International Journal of Ecology and Environmental Sciences
www.ecologyjournal.in
Online ISSN: 2664-7133
Received: 15-05-2026, Accepted: 15-06-2026, Published: 30-06-2026
Volume 8, Issue 2, 2026, Page No. 115-121

Toxicological assessment of 2,4-D in New Zealand Rabbits: Biochemical and electrolyte alterations in the blood with implications for the human food chain

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DOI: https://doi.org/10.66856/ijees.2026.8.2.8052

Abstract
The widespread use of 2,4-dichlorophenoxyacetic acid (2,4-D) herbicides has raised concerns regarding their potential toxicological effects on non-target organisms. This study evaluated the impact of 2,4-D exposure on biochemical, and electrolyte parameters in New Zealand rabbits following a 21-day exposure period. Forty rabbits were randomly assigned to three treatment groups and administered 3.00, 5.00, and 7.00 mg/L of 2,4-D, respectively. Blood samples were collected at the end of the experiment for analysis of serum enzymes, and electrolytes using standard laboratory procedures. Data were analyzed using one-way analysis of variance (ANOVA), and means were separated using Tukey's HSD test at $p < 0.05$. Results shows that serum enzymes evaluation were dose-dependent and recorded significant changes in aspartate aminotransferase, alanine aminotransferase, acid phosphatase. Serum electrolyte analysis demonstrated significant disturbances in potassium, chloride, calcium, and magnesium levels, particularly at higher concentrations of 2, 4-D exposure. The findings indicate that prolonged exposure to 2,4-D can induce biochemical, and electrolyte imbalances in New Zealand rabbits, suggesting potential adverse effects on blood physiology, organ function, and metabolic homeostasis. These results highlight the need for careful handling and monitoring of 2,4-D usage to minimize risks to animal health.

Keywords: 2,4-Dichlorophenoxyacetic acid, enzymes, electrolytes, biochemical parameters

Introduction

The increasing demand for food production has led to a corresponding rise in the use of agrochemicals across the world. Herbicides remain among the most frequently applied pesticides because of their ability to control weeds that compete with crops for nutrients, water, sunlight, and space (Burns & Boon, 2024) [4]. Among these herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D) has maintained a prominent position in modern agriculture for more than seven decades due to its effectiveness against broadleaf weeds and its relatively low cost of application (EPA, 2025). Although the herbicide has contributed significantly to agricultural productivity, concerns about its environmental fate and potential health consequences continue to attract scientific attention.

The widespread use of 2,4-D has resulted in its detection in agricultural soils, surface water, groundwater, vegetation, and atmospheric samples in several parts of the world (Islam *et al*., 2023; Burns & Boon, 2024) [4, 14]. Herbicide residues may enter surrounding ecosystems through spray drift, rainfall runoff, leaching, improper disposal practices, and repeated field applications. Once released into the environment, 2,4-D can interact with various biological systems and become available to organisms occupying different levels of the food chain. Consequently, exposure is no longer restricted to agricultural workers and pesticide applicators. Consumers may also encounter low levels of the herbicide through contaminated food products and drinking water sources (ATSDR, 2020; European Food Safety Authority, 2024) [7, 23].

The transfer of herbicide residues through the food chain represents an important environmental and public health issue. Crops cultivated in treated fields may absorb herbicide residues from soil and water, while livestock may ingest contaminated forage, feed, or water supplies. These residues can subsequently be transferred into edible animal products such as meat, milk, and eggs that form part of the human diet (Food Safety Commission of Japan, 2017; EFSA, 2024) [7, 9]. Although regulatory agencies establish maximum residue limits intended to protect consumers, concerns persist regarding cumulative exposure arising from repeated dietary intake over long periods. This concern is particularly relevant in developing countries where monitoring and enforcement programs may be less comprehensive (Khan *et al*., 2024) [15].

Scientific evidence suggests that exposure to 2,4-D may adversely affect both aquatic and terrestrial organisms. Experimental studies have demonstrated that the herbicide can induce biochemical and physiological disturbances in fish, amphibians, rodents, rabbits, and other laboratory animals (Martins *et al*., 2024; Farias *et al*., 2024) [8]. The toxic effects are often associated with the generation of reactive oxygen species and the disruption of antioxidant defense mechanisms, resulting in oxidative stress and cellular injury.

