Status

Superoxide dismutase (SOD) activity ((Misra and Fridovich (1972), reduced glutathione concentration (Anderson 1997) and malondialdehyde (MDA) level (Ohkawa et al., 1979) were determined to assess the antioxidant status in the kidneys and liver tissues.

### Statistical Analysis

Data were analyzed using GraphPad Prism 5.0 for one-way Analysis of Variance (ANOVA) at a significance level of $p < 0.05$.

## RESULTS

The total body weight after the entire doses were administered did not result in a significant change of the treated rats as compared with the body weight of control group (Table 2). As shown in (Figure 1); 0.41mg of 4-tOP/Kg and 0.62mg of 4-tOP/Kg in male and 0.62mg of 4-tOP/Kg female revealed a highly significant increase *(P<0.0001)* in urine trehalase activity as compared with the corresponding control. 0.2 mg of 4-tOP/Kg and 0.62mg of 4-tOP/Kg in male revealed a highly significant increase *(P<0.0001)* and 0.62mg of 4-tOP/Kg female revealed a significant increase *(P<0.003)* in serum urea level during the treatment period and as compared with the corresponding control (Figure 3). Urine creatinine, the 0.41mg of 4-tOP/Kg and 0.62mg of 4-tOP/Kg in male and 0.2mg of 4-tOP/Kg and 0.62mg of 4-tOP/Kg female showed significant increase *(P<0.0001)* as compared with the corresponding control in their levels throughout the treatment period (Figure 2). These biochemical results of trehalase, creatinine and urea was confirmed by the changes observed in kidney renal tubules and glomerular tufts as described in histological assay (Figures 13a - 14b).

Figure 4-6 illustrate the effect of the three tested doses of 4-tOP on AST, ALT and quinine oxidase. The regimens used of 4-tOP at 0.62mg of 4-tOP/Kg showed

**Table 1.** Animals grouping

| | Animal groups (n = 5) | Treatment |
|---|---|---|
| A | Control Male | 1ml of DMSO |
| B | Control Female | 1ml of DMSO |
| C | Male | 0.62mg of 4-tOP/Kg body weight (High) |
| D | Female | 0.62mg of 4-tOP/Kg body weight |
| E | Male | 0.41mg of 4-tOP/Kg body weight (Medium) |
| F | Female | 0.41mg of 4-tOP/Kg body weight |
| G | Male | 0.2mg of 4-tOP/Kg body weight (Low) |
| H | Female | 0.2mg of 4-tOP/Kg body weight |

**Table 2.** Body Weight Change (n=5) (Mean ± S.D) of the Experimental Animals (– = weight loss)

| **Group** | **Body weight change (Mean ± SD)** |
|---|---|
| A | -1.52 ± 0.14 |
| B | -2.46 ± 1.41 |
| C | -3.07 ± 2.98 |
| D | -5.13 ± 1.99 |
| E | -2.11 ± 0.71 |
| F | -3.14 ± 2.03 |
| G | -2.06 ± 1.20 |
| H | -3.02 ± 2.13 |

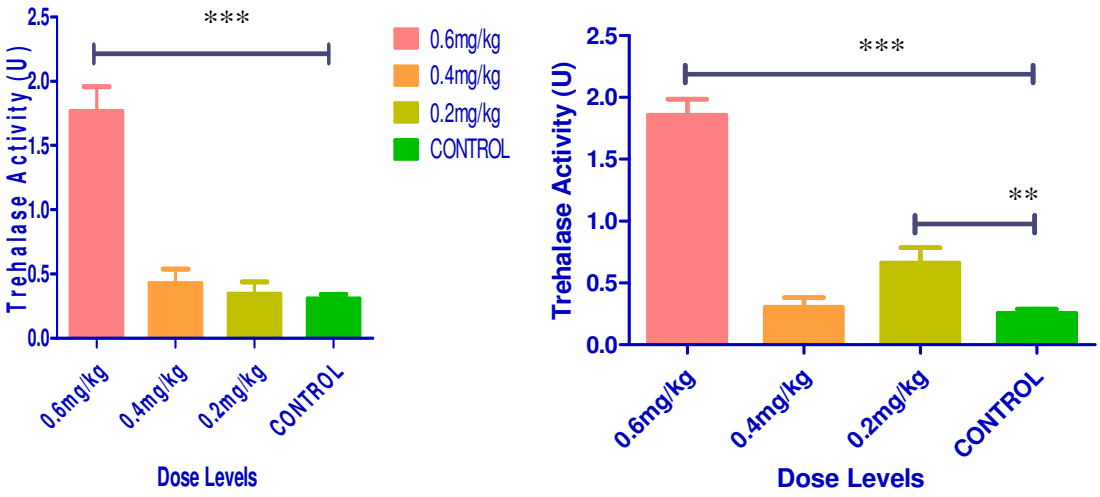


**Figure 1.** A bar histogram showing the effect of administration of high, medium and low doses of 4-tOP on the kidney function in male and female Wistar rats.

