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The Pharmaceutical Toxicity-Induced Oxidative Stress and Liver Enzyme Dysfunction in Albino Rats

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Abstract:
Introduced in 1952, the synthetic antibiotic isoniazid remains a critical first-line medication for tuberculosis. Its effectiveness is largely attributed to its high selectivity and potent activity against Mycobacterium tuberculosis. The toxicological effects of isoniazid on albino rats are investigated in this work. Albino rats were used in the controlled laboratory setting for the investigation. the LD_{50} was calculate by use probit analysis, the LD_{50} vale was 1500 mg/kg. the chronic exposure period was 30 days, using the sub lethal concentration 150 mg/kg (10% of LD50) of isoniazid, rats were split into two groups: control, and treatment, by use three replications for each, each replication contain 5 rats. The liver function enzymes (ALT, AST, and ALP), environmental stress enzymes (MDA), and oxidative stress enzymes (CAT and SOD) were measured at 1, 15, and 30 days of experiment. Results demonstrated that isoniazid exposure led to significant increases in antioxidant enzymes like CAT in rats shows a significant increase in all days of the experiment at ($p < 0.001$), while SOD shows non-significant decrease in the 1st day, and non-significant increased 15th day of the experiment and recorded increased non-significant in last days. MDA shows non statistically change through all experimental days at ($p < 0.05$). Liver function values such as AST and ALP which show non-significant decrease in all days of experiments, while the ALT value shows non-significant increase in experiments, at ($p < 0.05$). The study concluded that exposure to low amounts of Isoniazid leads to liver damage, Isoniazid induces oxidative stress in rats. which reduces rats' health.
Keywords: *Antioxidant Enzymes, Isonzaide, Lipid Peroxidation, Liver function enzymes, toxicology.*

INTRODUCTION:

Pharmaceutical compounds and their breakdown products are entering the environment primarily through human and animal excretion after drug use (Delgado et al., 2023). Among these, antibiotics, reproductive hormones, and hormone-mimicking estrogens are particularly concerning due to their high concentrations in the surface waters of lakes and rivers globally (Kakkar & Kumar, 2024). The detection of these pharmaceuticals, even at low concentrations, in complex chemical mixtures in surface waters highlights significant environmental risks (Hamzah, et al., 2023) This underscores the urgent need for robust ecological risk assessment methods to evaluate their impact on aquatic ecosystems and water quality (Liu, et al., 2025).

Isoniazid (INH), a synthetic antibiotic, has been a cornerstone of tuberculosis treatment since 1952 due to its potent and selective action against Mycobacterium tuberculosis (Gegia et al., 2017; Dartois & Rubin, 2022). Pharmaceutical analysis and therapeutic drug monitoring of INH in both pharmaceutical and biological samples are essential for understanding its bioavailability, bioequivalence, and for effective therapeutic management during patient follow-up (Phanouvong & Sriphong, 2023).

Despite its efficacy, INH poses significant risks to non-target species, particularly mammals in terrestrial environments. Studies in male albino mice indicate that INH therapy can induce hepatotoxicity, disrupting liver architecture (Humayun et al., 2017), and may also have immunosuppressive effects (Brindha, 2016). Histological findings in most mouse studies suggest an early onset of steatosis (Peng et al., 2020). Research in rats demonstrates that co-administering a low dose of vitamin C can mitigate INH-induced liver injury by reducing oxidative stress, with high doses not offering additional antioxidant benefits (Ergul et al., 2010). Oxidative stress and lipid peroxidation are commonly assessed using biomarkers such as malondialdehyde (MDA) levels, catalase (CAT) activity, and superoxide dismutase (SOD) activity (Carmo de Carvalho e Martins et al., 2022). This underscores the critical need to evaluate chronic exposure to sublethal doses of environmental contaminants, like pharmaceutical waste, to fully comprehend their cellular damaging mechanisms and associated risks.

