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Oxidative Stress Responses as A Marker of Toxicity in Mice Exposed to Water Deprivation

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Abstract

Water is essential for life, and its deprivation can have severe consequences on human health. Water scarcity is a growing concern globally, affecting millions of people worldwide. Understanding the toxic effects of water deprivation on vital organs is crucial for developing effective strategies to mitigate its impact. This study aimed to investigate the toxicity of water deprivation on oxidative stress in the kidney, histological changes in the kidney, and genotoxicity and cytotoxicity in bone marrow. Forty male albino mice were divided into four groups: control and three water-restricted models (timed, continuous, and intermittent). The results showed significant decreases in antioxidant enzyme activity and increased oxidative stress in the kidney, accompanied by histopathological damage, including tubular necrosis and glomerular hyperplasia. The micronucleus test revealed increased genotoxicity and cytotoxicity in bone marrow, disrupting erythropoiesis. The findings highlight the detrimental effects of water deprivation on kidney and bone marrow function, emphasizing the importance of adequate hydration for maintaining organ integrity. Water deprivation induced significant oxidative stress, renal toxicity, and genotoxic effects, particularly in the intermittent water restriction group. These results underscore the need for awareness about the health effects and toxicity of water deficiency. Based on the study's findings, we recommend prioritizing access to clean drinking water, promoting water conservation practices, and supporting research into innovative solutions for addressing water scarcity. Additionally, developing educational programs to raise awareness about the health risks of water deprivation and fostering community-based initiatives to support affected populations are essential steps towards mitigating these effects. By acknowledging the toxicity of water deprivation and taking proactive measures, we can work towards reducing the associated health risks and promoting overall well-being. Current results have significant implications for public health policy, emphasizing the need for sustainable water management practices and strategies to protect vulnerable populations from the adverse effects of water scarcity. By addressing the health risks associated with water deprivation, we can promote health, well-being, and sustainable development.

Key Words: Water deprivation, oxidative stress, kidney toxicity, micronucleus test, mice.

1. Introduction:

Water is the foundation of life, playing a crucial role in various physiological and ecological processes. In animals, water is necessary for maintaining cellular homeostasis, metabolic functions, thermoregulation, and overall survival. At the cellular level, water facilitates biochemical reactions, nutrient transport, and waste elimination, while also maintaining osmotic balance and preventing cellular dehydration or lysis (Boukersi *et al.*, 2021).

Dehydration, on the other hand, can have severe consequences on animal health, including reproductive issues, endocrine disruptions and impaired kidney function. Water deprivation can also lead to oxidative stress, which can cause DNA damage, genetic instability, and various diseases. In laboratory settings, water restriction is a common method used to motivate animals to engage in specific behaviors. However, this practice requires careful monitoring of the animals' health and hydration, and it can be

challenging for some animals to tolerate. Studies have shown that water intake varies between strains, ranging from 3.9 to 8 mL per mouse per day in static open-top cages (Nicolaus *et al.*, 2016).

The effects of dehydration on animal health are multifaceted. Impaired kidney function can lead to a decrease in renal blood flow and an increase in medullary interstitial osmolality, leading to kidney damage and potentially even kidney failure (Turner *et al.,* 2010). Reproductive issues can also arise, with water deprivation causing a decrease in testicular weight, affecting hormone synthesis and reproductive function (Hirano *et al.,* 2015). Endocrine disruptions can also occur, with dehydration disrupting the hypothalamic-pituitary-thyroid (HPT) axis, leading to alterations in thyroid hormone levels and metabolic dysfunction (Abd El Moghny & Ashour, 2016).

Oxidative stress, which arises from an imbalance between the production of reactive oxygen species (ROS) and the capacity of antioxidant defenses, is a recognized key factor in inducing DNA damage. This damage includes base modifications, strand breaks, and chromosomal aberrations, all of which are pivotal in the pathogenesis of various diseases, including cancer and neurodegenerative disorders (Valavanidis, Vlachogianni, & Fiotakis, 2009). Damage to DNA caused by free radicals can lead to genetic instability and subsequent diseases, including carcinogenesis, if not corrected (Davidson, Guo, & Loeb, 2002; Wallace, 2002; Evans, Dizdaroglu, & Cooke, 2004; Friedberg *et al*., 2006; Loeb, 2011).

Cells have developed various antioxidant defenses to counteract the effects of oxidative stress. These defenses include enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which work to degrade ROS and prevent DNA damage (Hunt *et al*., 1998). However, chronic oxidative stress can lead to a range of negative effects, including genomic instability, cancer, and neurodegenerative disorders (Limoli *et al*., 1997; Hunt *et al*., 1998).

The need for further research in this area is evident. Despite the importance of water in maintaining animal health, there is still much to be learned about the effects of dehydration and oxidative stress on animal health. Further research is needed to fully understand the mechanisms by which dehydration and oxidative stress affect animal health, and to develop effective strategies for preventing and treating these conditions.

This study aimed to investigate the toxic effects of water deprivation on kidney and bone marrow of mice. The investigation utilized oxidative stress marker analysis and histopathological analysis of the kidney to assess renal toxicity. Additionally, the study evaluated the cytotoxicity and genotoxicity of the bone marrow using the micronucleus test. By examining the impact of water deprivation on these critical organs, this research seeks to contribute to a better understanding of the importance of water in maintaining animal health.

This study aimed to investigate the toxic effects of water deprivation on kidney and bone marrow of mice. The investigation utilized oxidative stress marker analysis and histopathological analysis of the kidney to assess renal toxicity. Water deficiency has been shown to disturb hematopoiesis by impairing erythropoiesis and decreasing the production of bone marrow-derived cells, including immune and red blood cells, which compromises the body's ability to maintain

oxygen delivery and immune surveillance. Additionally, dehydration-induced oxidative stress can suppress bone marrow function, reduce leukocyte counts, and increase the susceptibility to infections and inflammation through immune dysregulation (El-Alfy *et al*., 2024). To assess genetic and cytotoxic effects on bone marrow, the micronucleus test was applied, as it is a sensitive and widely accepted method for detecting chromosomal alterations and evaluating cellular damage through the frequency of micronucleated erythrocytes and changes in erythropoiesis (El-Alfy *et al.,* 2024). By examining the impact of water deprivation on these critical organs, this research seeks to contribute to a better understanding of the importance of water in maintaining animal health. It is considered the first part of the research. Therefore, it should be well-built, cohesive and clearly stated to draw the reader's attention

2. Theoretical Framework:

Smith (2020) highlighted that global water usage trends are deeply entwined with sustainable development and economic advancement, emphasizing the need for adaptive planning. Hanasaki et al. (2012) linked water scarcity with broader environmental stressors such as pollution and climate instability, while Hanjra & Qureshi (2010) identified water scarcity as a growing threat to food security and social stability.

Mekonnen et al. (2020) demonstrated a correlation between water shortages and violent conflicts, a link echoed by Ward & Ruckstuhl (2020), who noted the strategic targeting of water infrastructure in conflict zones. Zhang et al. (2021) stressed that inefficient water use could hinder socio-economic progress and emphasized the need for skilled professionals to manage water resources sustainably.

Dehydration compromises essential physiological functions. Smith et al. (2020) found it disrupts cellular metabolism, while Williams & Clark (2018) associated chronic water deficiency with kidney and liver dysfunction. Gomez & Lee (2019) and Zhang et al. (2020) confirmed its impact on circulatory health and tissue integrity, respectively.

Gottlieb et al. (2006) identified water deprivation as a state activating the renin-angiotensin system, while Shen et al. (2007) and Levey et al. (1986) observed hematological changes including reduced erythropoiesis under deprivation. These changes may predispose to anemia and immune suppression, key indicators of systemic toxicity.