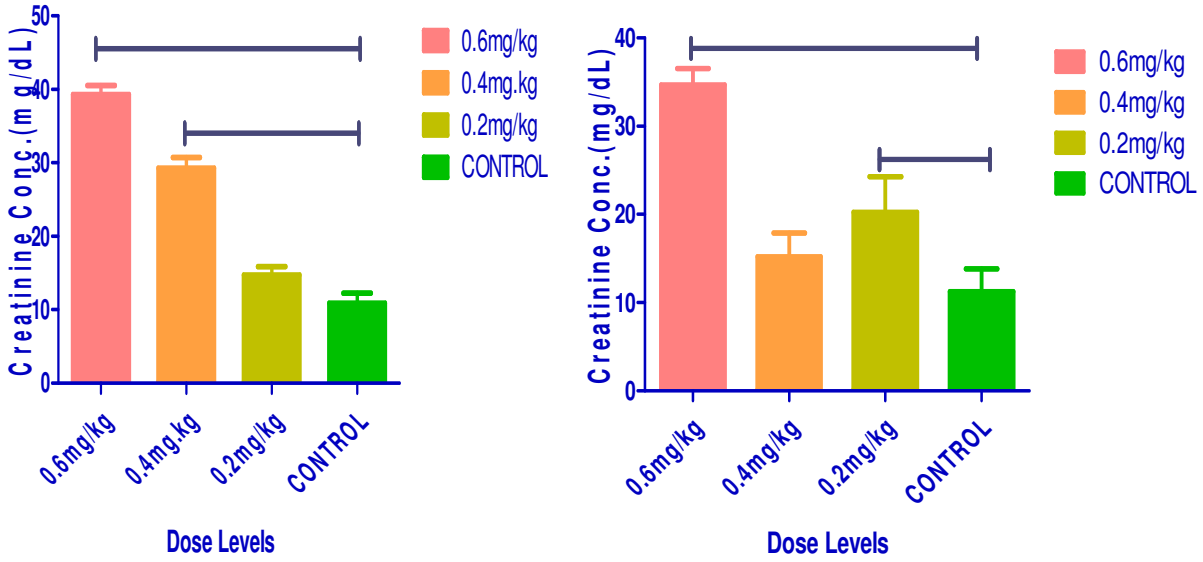


**Figure 2.** A bar histogram showing the effect of administration of high, medium and low doses of 4-tOP on the kidney function in male and female Wistar rats.

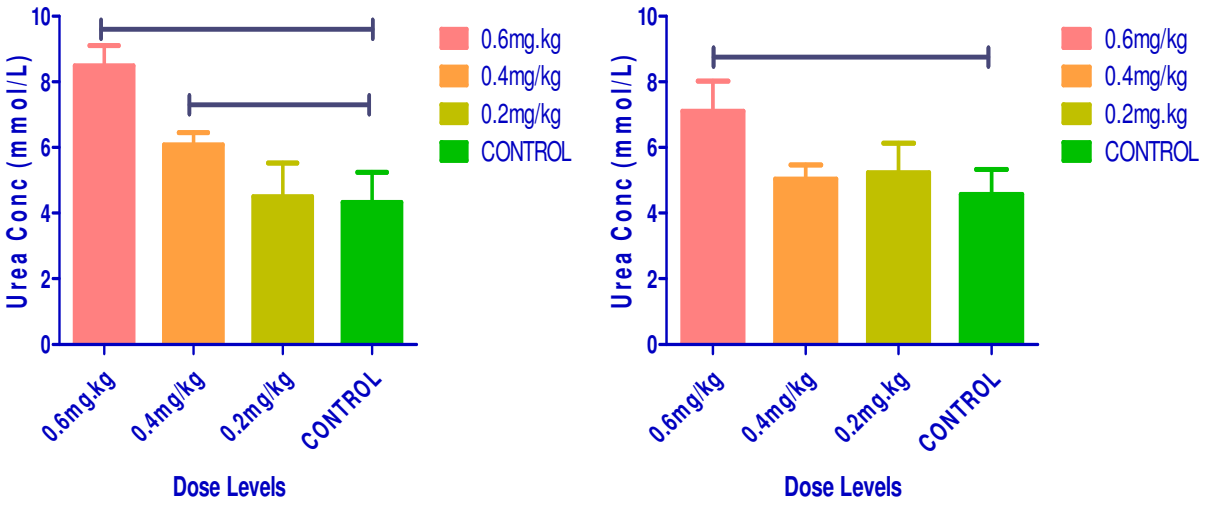


**Figure 3.** A bar histogram showing the effect of administration of high, medium and low doses of 4-tOP on the kidney function in male and female Wistar rats.