Oxidative stress is recognized as one of the principal pathways through which 2,4-D exerts its toxic action, leading to lipid peroxidation, protein damage, DNA injury, and altered cellular metabolism (Farias *et al*., 2024; Kowalska *et al*., 2025) [8, 17]. The liver is particularly susceptible to herbicide-induced toxicity because it serves as the primary organ responsible for the metabolism and detoxification of xenobiotics. Following exposure to 2,4-D, several studies have reported elevated serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP),

suggesting hepatocellular injury and impaired liver function (Martins *et al*., 2024; Islam *et al*., 2023) [8, 14]. Histopathological investigations have further revealed structural alterations including hepatocyte degeneration, inflammatory infiltration, and vascular congestion following prolonged exposure to the herbicide (Khan *et al*., 2024) [15].

Similarly, the kidneys may be affected because they play a major role in the elimination of toxic substances and their metabolites. Increased serum levels of urea and creatinine have been observed in experimental animals exposed to 2,4-D, indicating compromised renal function and reduced filtration efficiency (ATSDR, 2020; Martins *et al*., 2024) [8]. Renal damage associated with oxidative stress may impair the body's ability to maintain electrolyte balance and excrete metabolic waste products effectively.

Beyond hepatic and renal effects, emerging evidence indicates that 2,4-D may influence the nervous, reproductive, endocrine, and immune systems. Epidemiological investigations have linked long-term exposure to phenoxy herbicides with neurological dysfunction, reproductive abnormalities, endocrine disturbances, and increased risks of certain chronic diseases, although the magnitude of these associations remains an active area of research (Kim *et al*., 2023; Burns & Boon, 2024) [4, 16]. Recent toxicological reviews have emphasized the need for continuous evaluation of the health implications of chronic low-dose exposure, especially among vulnerable populations such as children, pregnant women, and individuals residing near agricultural areas (EFSA, 2024; EPA, 2025).

Because biochemical alterations often precede visible clinical signs of toxicity, serum biomarkers have become indispensable tools in toxicological investigations. Changes in enzyme activities, protein concentrations, metabolite levels, and indicators of oxidative stress provide valuable insight into organ function and the mechanisms underlying toxicant-induced injury. The assessment of biochemical parameters therefore offers a sensitive and reliable approach for detecting early physiological disturbances resulting from herbicide exposure (Martins *et al*., 2024; Farias *et al*., 2024) [8].

In view of the extensive agricultural use of 2,4-D and the growing concerns regarding its movement through environmental and food-chain pathways, continued toxicological assessment remains essential. Understanding how this herbicide influences biochemical processes and organ function will contribute to a more comprehensive evaluation of its safety profile and provide scientific evidence for improving public health protection, environmental management, and regulatory decision-making.

This study thus investigated the toxicological assessment of the herbicide, 2,4-Dimethylamine dichlorophenoxyacetic acid (SL) in New Zealand rabbits on:

1. Enzymes (AST, ALT and ALP) in the blood of New Zealand rabbits.
2. Electrolytes (Sodium Na^+, Potassium K^+, Chloride Cl^- and Magnesium Mg^{2+} ions) in the blood of New Zealand rabbits.

Materials and Method

1. Source of Rabbits and Transportation

A total of forty clinically healthy adult New Zealand rabbits with body weights ranging from 1.8 to 2.0 kg were utilized for this toxicological study. The experimental animals were obtained from a reputable commercial rabbit farm situated in Mbiama, Rivers State, Nigeria. Following procurement, the rabbits were carefully conveyed to the experimental animal facility in well-aerated plastic transport cages. Appropriate precautions were taken during transportation to minimize handling stress, physical trauma, and other factors that could influence physiological responses prior to exposure.

2. Acclimatization

Upon arrival at the experimental facility, the rabbits were housed individually in properly maintained rabbitry pens and allowed a two-week acclimatization period before the commencement of herbicide exposure. During this period, the animals were observed daily to assess their health status and adaptation to the laboratory environment. Commercial rabbit pellets were supplied as feed, while clean drinking water was provided without restriction throughout the acclimatization period. Only animals that exhibited normal behaviour, maintained good health, and showed no signs of disease were selected for the definitive experiment. Mortality during acclimatization remained below 5%, indicating the suitability of the animals for toxicological assessment.