Singh et al. (2023) linked oxidative stress to premature erythrocyte damage, a central mechanism in anemia. Al-Farsi et al. (2022) and Vaziri et al. (2019) found that iron overload in renal dysfunction is exacerbated by oxidative stress and impaired hydration. Ali et al. (2019) confirmed that dehydration elevates oxidative biomarkers like malondialdehyde and glutathione, alongside morphological gastric damage.

Faraco et al. (2014) and Halliwell & Gutteridge (2015) demonstrated that water deprivation increases oxidative load in neural and systemic tissues. Podkowińska & Formanowicz (2020) showed its role in vascular and renal dysfunction, contributing to chronic kidney disease through redox imbalance.

Oxidative stress also affects DNA integrity. Kryston et al. (2011) and Marnett (2000) explained that prolonged ROS exposure damages DNA, resulting in genomic instability and carcinogenesis. Petersen et al. (1998), Halliwell et al. (1991), and Grollman et al. (1993) described ROS-induced base modifications and strand breaks, especially the mutagenic lesion 8-oxoguanine (8-oxoG).

Telomeric DNA, particularly vulnerable due to its guanine richness, is significantly affected by oxidative stress (Von Zglinicki et al., 2000; Opresko et al., 2005). These alterations disrupt telomerase activity and compromise genome maintenance (Counter, 1996; Bailey et al., 2006).

Micronuclei (MN), nucleoplasmic bridges (NPBs), and nuclear buds (NBUDs) represent visible biomarkers of genotoxic events, linked to chromosomal breakage or missegregation (Fenech & Crott, 2002; Shimizu et al., 2000). Studies by Krishna & Hayashi (2000), and Mavournin et al. (1990) have validated the micronucleus assay (MNA) in rodents as a reliable tool for detecting chromosomal damage during erythropoiesis.

Bonassi et al. (2007) and Samanta & Dey (2012) highlighted the clinical relevance of micronucleus frequency as a predictor of cancer risk. Krishna & Hayashi (2000) further explained the mechanisms behind micronucleus formation and how immunological markers (e.g., anti-kinetochore antibodies) distinguish between clastogenic and aneugenic events

3. Materials and Methods:

3.1. Animals:

Male CD-1 albino mice (*Mus musculus*), aged between 6 and 8 weeks and weighing around 25±5g, were sourced from Theodore Billiharz institute for research in Cairo. In this study, we used 40 mice, housed them in cages in our laboratory, and allowed them to acclimate to the surrounding environment before starting the experiment. During this period, we had unrestricted access to water and a standard pellet diet. The ambient conditions were maintained at 25 ± 2 °C, with a relative humidity of $55 \pm 10\%$, and a 12-hour light/dark cycle. The study and all experimental procedures received approval from the Experimental Animal Care and Research Ethics Committee of Aims Shams University.

3.2. Animal Housing and Maintenance:

Animals were accommodated in acrylic cages, maintained under controlled laboratory conditions, and photoperiod (12:12 light-dark cycle). Prior to experimentation, animals underwent a one-week acclimation period. A standard rodent pellet diet and water were provided ad libitum, with food and water refreshed every 48 hours to ensure optimal animal welfare and minimize experimental variability**.**

3.3. Experimental design:

40 mature male albino mice of nearly at the same age were individually weighed and randomly assigned to 4 group as the following: Group 1 (Control group): Members of this group received ad libitum water. Group 2 (Timed group): Members of this group received free access to water for 30 minutes daily for two weeks. Group 3 (continuous group): Members of this group received a daily water volume of 2ml for two weeks. Group 4 (intermittent group): Members of this group received a repeated schedule of 2ml of water per day for 5 days, followed by 2 days of free water access for 30 minutes, for a total of 2 weeks. Both the control and treated animals underwent cervical dislocation 24 hours after the final

treatment, followed by sample collection for analysis.

3.4. Body Weight Measurements

Each mouse's body weight was weighed using a digital scale at the beginning of the experiment, after one week of treatment and on the day of sacrificing**.**

3.5. Oxidative Stress Markers Analysis:

CAT test: Kidney were obtained and promptly fixed in formalin to ensure stabilization. Subsequently, the samples were processed and homogenized. The activity of catalase (CAT) was assessed spectrophotometrically by tracking the rate of hydrogen peroxide decomposition at a wavelength of 240 nm. The results were reported as enzyme activity per milligram of protein. The total protein concentration was quantified using the Bradford assay. Data analysis was conducted to compare oxidative stress levels between the water-deprived group and the control group**.**

SOD test: The nitroblue tetrazolium (NBT) reduction assay is a widely used method to measure superoxide dismutase (SOD) activity by assessing the enzyme's ability to inhibit NBT reduction by superoxide radicals. Key reagents include phosphate buffer with DETAPAC and BSA, along with catalase, xanthine, NBT, BCS, and sodium cyanide (to inhibit Cu, Zn-SOD and isolate MnSOD activity). Sample lysates are tested to determine the protein amount needed for 50% inhibition of NBT reduction. The reaction is initiated by xanthine oxidase, and absorbance is measured at 560 nm. SOD activity is calculated by comparing inhibition levels, making this assay a reliable tool for studying oxidative stress**.**

GPX test: Glutathione Peroxidase (GPX) is an essential antioxidant enzyme that protects cells by reducing hydrogen peroxide and lipid peroxides to harmless products, using reduced glutathione (GSH), which is converted into glutathione disulfide (GSSG)**.** Measuring GPX activity is useful for assessing oxidative stress. Two widely used methods include**:**

Rotruck *et al.* (1973): In this method, a biological sample is mixed with GSH and hydrogen peroxide. GPX catalyzes the reaction, and the remaining GSH is measured using Ellman's reagent, producing a yellow color read at 412 nm**.**

Kokatnur & Jelling (1993): This method adds glutathione reductase and NADPH to recycle GSSG back to GSH, allowing continuous measurement. NADPH consumption is monitored, as it correlates directly with GPX activity**.**

Both methods reflect how effectively the body can neutralize oxidative threats. While sensitive and informative, they require fresh, properly handled samples and can be affected by interfering substances**.**

3.6. Histopathological Analysis

Each group's kidney specimens were removed, fixed in 10% formalin, washed in 70% alcohol, dried with decreasing alcohol concentrations, embedded in paraffin, sectioned using a microtome at a 5 µm thickness, and stained with the following dyes: 1. For histological analysis, use the standard hematoxylin and eosin (H&E) stain. routine 2. To show collagen fibres, use Masson's trichrome stain. Every staining technique was carried out in accordance with Bancroft and Layton's guidelines (Bancroft and Layton, 2013). 3. IHC staining for P62-positive cell identification. Images of the inspected slides were captured with a digital camera attached to a Leica microscope.

3.7. Micronucleus Test:

In this study, we followed Schmid's standard micronucleus test procedure (Schmid, 1976) with a slight modification. Instead of using fetal calf serum, we used 5% bovine albumin (obtained from the National Research Center, Giza, Egypt) as the suspending medium for bone marrow collection (Narayan *et al*., 2002).