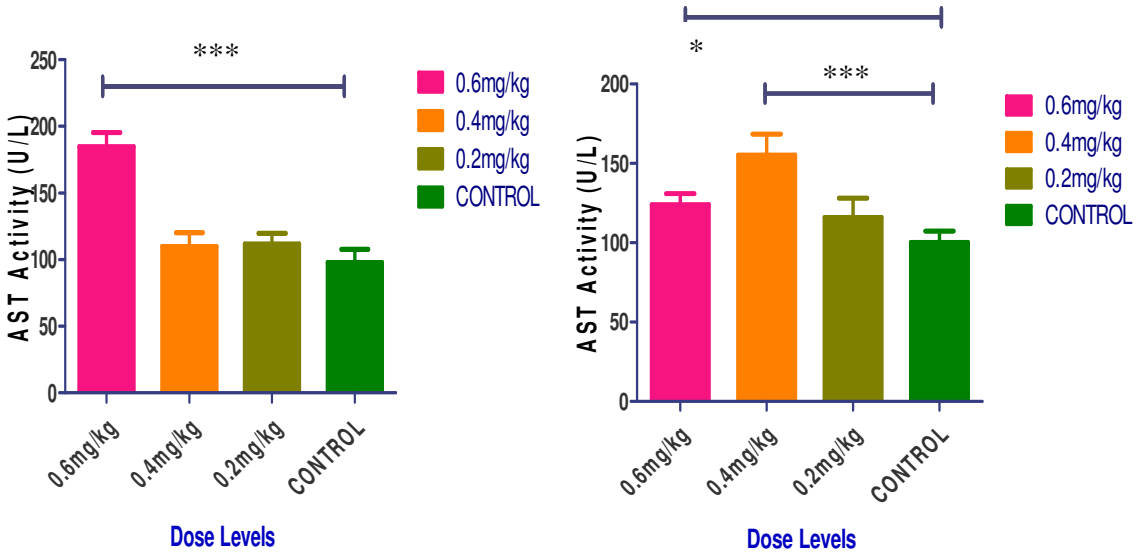


**Figure 4.** A bar histogram showing the effect of administration of high, medium and low doses of 4-tOP on the kidney function in male and female Wistar rats.

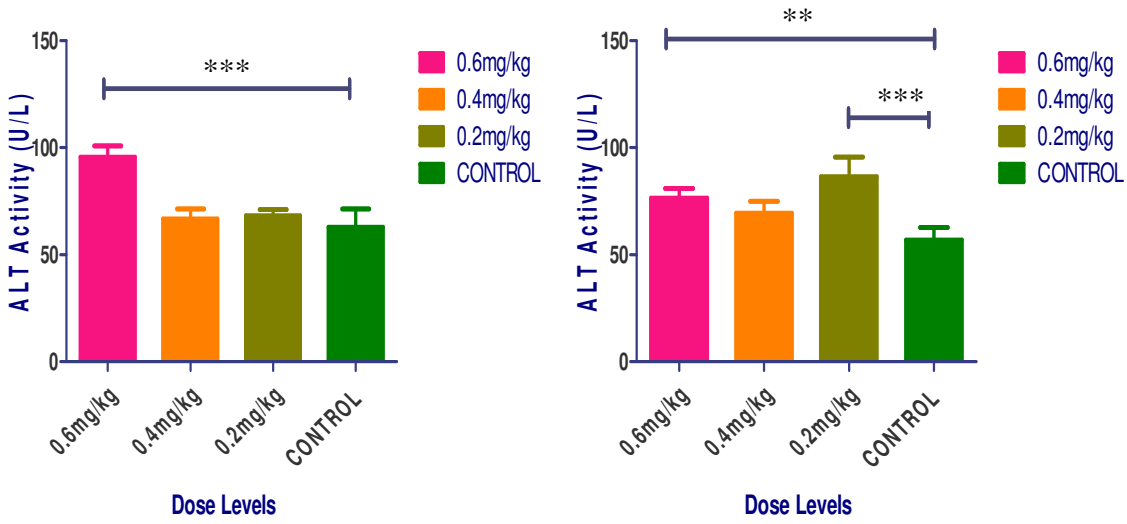


**Figure 5.** A bar histogram showing the effect of administration of high, medium and low doses of 4-tOP on the liver function in male and female Wistar rats.