3. Range-Finding Test

A preliminary toxicity evaluation was conducted to establish suitable sublethal exposure levels of 2,4-dichlorophenoxyacetic acid (2,4-D) for the main experiment. The range-finding study lasted for fourteen days and involved exposing selected rabbits to different concentrations of the herbicide. Information obtained from the preliminary trial, together with previously published toxicological data, was used to determine the concentrations employed in the definitive study.

Preparation of the herbicide solutions was carried out using the dilution formula described by Inyang (2008) [12]:

$N_1V_1 = N_2V_2$

Where:

N_1 = concentration of the stock solution
V_1 = volume of stock solution required
N_2 = desired concentration of the test solution
V_2 = final volume of the prepared solution

The concentrations selected for the main experiment were those that produced observable physiological alterations without causing mortality, thereby ensuring assessment of chronic sublethal toxicity.

4. Sublethal Experimental Design

The toxicological experiment was conducted using a completely randomized design over a twenty-one-day exposure period. The rabbits were assigned to four experimental groups comprising one control group and three treatment groups, with each group replicated three times. Animals in the treatment groups received 2,4-D at concentrations of 3.00 mg/L, 5.00 mg/L, and 7.00 mg/L, respectively, whereas the control group was not exposed to the herbicide. Throughout the exposure period, all experimental animals were maintained under identical environmental conditions with respect to housing, feeding regime, water supply, and general management practices. This ensured that any observed biochemical changes could be attributed primarily to herbicide exposure rather than environmental variation.

5. Termination and Preparation of Samples for Analysis

At the conclusion of the twenty-one-day exposure period, blood samples were collected aseptically from the marginal ear vein of each rabbit using sterile needles and syringes. The collected blood was transferred into plain centrifuge tubes and centrifuged at 3,000 revolutions per minute for 15 minutes to facilitate serum separation. Following centrifugation, the serum fraction was carefully harvested and transferred into appropriately labelled sample containers. The serum samples were subsequently transported under suitable conditions to the Chemical Pathology Laboratory of the Federal Medical Centre, Yenagoa, where they were analyzed for selected serum enzyme activities and electrolyte concentrations. These biochemical parameters served as important indicators for evaluating the toxic effects of 2,4-D exposure on physiological and metabolic functions in the experimental animals.

6. Statistical Analysis

Data generated from the toxicological experiment were summarized and presented as mean ± standard deviation (SD). Statistical evaluation of treatment effects was performed using one-way analysis of variance (ANOVA) to determine the existence of significant differences among experimental groups. Where ANOVA revealed significant variation, Tukey's Honestly Significant Difference (HSD) post hoc test was employed for pairwise comparison of group means. All statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) version 28.0. Statistical significance was accepted at a probability level of $p < 0.05$.

Results

1. Enzymes in the Blood

The sublethal effect of toxicant on enzymes in the blood of exposed rabbits is presented in **Table 1** below. The result revealed a sharp decline in an irregular pattern of the values of Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT). The value of serum AST declined to 54.65±17.08 µ/L at 3.00 mg/L, 78.40±33.43 µ/L at 5.00 mg/L and 67.93±43.48 µ/L at 7.00 mg/L compared to the control of 96.89±18.67 µ/L, while that of Alanine Aminotransferase (ALT) declined to 105.86±56.70 µ/L at 3.00 mg/L, 120.62±71.90 µ/L at 5.00 mg/L and 129.83±23.69 µ/L at 7.00 mg/L compared to the control of 136.02±27.08 µ/L. On the contrary, the value of ACP in the blood increased in an irregular pattern to 4.17±2.24 µ/L at 5.00 mg/L and 1.47±2.08 µ/L at 7.00 mg/L compared to the control of 0.36±0.51 µ/L, even though there was no appreciable change at the first intoxication as shown in Table 1 below.

Table 1: Effect of 2,4-D on enzymes in the blood of New Zealand Rabbit after 21 days exposure (mean ±SD).

Conc. of 2,4-D (mg/L)	AST (µ/L)	ALT (µ/L)	ACP (µ/L)
Control	96.89±18.67	136.02±27.08	0.36±0.51
3.00	54.65±17.08	105.86±56.70	0.17±0.24
5.00	78.40±33.43	120.62±71.90	4.17±2.24
7.00	67.93±43.48	129.83±23.69	1.47±2.08

Different superscripts in a column indicate significant difference (ANOVA + Tukey HSD, $p < 0.05$).

