

## Aniline induced Liver and Spleen Toxicity in experimental Albino rat

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### Abstract

In the present study, the effects of aniline administration on albino rats were examined. The experiment was divided into four groups from which group I as (control) and group II, III, IV (treated) rats were administered daily dose of 6 mg/kg bw, 18 mg/kg bw, and 36 mg/kg bw of aniline for duration of 21 days respectively. The findings revealed a notable inverse relationship between aniline dose and body weight, with higher doses corresponding to reduced body weight among treated rats. Conversely, liver and spleen weights showed a marked increase proportional to the aniline dose. Additionally, level of proteins was reduced, while cholesterol and level of SGOT and SGPT were increased in liver with increasing dose of aniline compared to group I (control). These results indicate that aniline exposure leads to substantial changes in body weight, liver and spleen weights, and a series of dose-dependent biochemical alterations. This suggests that aniline toxicity has measurable impacts on both physiological and metabolic parameters in albino rats.

**Key words :** Aniline, Methemoglobin, Liver, Spleen, Albino rat.

Aniline (C<sub>6</sub> H<sub>5</sub> NH<sub>2</sub>), an aromatic amine, and its derivatives are extensively used as chemical intermediates in the synthesis of various industrial products, including pesticides, herbicides, rubbers, synthetic fibers, dyes, and diphenylamine. They also serve as precursors for azo dyes used in polyester fabrics and textiles, as well as in the pharmaceutical industry, particularly in the production of paracetamol (acetaminophen), one of the most widely

consumed pharmaceutical compounds<sup>3</sup>. Despite its industrial significance, aniline is a toxic compound that poses significant ecological risks<sup>18</sup>. It is primarily released into the environment through industrial processes and can be found in industrial wastewater and leachates from disposal sites<sup>9</sup>. In India, aniline is commonly detected in the effluents of basic organic chemical manufacturing plants<sup>15</sup>.

In mammals, aniline is readily metabolized into several metabolites, including phenylhydroxylamine, 2-aminophenol, and 4-aminophenol<sup>6</sup>. Acute exposure to aniline in humans can lead to symptoms such as cyanosis resulting from methemoglobinemia, headaches, nausea, vomiting, confusion, vertigo, weakness, numbness, drowsiness, and even coma<sup>7</sup>.

Chronic exposure is associated with symptoms including loss of appetite, reduced body weight, and skin lesions<sup>22</sup>. Toxicological studies conducted on experimental animals have demonstrated that aniline exposure results in methemoglobinemia, hemolysis, and hemolytic anemia<sup>4</sup>. Furthermore, prolonged exposure to aniline can lead to liver damage and hepatocyte injury<sup>20</sup>. The spleen, an essential component of the lymphatic system, is responsible for producing lymphocytes, filtering the blood, storing blood cells, and destroying old blood cells. Exposure to aniline has been shown to cause oxidative and nitrosative stress<sup>5,21</sup>, which can potentially result in cancer in the spleens of rats.

Given the potential toxicity of aniline, the present study aims to investigate in detail the effects of aniline on the liver and spleen in experimental albino rats. This research is crucial for understanding the toxicological impact of aniline on mammalian physiology, particularly regarding liver and spleen function, and can contribute to the development of safety guidelines for handling aniline and its derivatives.

*Objectives :*

The objective of this study is to explore

the toxic effects of aniline, a common industrial chemical, on the liver and spleen of albino rats. Aniline is widely used in manufacturing but poses serious health risks upon exposure. The liver, responsible for detoxification, and the spleen, important for immune function, are likely to be impacted by aniline toxicity. By measuring biochemical markers such as enzyme activity, protein levels, and cholesterol, this study aims to quantify the damage caused by aniline. Additionally, it seeks to identify the risks associated with prolonged exposure, providing insights into how aniline affects these vital organs and the potential long-term health hazards.