MATERIALS AND METHODS

Rattus norvegicus var albinus, or albino rats, weighed 150 ± 5g. To become used to the new conditions, rats were brought to the lab in cages and kept in several plastic containers for 14 days. The temperature in the lab stayed at 19 ± 2 °C (de Boer & Koolhaas, 2024). The rats were fed commercial rat food during

the acclimation and testing period (Mohammed and Algburi., 2025). We conducted a static acute toxicity test after 14 days of acclimatization (Yuan et al.,2014). The 99% pure isoniazid that was sold by the General Company for the Manufacture of Medicines and Medical Supplies / Samarra was used to create isoniazid test solutions. To determine LD_{50} values, rats were exposed to six different isoniazid concentrations, including a control group (0, 250, 500, 1000, 2000 and 4000 mg/kg). Mortality rates were recorded at 24, 48, 72, and 96-hours' post-exposure. The LD_{50} value was subsequently calculated using Probit analysis (Finney, 1971). The sub-lethal toxicity test was conducted for 30 days. Rats were randomly divided into two groups, with three replicates per group and five individuals per replicate (total of 15 rats per group). The experimental groups were exposed to isoniazid at one sub-lethal concentrations: 150 mg/kg (10% of the determined LD_{50}), These concentrations were selected to evaluate the chronic effects of isoniazid exposure. The blood was collected 3 time through experiment period in (1,15,30) days of experiment for physiological parameters (Algburi, & AL-Amari, 2023).

The liver function AST,ALT.ALP estimate in serum, were carried out using DRI-CHEM NX500 fujifilm biochemistry analyzer (Mohsin, et al., 2024).

Catalase (CAT)

Catalase activity was determined using a continuous spectrophotometric rate assay. This technique measures the degradation of hydrogen peroxide (H_2O_2) by monitoring the decrease in absorbance at 240 nm. One unit of catalase is defined as the amount of enzyme that degrades 1.0 µmole of H_2O_2 per minute at pH 7.0 (Wiseman, 2010).

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity was determined spectrophotometrically using a colorimetric method adapted from Marklund and Marklund (1974). The reaction mixture comprised 20 µl of serum, 2 ml of Tris buffer, and 0.5 ml of 0.2 mM pyrogallol, with absorbance measured at 420 nm. A blank solution containing all components except serum, replaced by water, was used for calibration. One unit of SOD activity was defined as the amount of enzyme capable of inhibiting 50% of pyrogallol oxidation. SOD activity was calculated using the formula:

$$SOD\ Activity\ (unit) = (\%P/50\%) \times R/T$$

MDA

Malondialdehyde (MDA) levels, indicative of lipid peroxidation, were quantified using the Thiobarbituric Acid (TBA) assay. This spectrophotometric method relies on the reaction between two molecules of thiobarbituric acid and one molecule of malondialdehyde, resulting in a pink MDA-TBA complex. The absorbance of this complex is then measured at 535 nm (Rifai, 2017).

3. RESULTS

The LD_{50} was found to be 1500 mg/kg after exposed to isonizide

Aspartate aminotransferase (AST/GOT)

The result of AST enzyme value was highest on 1st day (120.33). Then AST value significantly increased by 15th day (136.670), with a slight continued decreased until last day (115.670). Despite these numerical changes, statistical analysis revealed no significant difference, indicating that the isonzaide did no significantly affect AST enzyme levels, as in figure (1).

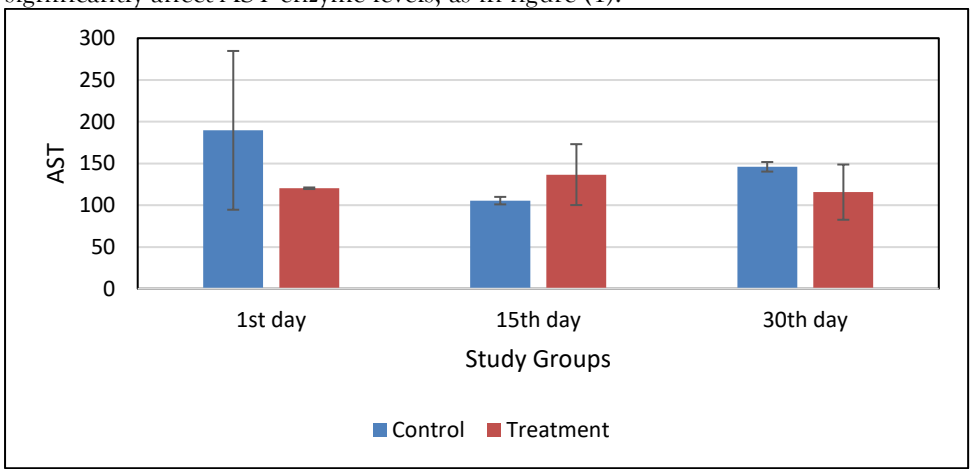

Fig:1 Aspartate aminotransferase of Rattus norvegicus after exposure to sub-lethal concentration of Isonzaide.