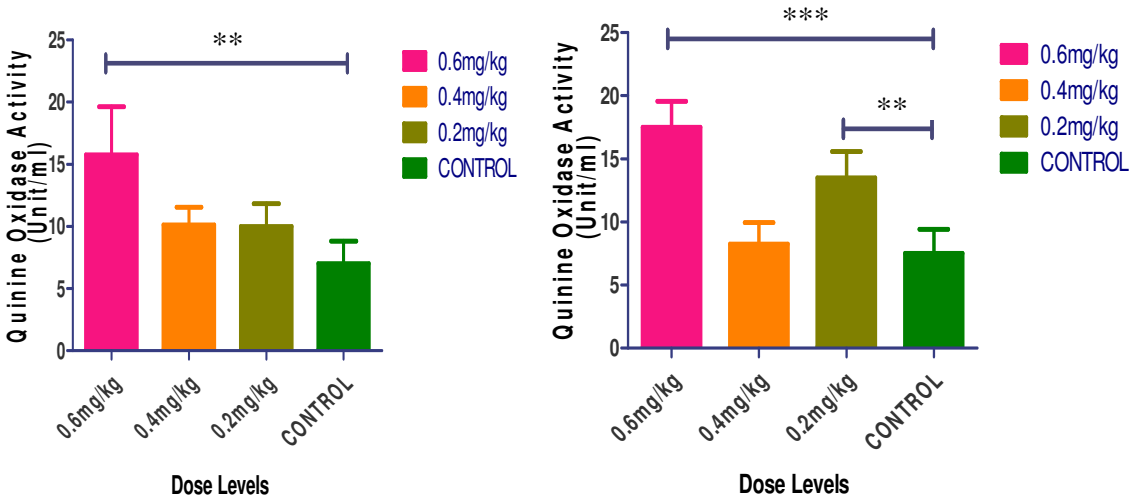


**Figure 6.** A bar histogram showing the effect of administration of high, medium and low doses of 4-tOP on the liver function in male and female Wistar rats.

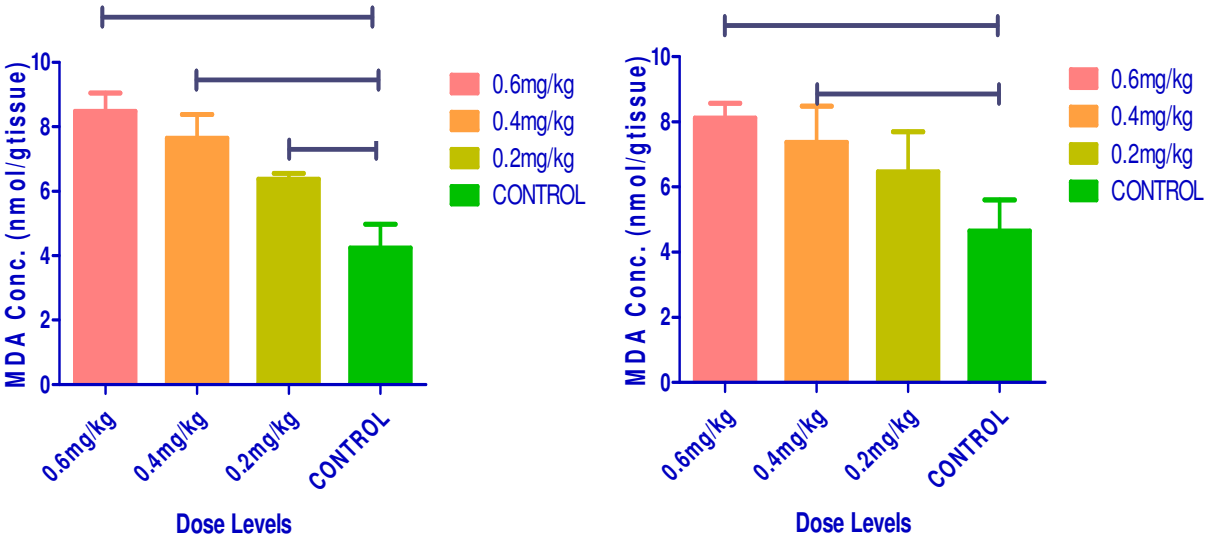


**Figure 7.** A bar histogram showing the effect of administration of high, medium and low doses of 4-tOP on the kidney function in male and female Wistar rats.