The study was conducted on albino rats (*Rattus norvegicus*) weighing between 200-250 g, sourced from Animal house. The rats were acclimatized and maintained under controlled environmental conditions with unrestricted access to food and water. A total of 24 rats were used in the experiment and were randomly divided into four groups, each comprising six rats. Group I served as the control group, while Groups II, III, and IV received oral doses of aniline at concentrations of 6 mg/kg bw, 18 mg/kg bw, and 36 mg/kg bw, respectively, for a duration of 21 days. The body weights of the rats were recorded before the start of the treatment and after sacrifice. Following the sacrifice, the liver and spleen were carefully dissected, cleaned, and weighed. Blood samples were collected in non-EDTA tubes for enzymatic analysis. Tissues were also collected, cleaned, and processed for biochemical analysis to determine protein and cholesterol levels using standard protocols. The levels of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were measured

using the UV-kinetic method, following the recommendations of the International Federation of Clinical Chemistry (IFCC). Protein content in liver tissues was estimated using Lowry's method<sup>16</sup>, and cholesterol levels were determined using Zake's method<sup>24</sup>. Data obtained from these analyses were statistically evaluated using the Student's t- test, with significance levels set at  $p < 0.05$  (\*),  $p < 0.001$  (\*\*), and  $p < 0.0001$  (\*\*\*)

*Body and Organs Weight :*

Table-1. Body and organs weight of control and aniline treated albino rats for 21 days duration

Groups	Initial body weight (g) before experiment	Final body weight (g) after experiment	Liver weight (g) after experiment	Spleen weight (g) after experiment
Group I (Control)	238.33±3.33	245±2.24	7.09±0.07425	0.68±0.0073
Group II (Treated) (6 mg/kg b. w. Aniline)	238±3.51	229.17±3.27 <sup>NS</sup>	7.52±0.07425 <sup>NS</sup>	0.76±0.01113*
Group II (Treated) (18 mg/kg b. w. Aniline)	239.17±2.39	221.67±2.47**	8.65±0.7405**	0.79±0.00712**
Group IV (Treated) (36 mg/kg b. w. Aniline)	237.5±3.1	216.67±2.47***	9.50±0.7416***	0.82±0.00704***

Values are presented as Mean ± SE, N = 06, For each group,  $p < 0.05$ \*,  $p < 0.001$ \*\*,  $p < 0.0001$ \*\*\*, NS = Not Significant.

The table-1 presents data on body weight, liver weight, and spleen weight across four groups, a control group and three groups treated with varying doses of aniline (6 mg/kg, 18 mg/kg, and 36 mg/kg body weight). The initial body weights were comparable among all groups before the experiment. Post-experiment, the control group exhibited a slight increase in body weight. In contrast, all treated groups demonstrated a dose-dependent decline in body weight. The group treated with 6 mg/kg of aniline showed a modest decrease which is not statistically significant, while the 18 mg/kg and 36 mg/kg groups experienced significant reductions. Additionally, liver and spleen weights increased in all treated groups compared to the control group after the experiment. The data suggest that exposure to escalating doses of aniline induces substantial physiological changes, notably in body weight, liver weight, and spleen weight. Higher doses of aniline result in a pronounced reduction in body weight and a marked increase in liver and spleen weights, indicating potential toxic effects that are dose-dependent. These findings underscore the necessity of understanding aniline's toxicity and its profound impact on body's organs.

Table-2. Enzymes (SGOT and SGPT) concentration of control and aniline treated serum of albino rats for 21 days duration

Groups	SGOT (IU/L)	SGPT (IU/L)
Group I (Control)	41.74±0.3026	32.02±0.3046
Group II (Treated) (6 mg/kg b. w. Aniline)	42.74±0.542*	32.85±0.1485 <sup>NS</sup>
Group II (Treated) (18 mg/kg b. w. Aniline)	43.90±0.2787**	33.47±0.1635**
Group IV (Treated) (36 mg/kg b. w. Aniline)	44.41±0.3952***	34.62±0.1676***

Values are presented as Mean ± SE, N = 06, For each group, p<0.05\*, p<0.001\*\*, p<0.0001\*\*\*, NS = Not Significant.

The table-2 presents data on the levels of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) across four groups: a control group and three groups treated with varying doses of aniline (6 mg/kg, 18 mg/kg, and 36 mg/kg body weight). In the group treated with 6 mg/kg of aniline, there was a slight increase in SGOT and SGPT levels compared to the control group. With increasing doses of aniline, both SGOT and SGPT levels exhibited further elevations. The group treated with 18 mg/kg of aniline showed a significant rise in both SGOT and SGPT levels. The group receiving

the highest dose of 36 mg/kg demonstrated the most pronounced increase in these enzyme levels. These findings indicate that escalating doses of aniline lead to a significant, dose-dependent elevation of SGOT and SGPT levels, suggesting that aniline exposure, especially at higher doses, may induce liver dysfunction or damage, as reflected by the increased levels of these liver enzymes. The data underscore the potential hepatotoxic effects of aniline and highlight the importance of monitoring liver enzyme levels to assess liver health and the risk of toxicity.