The study result findings align with previous research indicating that isoniazid commonly leads to a small, temporary increase in liver enzymes, specifically AST/ALT, during the initial weeks of treatment. Importantly, these elevations do not typically reach levels that suggest disease or become permanent (National Library of Medicine, 2024; LiverTox, 2023). This observation is further supported by clinical reviews, such as that by Chalasani et al. (2007), which similarly report that AST elevations are often brief and not linked to a decline in liver function.

Alanine Aminotransferase (ALT/GPT)

The result of ALT enzyme value highest on the 1st day (44.670 ±18.0), the ALT levels were similar between the control and treatment groups, with the treatment group showing a slightly higher value. On the 15th and 30th days, there is a significant increase in the ALT levels in the treatment group compared to the control group. Indicating that the result of enzyme remained relatively high during the first half of the study period and decreased in last days. suggesting a potential effect of the treatment in reducing this enzyme throughout the study period, as in figure (2).

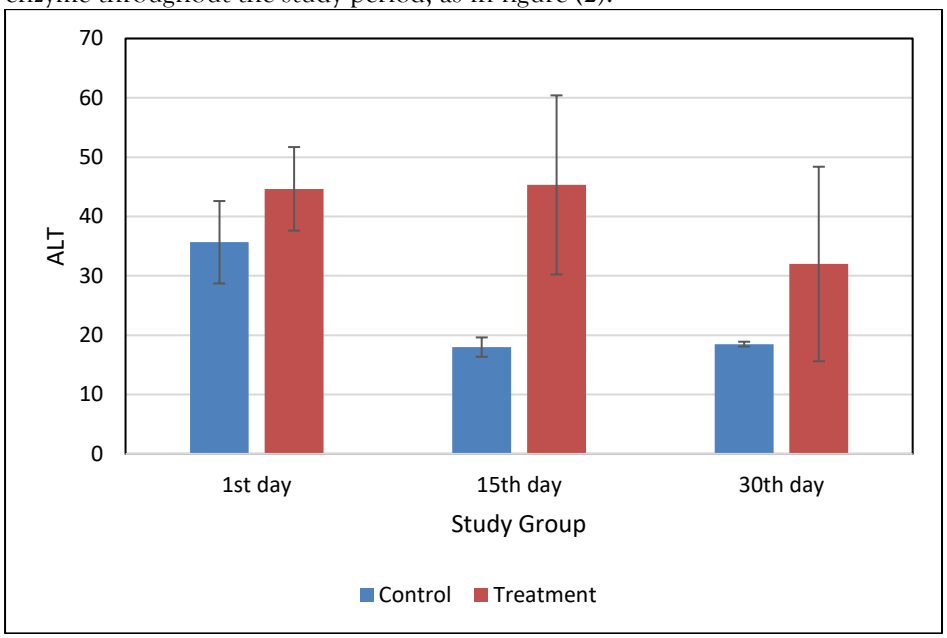

Fig:2 Alanine Aminotransferase of Rattus norvegicus after exposure to sub-lethal concentration of Isonzaide.

ALT is an enzyme primarily found in liver cells. Elevated levels of ALT in the blood are a key indicator of liver damage or disease. The leakage of this enzyme into the bloodstream signifies injury to the liver tissue. The elevated ALT levels in the treatment group throughout the study, especially on the 15th and 30th days, suggest that the pharmaceutical compound is causing hepatotoxicity, which is liver damage. This increase indicates that the liver cells in the treated rats are being injured, causing the ALT enzyme to be released into the bloodstream at a higher rate than in the control group. The continued elevation over time suggests a persistent toxic effect of the compound on the liver (Kaizal, et al., 2024).