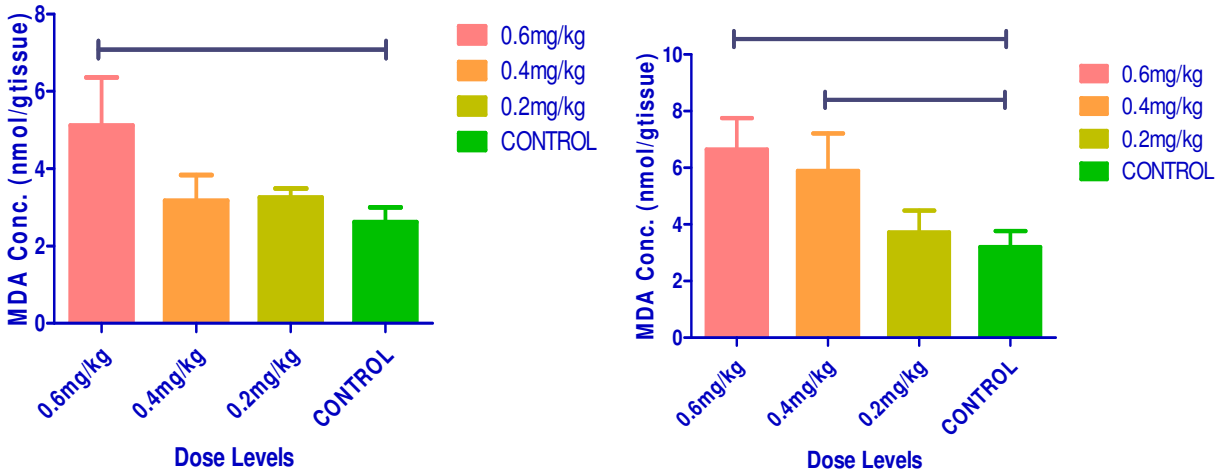


**Figure 8.** A bar histogram showing the effect of administration of high, medium and low doses of 4-tOP on the liver function in male and female Wistar rats.

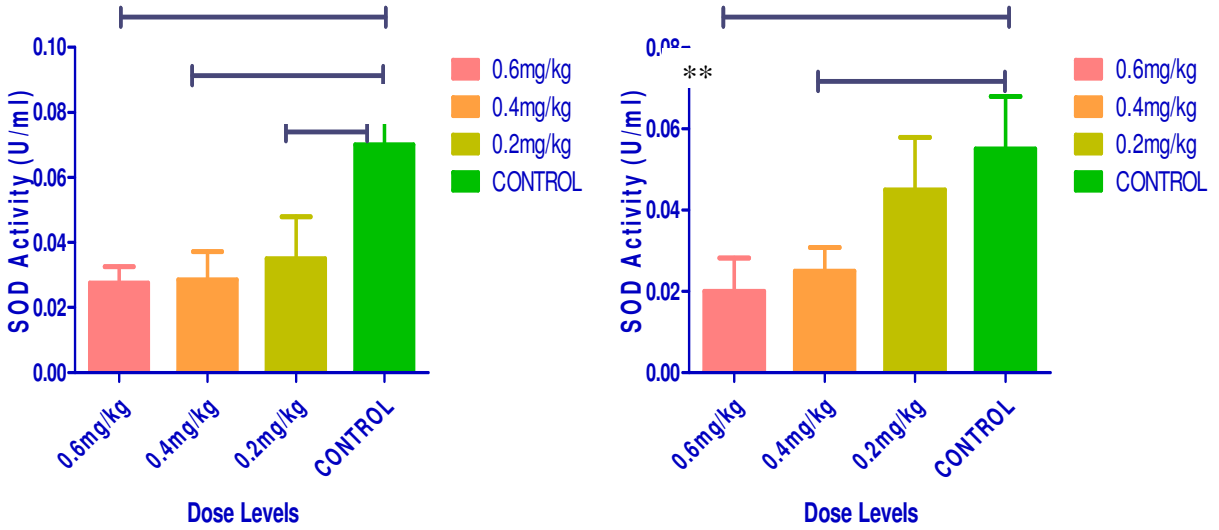


**Figure 9.** A bar histogram showing the effect of administration of high, medium and low doses of 4-tOP on the kidney function in male and female Wistar rats.