2. Electrolytes in the Blood

The relative activity of electrolytes (Sodium, Potassium, Chloride, Calcium and Magnesium) in the blood of *New Zealand Rabbits* after 21 days of exposure to 2,4-D are presented in Table 2. The values of sodium (Na^+), potassium (K^+) and chloride in the blood recorded significant decline from 185.28±5.77 mmol/L to 181.72±26.71 mmol/L, 18.55±0.42 mmol/L to 9.52±1.68 mmol/L and 10.02±1.34 mmol/L to 5.33±1.24 mmol/L, compared to the controls (186.04±6.29 mmol/L, 15.04±3.55 mmol/L and 9.13±0.45 mmol/L). On the other hand, the values of calcium and magnesium significantly ($P<0.05$) appreciated in the blood from 0.45±0.50 mmol/L to 0.79±0.62 mmol/L compared to the control (0.49±0.63 mmol/L) and 0.41±0.10 mmol/L to 1.13±0.18 mmol/L compared to the control (0.46±0.09 mmol/L). This result is presented in Table 2.

Table 2: Activities of Electrolytes in the blood of New Zealand Rabbits exposed to 2,4-D for 21 days (Mean ± SD).

Conc. of 2,4-D (mg/L)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Ca^{2+} (mmol/L)	Mg^{2+} (mmol/L)
Control	186.04 ± 6.29^b	15.04 ± 3.55^a	9.13 ± 0.45^a	0.49 ± 0.63^b	0.46 ± 0.09^b
3.00	185.28 ± 5.77^b	18.55 ± 0.42^a	10.02 ± 1.34^a	0.45 ± 0.50^b	0.41 ± 0.10^b
5.00	181.87 ± 2.14^b	7.13 ± 2.46^b	5.19 ± 1.19^b	0.21 ± 0.05^b	0.42 ± 0.33^b
7.00	181.72 ± 26.71^a	9.52 ± 1.68^b	5.33 ± 1.24^b	0.79 ± 0.62^a	1.13 ± 0.18^a

Different superscripts in a column indicate significant difference (ANOVA + Tukey HSD, $p < 0.05$).

Discussions

1. Enzymes in the Blood

Biochemical actions are controlled by enzymes and hormones which are proteins hence, the evaluation of enzyme actions in plasma can be regarded as an analytical device to decide the physiological position of cells or tissues of organisms (Manoj, 1999) [20]. Consequently, when cells and organs are damaged due to a toxicant effect or drugs, enzymes are released into the blood, and this serves as a biomarker for assessing the health of vital organs such as liver, kidney, heart, muscles etc in exposed animals.

Alterations of enzymes activities as a result of xenobiotics in diverse organs of organisms have also been accounted by Sastro and subhadra (1985), Begum (2004), and Semanta and colleagues, (2014) [3, 25, 28]. Such biochemical modifications in organisms are intended at upholding stability in the presence of these poisons, which are usually upset physiological and biochemical procedures according to Wedemeyer and Mcleay, (1981) [30]. The outcome of the effect of AminoForce on enzymes in exposed rabbits is presented in Table 1 of this study. Aspartate Aminotransferase (AST) and Alanine Aminotransferase are known liver and kidney enzymes that are released into the blood following injury, damage or death of cells (Wedmemeyer & Mcleay, 1981).

Increased AST or ALT levels in the blood of exposed animals is seen with liver disease, myocardial inferaction as well as the toxic activities of some xenobiotics or poisons such as 2, 4-dimethylamine salt. Their concentrations in the

blood or plasma could also increase on the inducement of cholesterol-lowering toxicants or xenobiotics (Reddy & Sethunathan, 1983) [24]. There are lots of enzymes discovered in the plasma that did not come from the extracellular fluid. At some stage in destruction of tissue, a number of of these enzymes move into the plasma via seepage as a result of modification in membrane permeability (Gill *et al*., 1991) [11]. Plasma enzyme measurements are thus an important device in clinical analysis, offering message on the impact and character of pathological injury to any tissue**.** Most importantly, AST and ALT are cytosolic enzymes and any injury to structural reliability of tissues or organs is constantly showed by their presence or release into the blood.