Alkaline phosphatase (ALP)

Alkaline phosphatase (ALP) results show a decrease in ALP levels in the treatment group compared to the control group on the 1st day, followed by a recovery and increase over the 15th and 30th days. On the 1st day, the control group had an average ALP level of approximately 225 U/L, while the treatment group had an average of about 155 U/L. This represents a significant initial decrease in ALP levels in rats exposed to the pharmaceutical compound. This initial drop could be due to the compound's direct inhibitory effect on the enzyme or a sudden disruption of the metabolic processes that produce it. By the 15th day, the ALP levels in the treatment group rose to an average of about 208 U/L, approaching the control group's level of roughly 205 U/L. On the 30th day, the treatment group's ALP levels continued to rise to an average of around 245 U/L, which is slightly lower than the control group's average of 265 U/L, as in figure (3).

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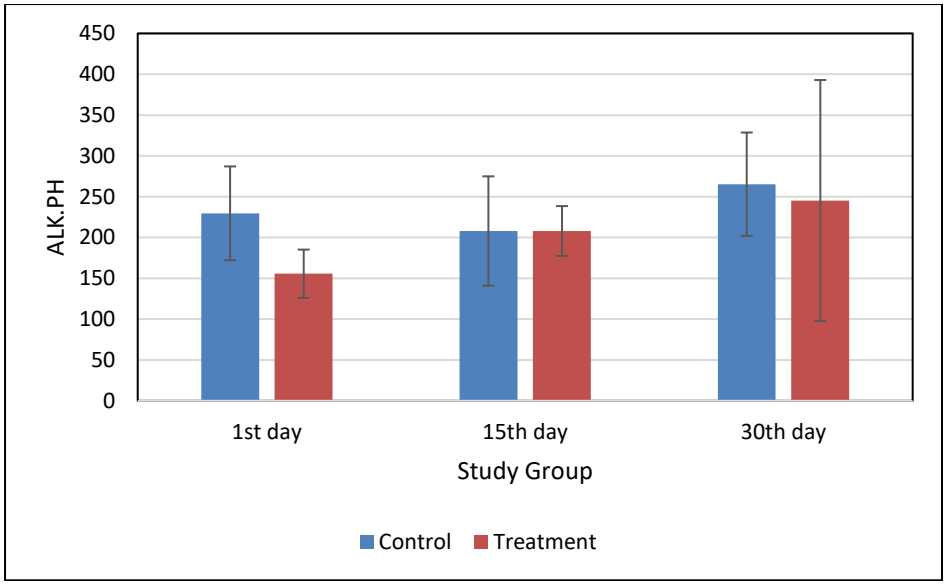

Fig:3 Alkaline Phosphatase of Rattus norvegicus after exposure to sub-lethal concentration of Isonzaide 1, 15, 30 days.

The observed pattern—an initial decrease followed by a subsequent rise—suggests that the pharmaceutical compound may have an acute inhibitory effect on ALP, which the body then compensates for over time. The body may have developed a tolerance to the compound, or the drug could have been metabolized, leading to a reduction in its inhibitory effect. Another possibility is that the initial drop in ALP triggered a compensatory mechanism, leading to an overproduction of the enzyme as a protective response. This compensatory rise in ALP could also indicate subclinical liver or bone stress, as ALP is an important marker for both. For example, damage to liver cells can release ALP into the bloodstream, and increased bone turnover (which can be a stress response) also elevates ALP. The wide variation on the final day highlights the need for a larger sample size and further statistical analysis to draw definitive conclusions.

Catalase (CAT)

CAT enzyme value was notably high on first day (150.56), a finding that was statistically significant (0.01). This elevated level was largely maintained through 15th day (148.89), with continued statistical significance (0.008), suggesting the treatment's effectiveness in sustaining high enzyme levels during this initial period. However, by last day, the enzyme concentration decreased significantly to (30.60), indicating a decline in the treatment's effect or a potential exhaustion of its ability to elevate enzyme levels with prolonged exposure, as in figure (4).