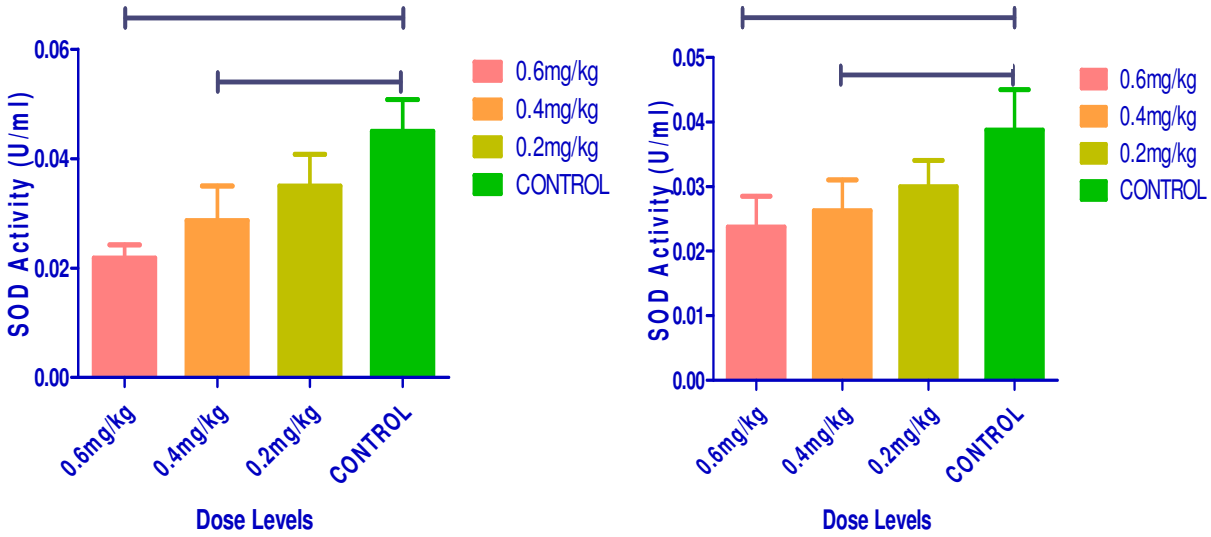


**Figure 10.** A bar histogram showing the effect of administration of high, medium and low doses of 4-tOP on the liver function in male and female Wistar rats.

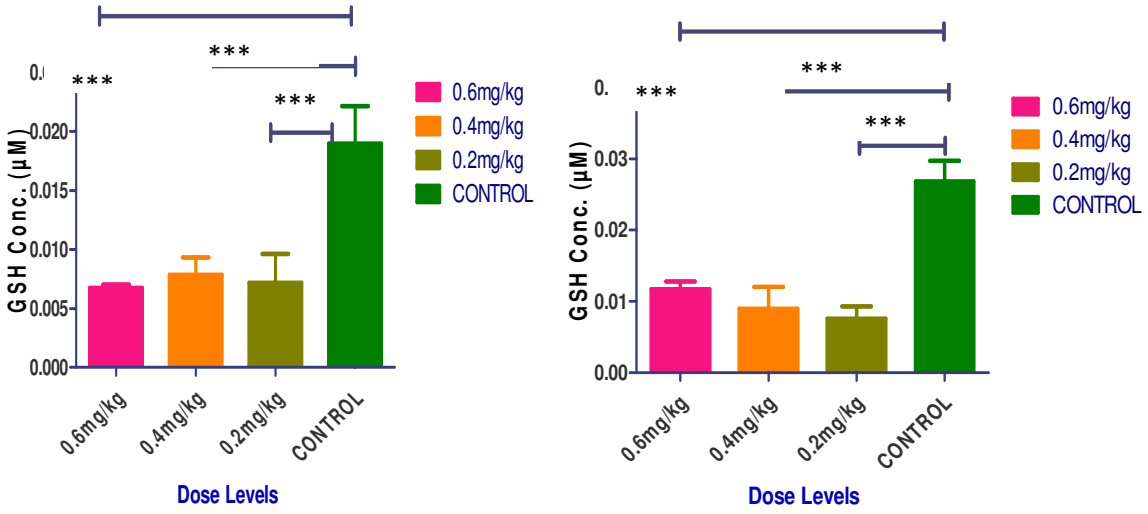


**Figure 11.** A bar histogram showing the effect of administration of high, medium and low doses of 4-tOP on the kidney function in male and female Wistar rats.