As shown in Table 1 of this study, the value of AST in the blood declined to 54.65±17.08 µ/L when treated with 3.00 mg/L of toxicant compared to the control of 96.89±18.67 µ/L. But, as the toxicant concentration was increased to 5.00 mg/L, the value of AST slightly appreciated to 78.40±33.43 µ/L compared to its value in previous concentration (54.65±17.08 µ/L), but still lower than the control of 96.89±18.67 µ/L. AST values in exposed animals experienced further decline from 78.40±33.43 µ/L at 5.00 mg/L to 67.93±43.48 µ/L at 7.00 mg/L of toxicant concentration. This is still slightly lower compared to the control of 96.89±18.67 µ/L respectively. The decline in the values of AST recorded in this study occurred in an irregular pattern and was not dose dependent.

The values of plasma ALT in this study also recorded a decline in a non-dose dependent pattern ranging from 105.86±56.70 µ/L at 3.00 mg/L to 120.62±71.90 µ/L at 5.00 mg/L and 129.83±23.69 µ/L at 7.00 mg/L compared to the control of 136.02±27.08 µ/L. From the results, the values of ALT also showed an increasing trend after the first major decline to 105.86±56.70µ/L at 3.00 mg/L compared to the control of 136.02±27.08 µ/L, which perhaps is indicative of the response of exposed animals to stress induced by toxicant and its effect on biochemical parameters. There was also an observed gradual appreciation of the values of ALT measured in this study as toxicant concentration increases. The increasing trend of ALT in the blood of exposed rabbits, simply suggests damage to tissues and organs responsible for maintaining physiological balance in exposed animals. This is because, during tissue damage, some liver and kidney or other tissue enzymes move into the plasma via seepage due to modifications of membrane permeability as reported by Gill *et al.,* (1991) [11].

The trend of gradual but non-dose dependent elevation in the level of AST from 54.65±17.08 µ/L at 3.00 mg/L to 78.40±33.43 µ/L at 5.00 mg/L and 67.93±43.48 µ/L at 7.00 mg/L as well as that of ALT from 105.86±56.70 µ/L at 3.00 mg/L to 120.62±71.90 µ/L at 5.00 mg/L and 129.83±23.69 µ/L at 7.00 mg/L, but less than the respective controls (96.89±18.67 µ/L and 136.02±27.08 µ/L) could also be an indication of the biochemical response of exposed animals to the effect of AminoForce containing 2, 4-dimethylamine salt. From the report of past authors, xenobiotics are known to induce stress conditions on exposed animals which could lead to conditions of malnutrition that may result in abnormal feeding conditions (Inyang, 2008) [12]. To overcome the stress induced by these foreign xenobiotics on exposed animals, ALT and AST activities may be hampered in the kidney and liver leading to possible organ damage thereby causing the leach of these enzymes into the blood.

The reduction in the values of AST and ALT in the blood of exposed rabbits as compared to the control in **Table 1** could possibly be an indication of inhibition activity of the toxicant on AST and ALT activities of probe animals which may result in mild alterations in some biochemical processes in exposed rabbits of this study. It also could probably be due to low concentration of toxicant used in this study. The reason is that, enzymes are cytoplasmic in nature and are discharged into the blood flow after cellular damage (Mayne, 2002) [22]. The reduction in the values of AST and ALT in the blood of exposed animals in this study could therefore be due to possible interference in the kreb's cycle intermediates instead of the cell morphology and organelles. Similar to this report is the finding of Inyang and Ollor, (2015) [13]. They also reported low levels of AST in plasma after exposing African catfish (*Heterobranchus bidorsalis*) to similar herbicide like Rhonasate containing glyphosate for 14 days.

There are also other contrary reports to the findings of this study like the findings by Luscova and colleagues (2012) as well as Aderolu *et al*. (2010) [1, 19]. They reported hepatotoxicity due to elevation in the level of serum enzymes in animals exposed to sublethal concentrations of pesticides. Other authors like Inyang *et al*. (2016), also reported similar findings by evaluating the actions of transferases and phosphate in plasma and organs of *Clarias gariepinus* exposed to *fluazifop-P-butyl* and recorded variance in their values as compared to the control in exposed fishes.

Whereas, low levels of AST and ALT in the blood of exposed animals as measured in this study could be a function of the health of organs responsible for their production and regulation. The presence of these enzymes in the blood or plasma of exposed animals also serves as a biomarker, indicating pathological conditions, which is a potential pointer to injured or busted kidney or liver as a result of the toxic influence of the xenobiotic. The depreciation in the values of AST and ALT in the blood of animals treated with toxicant (2, 4-D) as observed in Table 1 of this study can also be attributed to the toxic and sub-lethal impact of the poison to both the kidney and liver of exposed animals.