1. At the end of the experiment, the mice were sacrificed, and their femurs were removed. The ends of the bones were trimmed, and a blunt needle was inserted to access the bone marrow **.**
2. The bone marrow was flushed out using a syringe filled with 5% bovine albumin to create a fine suspension. This suspension was then centrifuged at 1000 rpm for 8 to 10 minutes. After centrifugation, the liquid supernatant was discarded, and a small amount of fresh suspending medium was added. The mixture was thoroughly mixed using a Pasteur pipette.
3. A small drop of the bone marrow suspension was placed on a slide, and a smear was made (3–4 slides per animal). The slides were air-dried overnight, then fixed in methanol for 5 minutes. After drying, they were stained first with May-Grunwald stain, followed by a combination of May-Grunwald stain and phosphate buffer (pH 6.8). This helped differentiate polychromatic erythrocytes (PCEs) from normochromatic erythrocytes (NCEs).
4**.** The slides were then stained with Giemsa stain and a buffer solution (pH 6.8) to highlight micronuclei. After washing with distilled water and buffer, the slides were dried and mounted for analysis.
5. For each animal, 2000 polychromatic erythrocytes (PCEs) were examined under a microscope, and the number of micronucleated PCEs (MNPCEs) was recorded. Normochromatic erythrocytes (NCEs) were also counted, and the percentage of MNPCEs and the PCE/NCE ratio were calculated for each animal**.**

3.8 Data Analysis:

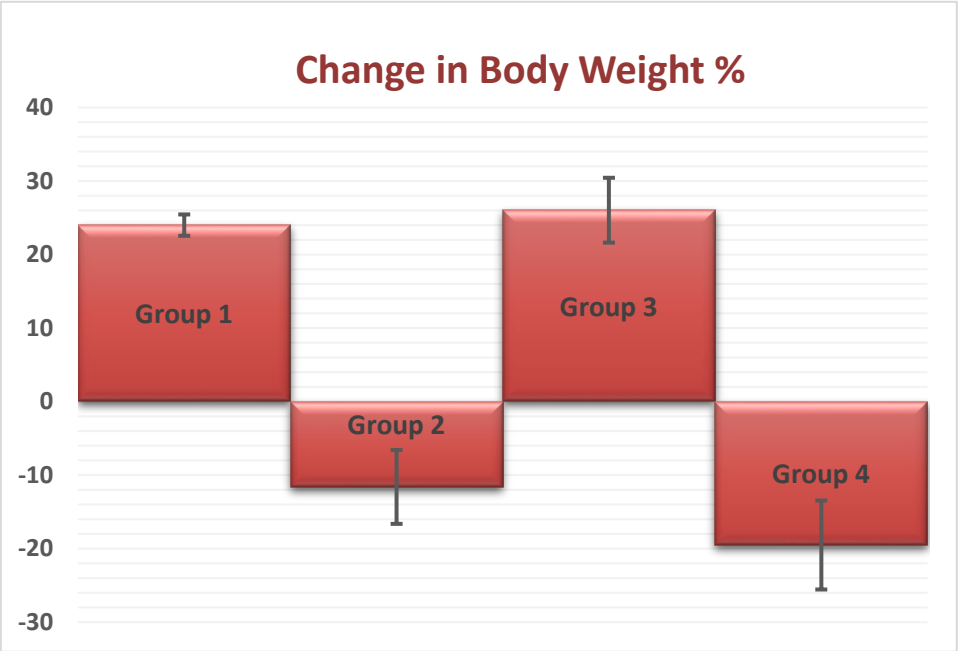
Statistical analysis was performed using SPSS software (version 16.0) on a personal computer. Data are presented as mean ± standard deviation. To determine significant differences between groups, independent samples T-tests were conducted. A p-value < 0.05 was considered statistically significant, while $p < 0.01$ indicated a stronger significance level. Non-significant results were characterized by $p > 0.05$. Data visualization was created using Excel 2019 .

4. Results

4.1 Body Weight

The body weight of the albino rats during the study was significantly different between the groups. The highest increase in body weight was observed in Group 3, while the lowest final body weight was recorded in Group 4, being approximately 26.03% and -19.51%, respectively. There was a significant increase in body weight in Group 3 ($p<0.001$) compared to Group 2. On the other hand, there was a more significant increase in Group 1 ($p<0.05$) compared to Group 3 (Table-2). This study demonstrated an increase in the percentage of body weight increase in the treated groups compared to the control group. body weight significantly increased with a percentage of 26.03± 4.40946 in group 3 when compared to the control 24 ± 1.45258. However, it decreased insignificantly with a percentage of -11.61 ± 5.01877 In Group 2 and significantly with a percentage of -19.5067± 6.04914 in Group 4 in comparison to the control.

Figure 1. Percentages of changes between nitial and final body weights in the control and treated groups.



4.2 oxidative stress markers analysis

The activities of oxidative stress enzymes in kidneys of albino mice *Mus Musculus* in the control and treated groups, values expressed as (mean ± SD). Their activity expressed as unit U mg-1 protein. (n=3 animals for each four groups). SOD=Superoxide dismutase, CAT=Catalase, MDA=Malondialdehyde, GPx=Glutathione peroxidase.

The treated groups (Group 2, Group 3, and Group 4) showed a marked decrease ($p<0.05$) in the activity of antioxidant enzymes compared to the control group (Group 1). Specifically, the catalase (CAT) activity decreased significantly in Group 2 (3.3433 ± 0.09504), Group 3 (2.9767 ± 0.12220), and Group 4 (2.0700 ± 0.09539) when compared to Group 1 (4.6733 ± 0.14048). Similarly, SOD activity was significantly reduced in the treated groups—Group 2 (2.8667 ± 0.09504), Group 3 (0.13051 ± 2.4033), and Group 4 (2.0433 ± 0.13317)—as compared to the control (3.5900 ± 0.15524). A parallel trend was observed for GPx activity, which decreased in the treated groups: Group 2 (5.1000 ± 0.10536), Group 3 (4.2667 ± 0.24090), and Group 4 (2.9500 ± 0.18330) relative to the control (6.2800 ± 0.25515). Among all treated groups, Group 4

consistently exhibited the lowest enzyme activity levels across CAT, SOD, and GPx, suggesting a stronger oxidative stress burden relative to the other groups. The statistical analysis revealed that changes in enzyme levels were highly significant in Group 2 ($p<0.001$) and significant in Groups 3 and 4 ($p<0.05$), indicating a dose-dependent or treatment-related modulation in oxidative stress response.

The activity of oxidative stress enzymes in the kidneys of albino mice showed significant variation across the experimental groups. The SOD, CAT, and GPx levels were observed to be highest in Group 1 (control), and progressively decreased in the other groups depending on the treatment received.

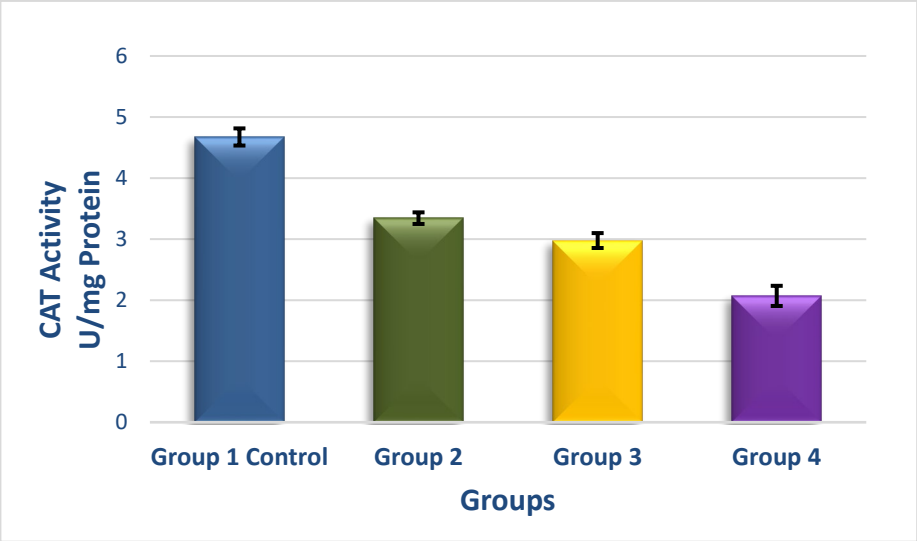


Figure 2. Catalase (CAT) activity in kidneys of albino mice *Mus Musculus*, (mean ± SD). Superoxide dismutase activity expressed as unit U mg^{-1} protein.