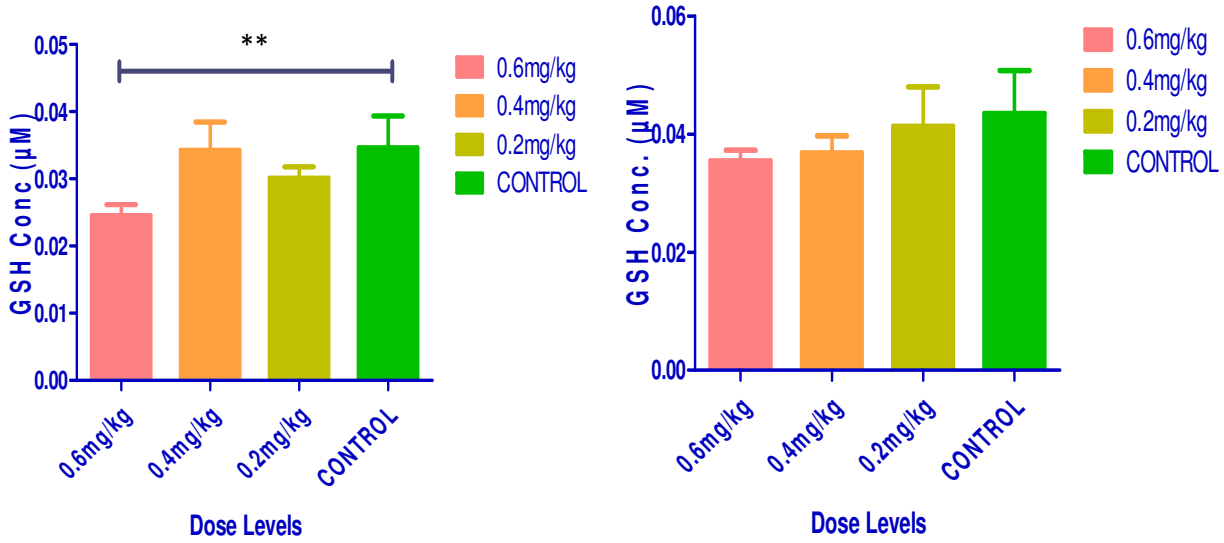


**Figure 12.** A bar histogram showing the effect of administration of high, medium and low doses of 4-tOP on the liver function in male and female Wistar rats.

highly significant increase *(P<0.0001)* in male, for female at 0.41mg of 4-tOP/Kg and 0.62 mg of 4-tOP/Kg it show highly significant increase (*P<0.0001*) AST activity as compared with the corresponding control. Similarly, the high dose of 4-tOP resulted in highly significant elevation in the male ALT (*P<0.0001*) while female 0.62 and 0.2 mg of 4-tOP/Kg show a highly significant difference when compared to control. Quinine oxidase shows a significant increase in both gender, male at 0.62mg of 4-tOP/Kg and female 0.62 and 0.2 mg of 4-tOP/Kg

Concerning the tissue perioxdation product (MAD) (Figures 7-8) and antioxidant defense system GSH; at all dose level there was a highly significant increase in treated group as compared to control in kidney, while in liver GSH only 0.41 group male shows a significant increase. For SOD (Figure 9-10), the 0.2mg of 4-tOP/Kg, 0.41mg of 4-tOP/Kg and 0.62mg of 4-tOP/Kg in kidney induced highly significant *(P<0.0001)* elevation but for female only the 0.41mg of 4-tOP/Kg and 0.62 mg of 4-tOP/Kg was significantly *(P<0.0018)* elevated, at 0.62 mg of 4-tOP/Kg in male liver there was a significant *(P<0.0024)* increase, for female there was a significant increase at 0.62 and 0.41 mg of 4-tOP/Kg in the level of the oxidative stress marker MAD as compared with control. On the other hand as a consequence of 4-tOP treatment; the generation of the antioxidant and free radical in kidney and liver tissue was affected and reduced significantly as compared with control. In all treatment group in kidney tissue, male showed a highly significant *(P<0.0001)* decrease, female (high dose) as well decreased significantly *(P<0.0013)* in SOD activity. SOD activity decreased significantly in male *(P<0.0003)* and female *(P<0.0064)* (high and medium dose) treatment group.

## DISCUSSION

Many phenolic xenoestrogens as 4-tert-octylphenol (4-tOP), have been found to simulate estrogenic effects which harmfully affect the health of animals and human (Tran *et al.,* 2020). Gender has been known to play an important role in drug metabolism; emphasizing the real reason why the present project was embarked upon. More so, gender-linked metabolism of 4-tOP has not been intensively studied. Therefore, this study investigated the gender effects of 4-tOP in the exposed Wistar rats.

Trehalase an enzyme which hydrolyzes the disaccharide trehalose, yielding glucose (Shrestha *et al.,* 2024). It is expressed in the intestine and kidney and act as marker of renal tubular damage in kidney. Trehalase, a lysosomal enzyme located in the brush border membrane of renal proximal tubules is a recommended biomarker of nephropathy (Liu *et al.,* 2021). Elevated levels of trehalase in the urine of female rats than in male rats may be an indication of gender sensitivity to renal toxicity by 4-tOP exposure.