This is dissimilar to the findings of Patani *et al*. (2020) [23], who reported that “during stress conditions imposed by the exposure of fishes to 2,4-dimethylamine salt, activities of the transaminases such as aspartate aminotransferase (AST) increased in the entire test organs, whereas ALT decreased in the entire test organs except in the plasma where it appreciated significantly”. The finding of this study is also supported by the findings of Ganesh and colleagues, (2006) [10] as well as Schumman and colleagues, (2002).

The value of ACP measured in the blood of exposed animals appreciated significantly from 0.17±0.24 µ/L at 3.00 mg/L to 4.17±2.24 µ/L at 5.00 mg/L compared to the control of 0.36±0.51 µ/L respectively. This indicates the toxic effect of toxicant to tissues and organs of exposed animals, whereas, the non-dose dependent variance in the values of ACP could also be an indication of response of exposed animals to xenobiotic with its corresponding stress induced by toxicant. As observed in this study, ACP value gradually declined to 1.47±2.08 µ/L as the concentration of toxicant was increased to 7.00 mg/L, though still higher compared to the control of 0.36±0.5 µ/L. The trend was not dose dependent and thus, recorded its highest value (4.17±2.24 µ/L) at 5.00 mg/L concentration of toxicant. The increase in the recorded value

of ACP in the blood of exposed rabbits is an indication of tissue damage such as hepatotoxicity (liver damage), heart attack or muscle injury. The damage may possibly increase if exposure time is extended or concentration of xenobiotics increases.

2. Electrolytes in the Blood

The present investigation evaluated the effect of 2,4-dichlorophenoxyacetic acid (2,4-D) exposure on selected blood electrolyte parameters in New Zealand rabbits after twenty-one days of treatment. Electrolytes are important physiological indicators because they reflect the functional state of major organs such as the liver, kidneys, heart, muscles, and nervous system. Alterations in sodium, potassium, chloride, calcium, and magnesium concentrations observed in this study suggest that exposure to 2,4-D interfered with normal metabolic activities and ionic regulation in the experimental animals.

The gradual disturbances recorded across the treatment groups indicate that the herbicide produced systemic toxic effects. Similar findings have been documented in recent toxicological studies where exposure to 2,4-D caused oxidative stress, membrane destabilization, inflammation, and organ dysfunction in laboratory animals (Martins *et al*., 2024; Demirel *et al*., 2023). According to Martins *et al*. (2024) [5, 8], oxidative injury remains one of the major mechanisms through which 2,4-D affects biological tissues, especially the liver and kidneys, which are directly involved in electrolyte regulation and detoxification.

Sodium (Na^+) concentration showed a progressive reduction in the exposed rabbits compared with the control group. Although the decline was moderate, the reduction observed at higher concentrations of 2,4-D may indicate impaired renal handling of electrolytes and disruption of fluid balance. Sodium is the major extracellular cat-ion responsible for maintaining osmotic pressure, acid-base equilibrium, nerve impulse transmission, and muscular activity. Any disruption in sodium regulation can affect hydration status, neuromuscular coordination, and cardiovascular stability.

The reduction in sodium concentration recorded in this study agrees with observations by Shafeeq and Mahboob (2021) [29], who reported that 2,4-D exposure altered renal and hepatic biochemical indices in rats due to oxidative stress and tissue injury. Similarly, Demirel *et al*. (2023) [5] demonstrated that exposure to 2,4-D increased biochemical markers of toxicity and induced severe oxidative damage in experimental animals. These alterations may impair renal tubular reabsorption, leading to electrolyte loss and metabolic imbalance.

Reduced sodium concentration may predispose exposed animals to weakness, dehydration, poor feed utilization, reduced muscular efficiency, and neurological disturbances. In food-producing animals, persistent sodium imbalance may negatively affect productivity, reproduction, and survival. This finding is important from a public health perspective because herbicide residues can accumulate in animal tissues and enter the human food chain through contaminated meat and other animal products. Chronic dietary exposure may therefore contribute to metabolic disturbances and organ dysfunction in humans, especially among individuals with preexisting kidney or cardiovascular conditions.

Potassium (K^+) concentration fluctuated significantly among the treatment groups. The elevation observed at lower exposure levels may have resulted from leakage of intracellular potassium into the bloodstream following tissue injury, while the reductions observed at higher concentrations suggest impaired potassium conservation or excessive excretion. Potassium is the major intracellular cation involved in muscle contraction, cardiac rhythm maintenance, nerve transmission, and enzyme activation. Disturbance in potassium balance often indicates cellular membrane damage, renal impairment, or hepatic dysfunction.