The activity of catalase (CAT) showed a significant decrease ($p<0.05$) from 4.6733 ± 0.14048 U/mg protein in the control group to 3.3433 ± 0.09504 U/mg protein in Group 2, 2.9767 ± 0.12220 U/mg protein in Group 3, and reached the lowest value of 2.0700 ± 0.09539 U/mg protein in Group 4**.**

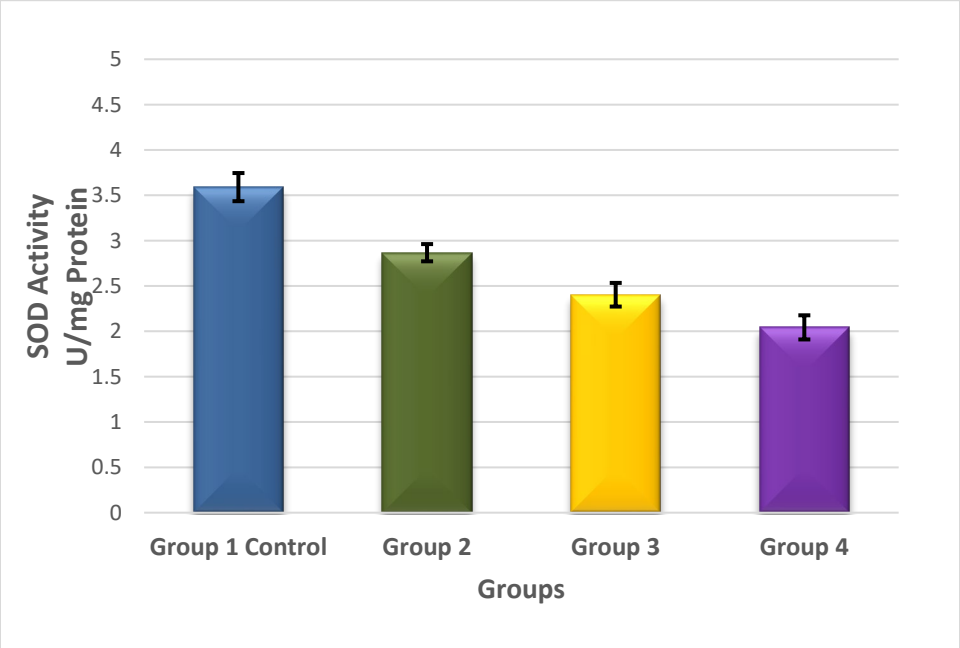


Figure 3: Superoxide dismutase (SOD) activity in kidneys of albino mice *Mus Musculus*, (mean ± SD). Superoxide dismutase activity expressed as unit U mg^{-1} protein.

Similarly, SOD activity was highest in the control group at 3.5900 ± 0.15524 U/mg protein and significantly declined across the treated groups, with values of 2.8667 ± 0.09504 in Group 2, 0.13051 ± 2.4033 in Group 3, and 2.0433 ± 0.13317 in Group 4 ($p<0.05$).

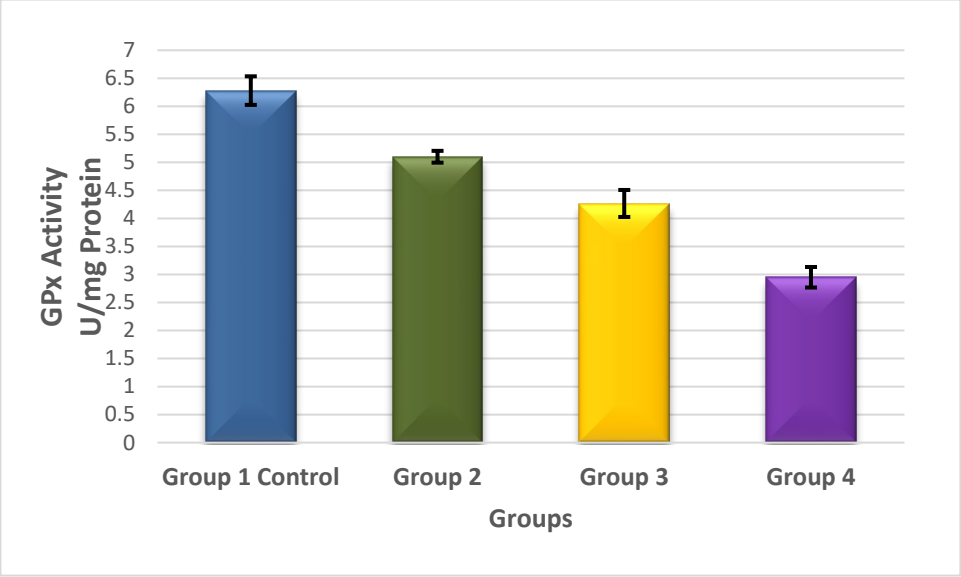


Figure 4: Glutathione Peroxidase (GPX) activity in kidneys of albino mice *Mus Musculus*, (mean ± SD). Superoxide dismutase activity expressed as unit U mg^{-1} protein.

The GPx enzyme followed the same trend. The control group (Group 1) had the highest GPx activity at 6.2800 ± 0.25515 U/mg protein, while Groups 2, 3, and 4 recorded significantly lower activities at 5.1000 ± 0.10536, 4.2667 ± 0.24090, and 2.9500 ± 0.18330 U/mg protein respectively ($p<0.05$, $p<0.001$).

These results indicate that exposure to the experimental conditions significantly influenced oxidative stress markers, with the greatest antioxidant activity retained in the control group, and a progressive decline observed in the treated groups.

4.3 Histopathological Analysis:

The kidney is a vital excretory organ responsible for filtering blood, regulating fluid and electrolyte balance, and eliminating metabolic waste. Histologically, it is divided into two main regions: the cortex and the medulla. The renal cortex contains the glomeruli, proximal and distal convoluted tubules, and associated blood vessels. The glomerulus is a compact network of capillaries encased in Bowman's capsule, forming the renal corpuscle—the core structure of the nephron, which is the functional unit of the kidney. Each nephron continues with a tubular system that includes the proximal tubule, loop of Henle, distal tubule, and collecting duct.

The glomerular filtration barrier consists of three key components: fenestrated endothelial cells, a specialized basement membrane, and podocytes with interdigitating foot processes. This barrier is essential for selective filtration of blood plasma. The renal tubules, lined by cuboidal epithelial cells, are responsible for the reabsorption of water, ions, and nutrients, and the secretion of waste products. The renal medulla, composed mainly of loops of Henle

and collecting ducts, plays a crucial role in urine concentration.

Group 1 (Control Group): The renal cortex exhibited a normal histological architecture. The glomeruli appeared well-formed with intact capillary tufts and clear Bowman's spaces. The surrounding renal tubules were lined by cuboidal epithelial cells showing uniform nuclei and preserved cytoplasm. There were no signs of necrosis, inflammation, or vascular congestion. The tissue structure indicated healthy kidney function with preserved filtration and reabsorption capacities.

Group 2: Sections of renal tissue revealed early pathological changes. Vacuolation was observed within the glomerular tuft, indicating the presence of cytoplasmic clear spaces due to cellular injury. Renal tubular epithelial cells showed signs of necrosis, such as cell swelling, nuclear fading, and cytoplasmic disintegration. Necrosis refers to a form of irreversible cell injury characterized by the breakdown of cellular structures, loss of membrane integrity, and uncontrolled cell death leading to tissue damage. Mild inflammatory infiltration was noted in the interstitial tissue, reflecting an initial immune response.