Creatinine is a byproduct of muscle movement that arises from the breakdown of creatine, a substance synthesized in the liver and delivered to the muscles to support muscle activity (Prabhu *et al.,* 2022). Elevated levels of creatinine in the urine typically signify compromised kidney function, as the kidneys are responsible for eliminating creatinine from the bloodstream (Lin 2023). Conversely, diminished levels of creatinine in the blood may be attributed to impaired liver function or decreased muscle mass, as reduced muscle mass naturally results in lower creatinine production (Casciola *et al.,* 2023). The female rats was observed to

be more sensitive to 4-tOP, as female were sensitive to the drug even at the lowest dose (Figure 2). Urea is a nitrogenous waste and byproduct of the breakdown of proteins and amino acids; it is excreted from the body through the urine. It is a biomarker of nephrotoxic profile (Griffin *et al.,* 2019).

The results of trehalase activity and creatinine concentrations which were higher in females than in males appeared to have shown that female rats were more prone to 4-tOP renal toxicity than the male rats at the given 4-tOP exposure (Lobna *et al.,* 2018; Ostermann and Joannidis 2016).

Quinine oxidase is present in negligible amount in the blood. However in cases of parenchymatous lesions of the liver, increased amounts could be detected in the blood. The test is specific for parenchymatous liver damage (Roman and Dulmanis, 1959). In general, the milder the clinical symptoms the lower the levels of quinine oxidases. The female were found to be more sensitive to 4-tOP toxicity.

In this study, it was observed that there is a significant increase in AST, ALT and quinine oxidase activity of rats' serum treated with 4-tert-octylphenol. This might be a defensive mechanism against the effect of 4-tOP. This may suggest damage to the membrane of liver cells and possibly disorder in the biosynthesis of these enzymes with modulation in the permeability of the liver membrane. Comparing gender toxicity, for aspartate aminotransferase, female were found to be more sensitive to 4-tOP effect at medium dose, while male treated rats were toxic to the drug at the highest dose. For Alanine aminotransferase female treated rats were found to be sensitive to the effect of 4-tert-octylphenol. Elevated level of serum AST and ALT has been linked with hepatic injury (Sulaiman *et al.,* 2014).

Superoxide dismutase (SOD) is a class of enzyme that catalyzes the breakdown of superoxide anion into oxygen and hydrogen peroxide (Karmakar *et al.,* 2022). Superoxide dismutase activities obtained from the results of this study indicated higher level of significance in male rats than as observed in females. The result is an indication of important microbial oxidative stress tolerance system more pronounced on the male rat system as compared to the females. It is an important metallo-enzymes meant for the degradation of free radical (Hekmat *et al.,* 2024). When SOD level is high, tissue is protected against oxidative stress.Low SOD level shows that oxidative stress is expressed. Reduction in the activity of this antioxidant enzyme might be as a result of depletion of antioxidant defense system following the over-generation of free radicals (Abdulrahman *et al.,* 2024).

Reduced Glutathione activities in liver tissue obtained from the results of this study showed a significance increase in male but were not significance in female. Contrariwise, result of reduced glutathione activity on renal appeared not to be gender based. Reduced glutathione (GSH) is a tripeptide glutamate, cysteine and glycine residues with an unusual peptide bond between the α-amine of cysteine and the side-chain carboxylate of glutamate (Senapati *et al.,* 2024). GSH is one of the most commonly used biomarkers for lipid peroxidation.

Following treatment with 4-tOP, decline level of SOD and GSH activity was observed in kidney and liver when compared with the control group. This is pathological; it ascertained the challenge posed on the body antioxidant status. The results of SOD and GSH which were higher in males than in females appeared to have shown that male rats were more prone to 4-tOP hepato toxicity than the female rats at the given 4-tOP exposure.

Malondialdehyde (MDA) is an end-product of lipid peroxidation (LPO) following the activity of oxidant which has been found to be genotoxic through interaction with DNA leading to a final disruption of DNA base pairing. It is a highly reactive dialdehyde produced upon the breakdown of peroxidated polyunsaturated fatty acid (PUFAs). It marks the end point of cellular damage. The observed increase in MDA concentration in the kidney and liver tissues of the rats suggested lipid peroxidation which may lead to functional changes in these tissues. Result of Hanioka *et al.,* (2017) suggested that 4-tOP toxicity in rat hepatic tissue arises from modify specific cytochrome P450 isoforms. The increased MDA concentration and loss of activity of GSH-dependent enzymes represent an inverse relationship between lipid peroxidation and GSH levels, an ingredient of oxidative stress in the tissues. The results of MDA which were higher in females liver than in males appeared to have shown that female rats were more prone to 4-tOP hepato toxicity than the female rats at the given 4-tOP exposure.

## CONCLUSION

The female rats were more sensitive to renal and hepatotoxicity of 4-tOP, probably mediated by oxidative stress. The policymakers should ensure strict regulations on the use of 4-tOP in consumer products and promote safer, more environmentally friendly alternatives.

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