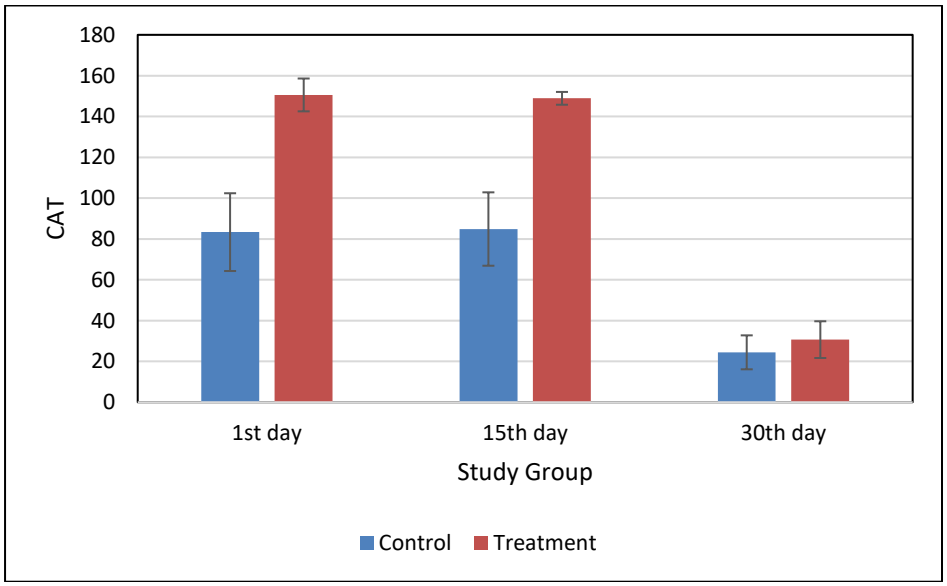

Fig:4 CAT activity of Rattus norvegicus after exposure to sub-lethalconcentration of Isonzaide.

The metabolism of Isonzaide can lead to the generation of reactive oxygen species (ROS), including superoxide radicals ($O_2^{\cdot}$). These radicals are produced early in the metabolic process, often within hours

of exposure (Girón-Pérez et al., 2021). Superoxide radicals are tuns into hydrogen peroxide by superoxide dismutase (SOD), an enzyme essential for managing oxidative stress (Oruc, 2010). The accumulation of (H_2O_2) triggers the upregulation of catalase (Domínguez et al., 2010).

The results also show that the treatment directly impacted the CAT enzyme early in the experiment, with its effect clearly diminishing and enzyme levels decreasing toward the study's end. This pattern might indicate either the exhaustion of the physiological effect or the body's adaptation to the compound after prolonged exposure.

Superoxide Dismutase (SOD)

Superoxide Dismutase (SOD) enzyme showed in result exhibited an upward trend across the study duration. Starting at 26.84 on day 1, levels increased to 38.670 by day 15, and ultimately reached their highest value of 44.440 on day 30. Despite this consistent increase, no statistically significant differences were observed between the various time points. This suggests that the observed changes in SOD levels may be attributable to natural biological factors rather than a definitive effect of the treatment, as in figure (5).

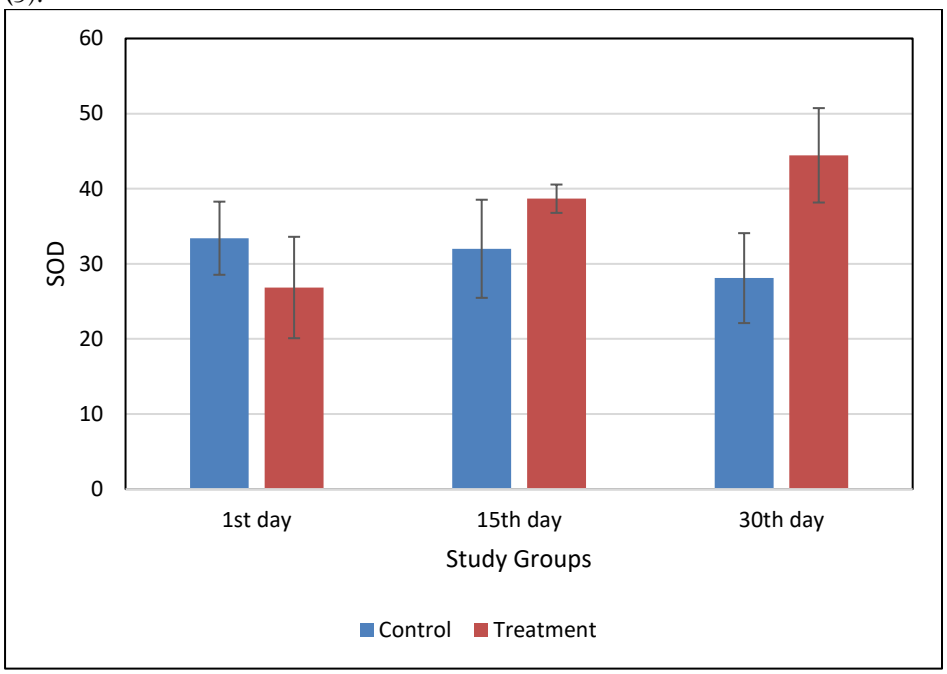

Fig:5 SOD activity of Rattus norvegicus after exposure to sub-lethal concentration of Isonzaide.