Group 3: Moderate structural damage was evident. The renal tubules exhibited coagulative necrosis, where the general outline of the cells was preserved despite the loss of nuclei and cytoplasmic integrity. Inflammatory cell infiltration increased within the interstitial spaces, suggesting progression of tissue injury. Interstitial blood vessels were markedly congested, indicating impaired circulation and possible hypoxic stress within the renal parenchyma.

Group 4: Severe histological alterations were noted. There was widespread tubular degeneration, with loss of cellular architecture and detachment of epithelial cells from the basement membrane. The glomerular tuft showed hyperplasia, characterized by an increased number of resident cells, likely as a response to sustained injury. Extensive necrosis affected nearly all tubular cells. Focal aggregates of inflammatory cells were clearly visible, pointing to localized immune reactions. Moreover, thickening of the blood vessel walls suggested chronic inflammation or fibrosis, possibly leading to compromised vascular function.

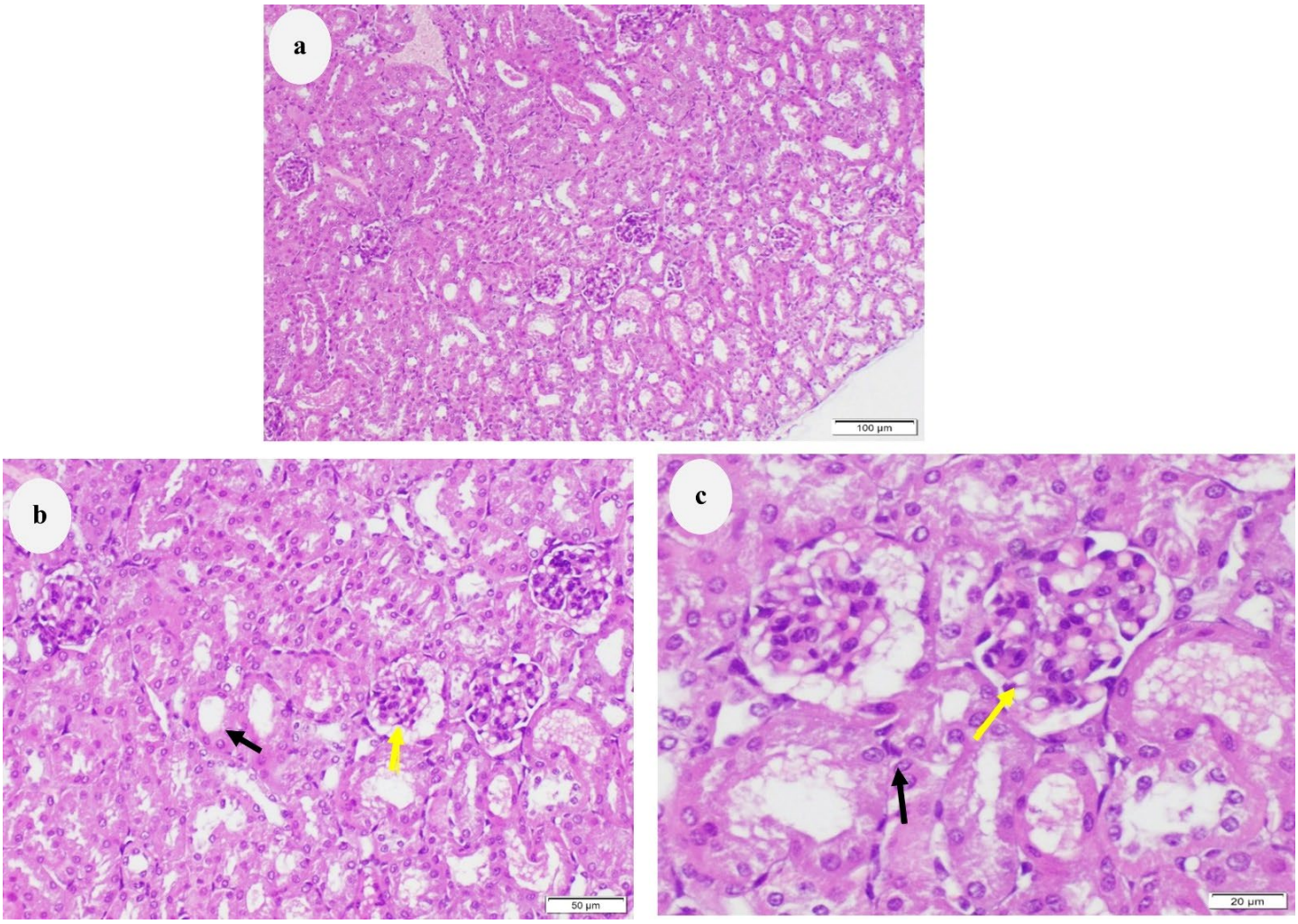


Figure 5: Histological alterations in kidney of mice: Photomicrographs of transverse sections of kidney stained with H&E. In (a), distinct renal formations are visible, including well-defined glomeruli and surrounding tubular structures (scale bar = 100 µm). In (b), the image highlights the organized epithelial lining of renal tubules reflecting preserved functional architecture (scale bar = 50 µm). Finally, in (c), notable features such as glomerular cells and surrounding components are identified (scale bar = 20 µm)