Table-3. Protein and cholesterol concentration of control and aniline treated liver and spleen of albino rats for 21 days duration

Groups	Liver Protein (mg/100mg)	Cholesterol (mg/100mg)	Spleen Protein (mg/100mg)	Cholesterol (mg/100mg)
Group I (Control)	29.49±0.1307	58.58±0.0973	17.41±0.1302	30.65±0.2903
Group II (Treated) (6 mg/kg b. w. Aniline)	28.75±0.1971*	59.17±0.2380*	16.75±0.1594**	31.67±0.1459*
Group II (Treated) (18 mg/kg b. w. Aniline)	27.46±0.1261***	60.14±0.0796**	15.82±0.1941***	32.47±0.1648***
Group IV (Treated) (36 mg/kg b. w. Aniline)	25.66±0.2997***	62.28±0.2632***	15.15±0.2936***	33.38±0.3416***

Values are presented as Mean ± SE, N = 06, For each group, p<0.05\*, p<0.001\*\*, p<0.0001\*\*\*

The table-3 presents data on liver and spleen protein and cholesterol levels in subjects exposed to varying doses of Aniline. The study includes a control group and three treated groups administered 6 mg/kg, 18 mg/kg, and 36 mg/kg body weight for 21 days. Exposure to Aniline led to a significant, dose-dependent reduction in liver protein content and an increase in liver cholesterol levels, suggesting potential hepatic dysfunction. While spleen protein levels were slightly reduced, they were not significantly different from those in the control group. However, the increase in spleen cholesterol levels indicates possible alterations in spleen lipid metabolism due to Aniline exposure.

The present study demonstrates that higher doses of aniline (18 mg/kg and 36 mg/kg body weight) result in significant reductions in body weight compared to the control group. This reduction is primarily due to metabolic disruptions and the onset of hemolytic anemia, which collectively reduce energy levels and suppress appetite. Additionally, aniline exposure generates oxidative stress, leading to impaired organ function and nutrient absorption. Similar findings have been reported by several studies<sup>6,12</sup>. However, studies by Wu *et al.*,<sup>23</sup> concluded that body weights were not statistically significantly affected in their investigations.

In our study, we observed an increase in liver weight following higher doses of aniline, likely due to inflammation, liver cell hypertrophy, and the accumulation of toxic metabolites. This liver enlargement occurs as the organ attempts to detoxify and metabolize the chemical. These results are consistent with findings of previous study<sup>13</sup>. Moreover, a significant increase in

spleen weight was observed in the treated groups compared to the control group, attributed to splenic congestion and the accumulation of damaged red blood cells resulting from hemolytic anemia. This leads to spleen enlargement as it works to filter damaged cells and manage the increased workload. Our results align with studies conducted by<sup>10,11,17</sup>, on aniline-induced toxic effects.

Aniline exposure also induces oxidative stress and hepatotoxicity, leading to liver cell damage. In our study, liver function was assessed by examining SGOT and SGPT levels in control and aniline-treated albino rats. The results confirmed elevated enzyme levels in treated animals, particularly at higher doses, indicating liver damage. This observation is consistent with findings by Abebe *et al.*,<sup>1</sup> and Amin *et al.*,<sup>2</sup>, corroborating the hepatotoxic effects of aniline exposure.

Furthermore, aniline-induced liver damage impairs protein synthesis by disrupting normal liver functions, leading to a reduction in overall protein levels. Our study corroborates this decrease in protein levels following higher doses of aniline, consistent with the findings of Pauluhn,<sup>19</sup>. Finally, liver dysfunction caused by aniline disrupts lipid metabolism, impairing cholesterol processing and leading to cholesterol accumulation. Our findings revealed a significant increase in cholesterol levels in the organs of animals exposed to higher doses of aniline, which is in agreement with the results of Kovacs *et al.*,<sup>14</sup>. This study highlights the toxicological impact of aniline on albino rats, particularly regarding body weight, liver and spleen enlargement, liver function, protein levels, and cholesterol accumulation.

The present study concludes that higher doses of aniline significantly impact body weight, liver function, and spleen size in treated animals due to metabolic disruption, hemolytic anemia, and oxidative stress. The findings demonstrate increased liver and spleen weights, along with elevated SGOT and SGPT levels, indicating liver damage. Furthermore, aniline exposure results in decreased protein levels and elevated cholesterol, reflecting impaired liver function and altered lipid metabolism. Overall, this study confirms the toxic effects of aniline on the liver and spleen of albino rats.

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