Superoxide dismutase (SOD) serves as a critical antioxidant enzyme that rapidly converts (O2-) into hydrogen peroxide (H2O2) to mitigate oxidative damage. This enzymatic activity represents a first-line defense against ROS-induced cellular damage. The initial rise in SOD activity after diazinon (Akturk et al., 2006).

A biological stress response is typically indicated by the pattern of SOD levels in the treatment group first declining and then gradually rising. Reactive oxygen species (ROS) were probably sharply increased by the pharmaceutical ingredient at first, overwhelming the body's natural antioxidant defenses. The body's systems, especially those that produce antioxidants, were elevated in response to the continuous oxidative stress. SOD levels significantly increased on days 15 and 30, which suggests that the body is actively attempting to repair the oxidative harm the chemical has produced. The goal of this protective, compensating process is to restore the equilibrium between pro-oxidants and antioxidants by increasing SOD.

Malondialdehyde (MDA)

MDA values initial level on first day was 5.08, rising slightly to a highest value of 5.61 on 15th day, before decreasing to a lowest of 4.92 on last day. Despite MDA result slight variations, no statistically significant differences were observed between the different time points, indicating that the treatment did not significantly impact MDA levels, as in figure (6).

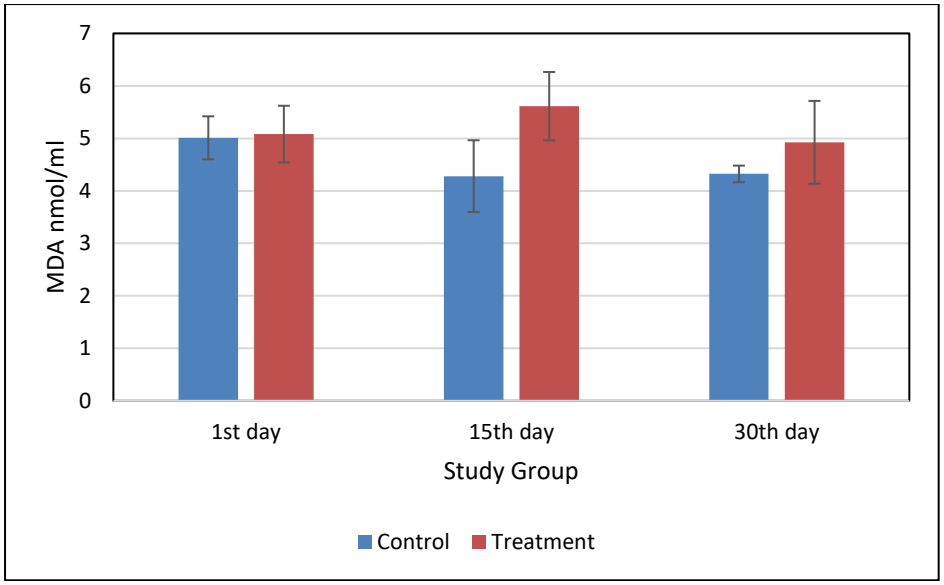

Fig:6 MDA activity of Rattus norvegicus after exposure to sub-lethal concentration of Isonzaide.

Isonzaide, induces oxidative stress by generating reactive oxygen species (ROS) in the body. ROS are highly reactive molecules that can damage cellular components, including lipids, proteins, and DNA (Hammad & Ziada, 2019).

The increase in ROS leads to lipid peroxidation, where unsaturated fatty acids in cell membranes are oxidized, resulting in the formation of malondialdehyde (MDA). MDA is a stable end product of lipid peroxidation and serves as a reliable marker for oxidative damage (El-Beltagi & Mohamed, 2013).

The most important finding from this data is the sustained elevation of Malondialdehyde (MDA) in the treatment group. MDA is a common and reliable biomarker of lipid peroxidation, a process that occurs when oxidative stress damages cell membranes. An increase in MDA levels is a direct indicator of cellular damage caused by free radicals.

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