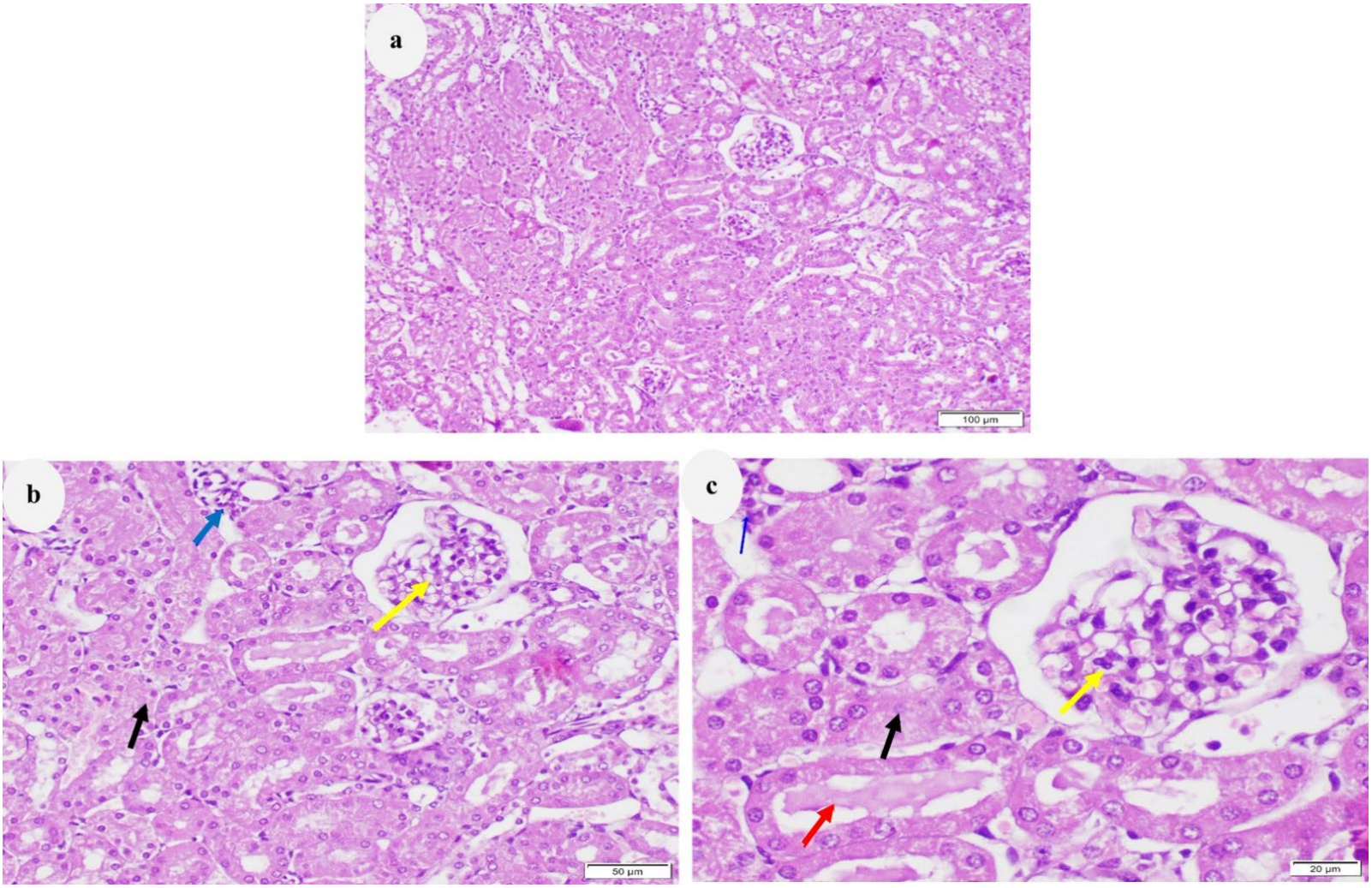


Figure 6: Histological alterations in kidney tissue of mice: Photomicrographs of transverse sections of kidney stained with hematoxylin and eosin (H&E) illustrate pathological changes. In panel (a), vacuolation of the glomerular tuft is visible (yellow arrow), accompanied by necrosis of renal tubules (black arrow), suggesting significant structural disruption (scale bar = 100 µm). In panel (b), mild inflammation is observed in the interstitial spaces (blue arrow), along with continued renal tubule damage, reflecting early inflammatory responses (scale bar = 50 µm). Panel (c) provides a closer view of these alterations, emphasizing vacuolation, necrosis, and inflammation with greater detail (scale bar = 20 µm)

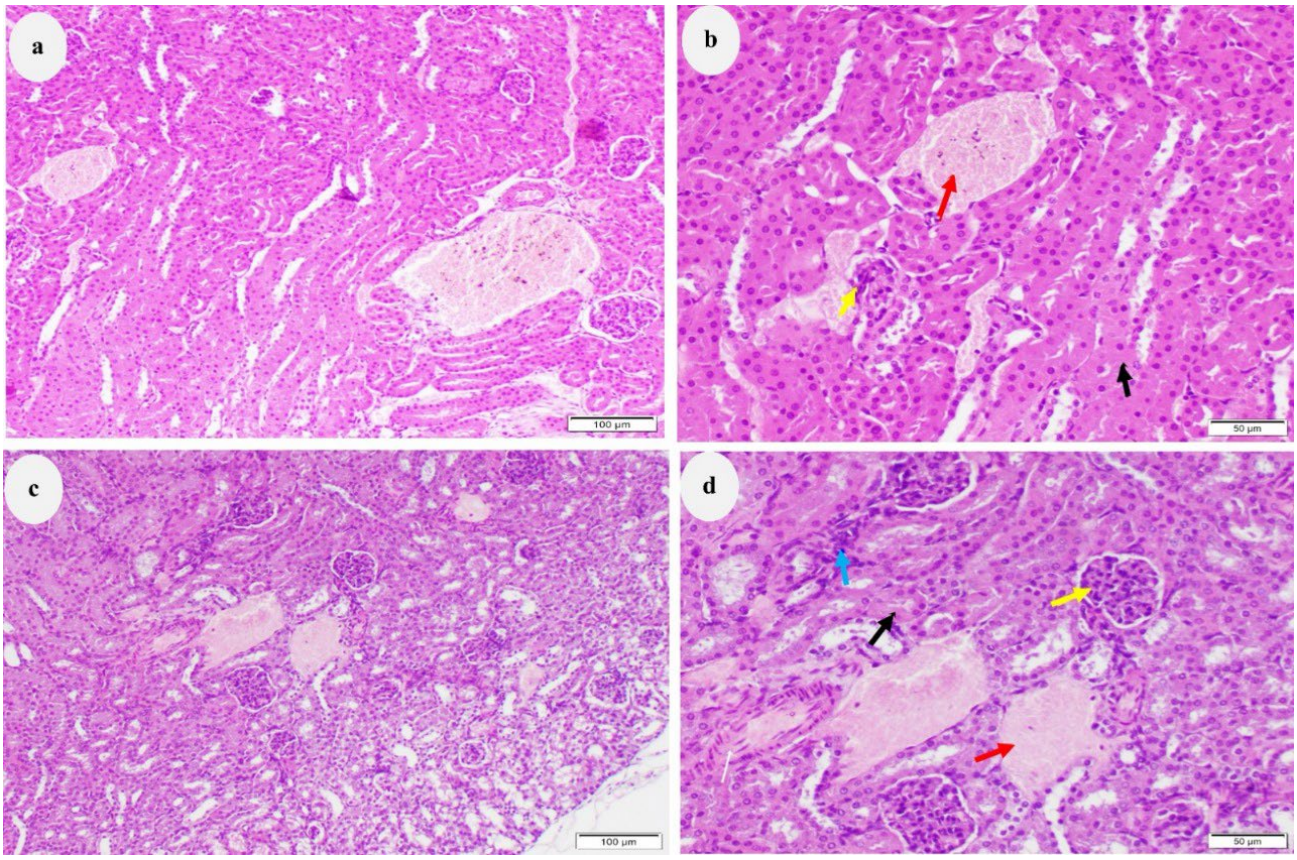


Figure 7: Histological alterations in kidney tissue of mice: Photomicrographs of transverse sections of kidney stained with hematoxylin and eosin (H&E) illustrate notable pathological changes. Group 3 (a, b) exhibits coagulative necrosis of the renal tubules (black arrow), intra-renal inflammation (yellow arrow), and congested interstitial blood vessels (red arrow). In (a), scale bar = 100 µm, and in (b), scale bar = 50 µm. In contrast, Group 4 (c, d) shows hyperplasia of the glomerular tuft (yellow arrow), necrosis of all tubular cells (black arrow), focal aggregation of inflammatory cells (blue arrow), and thickening of the blood vessel walls (white arrow). In (c), scale bar = 100 µm, and in (d), scale bar = 50 µm.

4.4. Micronucleus Test

Results of the micronucleus test are depicted in Table (1). As observed microscopically, normochromatic erythrocytes (NCEs) appeared with a dark blue stain, while polychromatic erythrocytes (PCEs) exhibited a light blue to violet coloration. Micronucleated polychromatic erythrocytes (MNPCEs) were identified as PCEs containing one or more small, dark blue, ring-shaped micronuclei. These structures represent acentric chromosomal fragments that failed to be incorporated into the daughter nuclei during the anaphase stage of cell division.

A significant ($P < 0.05$) increase in the frequency of micronucleated polychromatic erythrocytes (MNPCEs) was detected in all treated groups compared to the control group. The Timed group showed the highest percentage of MNPCEs (2.9500 ± 0.35000), followed closely by the Intermittent group (2.9333 ± 0.30551) and the Continuous group (2.8167 ± 0.30551), whereas the control group exhibited the lowest frequency (1.1833 ± 0.18930). These findings reflect the genotoxic potential of the administered treatments.

Additionally, the cytotoxic effect was assessed through the PCEs/NCEs ratio, indicating the degree of erythropoiesis disruption in the bone marrow. All treated groups showed a highly significant ($P < 0.001$) increase in the mean PCE/NCE ratio in comparison to the control group. The Intermittent group demonstrated the highest cytotoxic effect with a ratio of (1.4577 ± 0.03958), followed by the Timed group (1.3733 ± 0.01182), and then the Continuous group (1.3013 ± 0.02013). The control group recorded the lowest value (1.1836 ± 0.01182). These results confirm that the treatments not only induced genotoxic effects but also altered erythropoiesis, with the Intermittent group showing the most pronounced impact**.**

Table 1: represents the mean and standard deviation of micronucleated polychromatic erythrocytes (MNPCEs) and PCEs/NCEs ratio in 6000 polychromatic erythrocytes (PCEs) and corresponding normochromatic erythrocytes (NCEs) scored in the bone marrow of three mice of the control group and treated groups.

Groups	**Total scored cells/ No. of mice**	**Total MNPCEs**	**Micronuclei**	**Total NCEs**	**Cytotoxicity**
			MNPCEs/Total PCEs % **(Mean± SD)**		**PCEs/ NCEs** **(Mean± SD)**
Group 1 (Control)	6000/3	71	1.1833± 0.18930	5051	1.1836± 0.01182
Group 2 (Timed Group)	6000/3	177	2.9500± 0.35000*	4357	1.3733± 0.01182**
Group 3 (Continuous Group)	6000/3	169	2.8167± 0.30551*	4590	1.3013± 0.02013**
Group 4 (Intermittent Group)	6000/3	176	2.9333± 0.30551*	4110	1.4577± 0.03958**

* Significant ($P < 0.05$)

* Highly significant ($P < 0.001$)

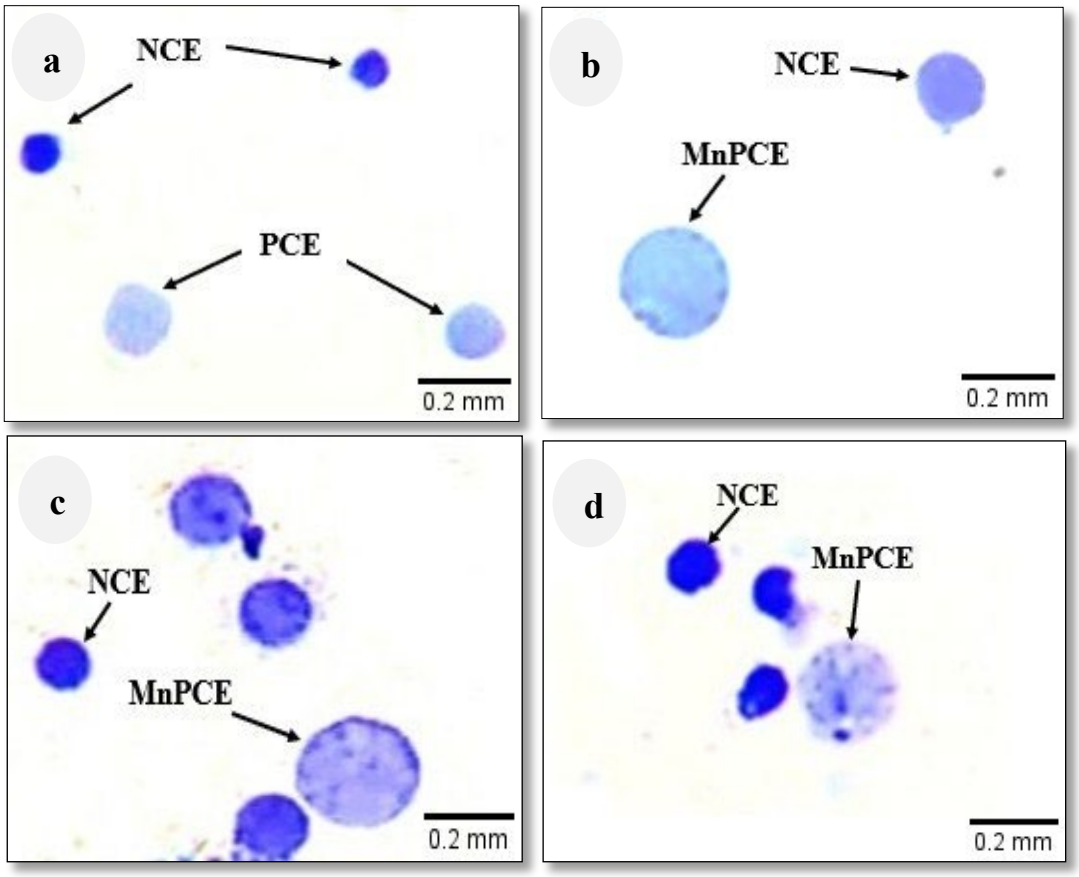


Figure 8: Bone marrow smears of Mus musculus showing polychromatic erythrocytes (PCE), normochromatic erythrocytes (NCE), and micronucleated polychromatic erythrocytes (MnPCE). (a) Control group, (b) Group 2, (c) Group 3, (d) Group 4. The scale bar is 0.2 mm.

5. Discussion

This study investigated the toxicological effects of water deprivation on renal function and bone marrow integrity in mice, focusing on oxidative stress markers, histopathological changes, and cytogenetic outcomes. The findings provide compelling evidence that restricted water intake significantly disrupts homeostatic balance, resulting in systemic physiological stress and cellular damage.

The recorded body weight variations among the experimental groups underscore the metabolic strain imposed by water deprivation. Group 3, which received a consistent yet limited water supply, exhibited an unexpected increase in body weight. This could be attributed to adaptive physiological mechanisms aimed at maximizing fluid retention and metabolic efficiency, as described in similar studies examining caloric and fluid restriction in rodents (Rossetti *et al.*, 2018). In contrast, the pronounced weight loss observed in Group 4 reflects metabolic decompensation caused by intermittent water availability. This aligns with findings from Turner *et al.* (2010), who

reported that inconsistent hydration patterns impair renal osmoregulation and trigger catabolic processes.

A key result of this study was the significant decline in the activities of antioxidant enzymes—catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx)—across all treatment groups. This observation corroborates the notion that dehydration amplifies oxidative stress by increasing the production of reactive oxygen species (ROS) while simultaneously depleting antioxidant reserves. Similar biochemical trends were described by Singh *et al.* (2023), who noted that oxidative stress leads to erythrocyte dysfunction and accelerates red blood cell turnover, contributing to anemia and tissue hypoxia.

Ali *et al.* (2019) demonstrated that prolonged dehydration elevates malondialdehyde (MDA) and nitric oxide levels while reducing CAT and GPx, which corresponds with our findings. Additionally, the renal oxidative damage observed may be influenced by disturbed iron metabolism—a known trigger of oxidative injury in the kidneys (Al-Farsi et al., 2022; Vaziri *et al.*, 2019). These effects cumulatively contribute to the deterioration of renal function and the progression of chronic kidney pathologies.

The histological analysis provided direct evidence of structural kidney damage following water deprivation. The progression from mild vacuolation and inflammation in Group 2 to widespread necrosis and glomerular hyperplasia in Group 4 illustrates a clear dose-response relationship. These pathological findings are consistent with earlier research by Podkowińska and Formanowicz (2020), who described oxidative stress-mediated disruption of glomerular and tubular structures in chronic kidney disease models. The observed blood vessel wall thickening and inflammatory infiltration also suggest the initiation of fibrotic and ischemic processes, potentially leading to irreversible renal dysfunction.

Furthermore, our findings resonate with Shen *et al.* (2007), who demonstrated that fluid deprivation in rodents induces hematological abnormalities, including increased hematocrit and decreased lymphocyte counts, due to plasma volume reduction and impaired renal clearance.

The results of the micronucleus test revealed a significant increase in micronucleated polychromatic erythrocytes (MNPCEs) across all treated groups, indicating genotoxic stress. The intermittent water restriction group demonstrated the most severe genotoxic and cytotoxic effects, as evidenced by the elevated MNPCE frequency and the disrupted PCE/NCE ratio. These cytogenetic markers suggest both chromosomal breakage (clastogenesis) and spindle malfunction (aneugenesis), mechanisms that have been well characterized in the literature (Krishna & Hayashi, 2000; Fenech, 2000).

Moreover, oxidative DNA damage caused by ROS—particularly 8-oxoguanine lesions—is implicated in the formation of micronuclei and subsequent chromosomal instability (Grollman & Moriya, 1993; Kryston *et al.*, 2011). Von Zglinicki et al. (2000) and Oikawa *et al.* (1999) have also highlighted the susceptibility of telomeric regions to oxidative damage, suggesting that water deprivation may compromise chromosomal integrity at multiple genomic levels.

The disruption of erythropoiesis, as evidenced by an altered PCE/NCE ratio, also supports previous work by Levey et al. (1986) and El-Alfy *et al.* (2024), who reported that hydration deficits impair bone

marrow functionality and reduce immune cell production. This may have broader implications for host defense and systemic inflammation under dehydrated conditions.

This research aligns with a growing body of evidence linking water scarcity to biological and public health crises. Global assessments by Hanjra and Qureshi (2010) and Mekonnen *et al.* (2020) have warned of the social, agricultural, and health consequences of water scarcity, particularly in vulnerable populations. Zhang *et al.* (2021) emphasized the need for efficient water management systems and policy interventions to prevent resource conflicts and maintain ecosystem stability.

From a molecular biology perspective, oxidative DNA damage and the resulting genomic instability are increasingly recognized as precursors to chronic diseases, including cancer and neurodegenerative disorders (Valavanidis *et al.*, 2009; Wallace, 2002). Our findings support the growing interest in using oxidative stress biomarkers as early indicators of tissue toxicity and potential targets for therapeutic intervention.

6. Conclusion

This study demonstrates that water deprivation has significant toxicological effects on mice, particularly targeting the kidney and bone marrow. The findings revealed that limited water intake induces oxidative stress, leading to measurable biochemical, histological, genetic, and cellular alterations. Notably, intermittent water deprivation exhibited the most severe impact, indicating that inconsistent hydration is more harmful than constant restriction. These results underscore the critical importance of adequate water intake in maintaining systemic and cellular health. Given the growing concern of water scarcity globally, our findings highlight the need for awareness about the health effects and toxicity of water deficiency. The study's results have significant implications for public health policy, emphasizing the need for sustainable water management practices and strategies to protect vulnerable populations. Based on our findings, we recommend prioritizing access to clean drinking water, promoting water conservation practices, and supporting research into innovative solutions for addressing water scarcity. Furthermore, developing educational programs and community-based initiatives can help mitigate the health risks associated with water deprivation. By acknowledging the toxicity of water deprivation and taking proactive measures, we can work towards reducing the associated health risks and promoting overall well-being, ultimately contributing to sustainable development and public health.

7. References

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Abbreviation

CAT: Catalase

DETAPAC: Diethylenetriaminepentaacetic Acid

eNOS: Endothelial Nitric Oxide Synthase

GPx: Glutathione Peroxidase

H&E: Hematoxylin and Eosin

HPT axis: Hypothalamic-Pituitary-Thyroid Axis

MDA: Malondialdehyde

MN: Micronuclei

MNPCEs: Micronucleated Polychromatic Erythrocytes

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

NBUDs: Nuclear Buds

NF-κB :Nuclear Factor kappa-light-chain-enhancer of Activated B Cells

NCEs: Normochromatic Erythrocytes

NPBs: Nucleoplasmic Bridges

8-oxoG: 8-Oxoguanine

PCEs: Polychromatic Erythrocytes

RAS: Renin-Angiotensin System

ROS: Reactive Oxygen Species

SOD: Superoxide Dismutase

SPSS: Statistical Package for the Social Sciences

المستخلص العربي

يُعدّ الماء عنصرًا أساسيًا لاستمرار الحياة، ويمكن أن يؤدي الحرمان منه إلى عواقب صحية وخيمة على الإنسان. ومع تفاقم أزمة ندرة المياه على مستوى العالم، والتي تؤثر على ملايين الأشخاص، تبرز الحاجة الشديدة إلى فهم التأثيرات السُمّية للحرمان من الماء على الأعضاء الحيوية، بهدف تطوير استراتيجيات فعالة للتخفيف من هذه الآثار. و لذلك تهدف هذه الدراسة إلى تقييم السُمّية الناتجة عن الحرمان من الماء من خلال قياس الإجهاد التأكسدي في الكلى، والتغيرات النسيجية فيها، بالإضافة إلى دراسة السُمّية الجينية والخلوية في نخاع العظم. استخدمت الدراسة أربعين فأرًا أبيض من الذكور، وُزّعوا على أربع مجموعات: مجموعة ضابطة، وثلاث مجموعات خضعت لأنماط مختلفة من تقييد الماء (مؤقت، مستمر، ومتقطع).أظهرت النتائج انخفاضًا ملحوظًا في نشاط إنزيمات مضادات الأكسدة، وزيادة في مؤشرات الإجهاد التأكسدي في الكلى، مترافقة مع تضرر نسيجي شمل تنكّسًا أنبوبيًا وتضخمًا في الحبيبات. كما أظهر اختبار النواة الدقيقة ارتفاعًا في مؤشرات السُمّية الجينية والسُمّية الخلوية في نخاع العظم، مما أدى إلى اضطرابات في عملية تكوّن كريات الدم الحمراء. وتُشير هذه النتائج إلى أن الحرمان من الماء يُحدث أضرارًا كبيرة في وظائف الكلى ونخاع العظم، وكان نمط التقييد المتقطع هو الأكثر تأثيرًا من حيث السُمّية. وتُبرز هذه النتائج خطورة نقص المياه على الصحة العامة، كما تؤكد أهمية الحفاظ على الترطيب الكافي لضمان سلامة الأعضاء الحيوية.استنادًا إلى نتائج الدراسة، نوصي بإعطاء أولوية قصوى لتوفير مياه شرب نظيفة وآمنة، وتعزيز ممارسات ترشيد استهلاك المياه، ودعم الأبحاث المبتكرة التي تسعى إلى إيجاد حلول فعالة لمواجهة ندرة المياه. كما نؤكد أهمية تنفيذ برامج توعوية تسلط الضوء على المخاطر الصحية المرتبطة بالحرمان من الماء، إلى جانب دعم المبادرات المجتمعية التي تساند الفئات المتضررة.إن إدراك السُمّية الناتجة عن الحرمان من الماء، واتخاذ الإجراءات الاستباقية اللازمة، يُعدّ أمرًا بالغ الأهمية للحد من آثاره الصحية، ويُساهم في تعزيز الصحة العامة وتحقيق أهداف التنمية المستدامة. وتُبرز هذه النتائج أهمية تبنّي سياسات فعالة ومستدامة في إدارة الموارد المائية، لحماية الفئات السكانية الأكثر عرضة للخطر من الأضرار الناجمة عن ندرة المياه.