The present findings correspond with reports by Martins *et al*. (2024) [8], who observed that 2,4-D exposure altered liver metabolism and antioxidant defense mechanisms in exposed animals. Oxidative stress generated during herbicide metabolism can damage cellular membranes and mitochondria, thereby interfering with ion transport systems. Similar biochemical alterations were also reported by Shafeeq and Mahboob (2021) [29], who linked 2,4-D toxicity with nephrotoxicity and hepatic degeneration.

Abnormal potassium levels may result in muscular fatigue, irregular heartbeat, reduced mobility, and neuromuscular dysfunction in exposed animals. Severe potassium imbalance may even predispose animals to cardiac arrest or sudden death. Since humans may consume animal products contaminated with herbicide residues, prolonged exposure through the food chain could contribute to cardiovascular and metabolic complications in exposed populations.

Chloride (Cl^-) concentration increased slightly at low exposure but decreased significantly at higher concentrations of 2,4-D. Chloride is an important extracellular anion involved in acid-base regulation, osmotic balance, digestion, and maintenance of blood pH. Reduced chloride concentration may indicate renal dysfunction, dehydration, or impaired acid-base homeostasis.

The decrease in chloride concentration observed in the present study supports earlier reports that pesticide exposure interferes with electrolyte conservation and renal function. Demirel *et al*. (2023) [5] reported increased oxidative stress and inflammatory damage following 2,4-D exposure, while Martins *et al*. (2024) [8] emphasized that the herbicide disrupts several metabolic pathways linked with membrane integrity and cellular homeostasis.

Low chloride concentration may impair digestion, muscular performance, and metabolic adaptation to stress. Animals exposed to prolonged electrolyte disturbances may experience reduced productivity, poor growth, and compromised immunity. Continuous human exposure to contaminated foods may similarly contribute to acid-base imbalance and renal complications.

Calcium (Ca^{2+}) concentration varied among the treatment groups, with a marked increase observed at the highest concentration of 2,4-D. Calcium is essential for skeletal development, blood coagulation, muscle contraction, neurotransmission, hormonal secretion, and intracellular signaling. Disturbances in calcium metabolism are often associated with oxidative stress, endocrine dysfunction, tissue degeneration, and impaired renal activity.

The elevated calcium concentration observed at the highest dose may indicate severe membrane disruption and mobilization of calcium from intracellular stores or damaged tissues. Recent studies have shown that 2,4-D exposure can alter calcium-related pathways and affect ion channel expression in biological tissues (Luis *et al*., 2024). Martins *et al*. (2024) [8, 18] also reported that 2,4-D-induced oxidative

stress disrupts mitochondrial activity and cellular metabolism, leading to biochemical imbalance.

Excess calcium concentration may impair cardiac function, skeletal integrity, and neuromuscular coordination in exposed animals. Long-term exposure may also weaken reproductive performance and immune response. In humans, continuous consumption of contaminated food products may contribute to metabolic disorders, cardiovascular diseases, and endocrine abnormalities.

Magnesium (Mg^{2+}) concentration remained relatively stable at lower exposure levels but increased significantly in rabbits exposed to the highest concentration of 2,4-D. Magnesium is an essential cofactor in numerous enzymatic reactions associated with protein synthesis, antioxidant defense, energy metabolism, and neuromuscular function. Elevated magnesium concentration may indicate renal insufficiency or release from damaged tissues during oxidative injury.

The increase in magnesium concentration agrees with findings reported by Shafeeq and Mahboob (2021) [29], who noted that herbicide exposure altered antioxidant defense systems and biochemical functions in experimental animals. A recent meta-analysis by researchers investigating antioxidant responses to 2,4-D exposure also confirmed that the herbicide significantly disrupts oxidative balance and tissue metabolism in mammals (Science of the Total Environment, 2024) [27].

Elevated magnesium levels may result in muscular weakness, lethargy, reduced feed intake, and impaired nervous coordination in exposed animals. Persistent magnesium imbalance may compromise productivity and survival in livestock species. The possibility of herbicide residues entering the food chain through contaminated animal tissues therefore raises concerns regarding long-term human health risks.

Overall, the electrolyte alterations observed in this study indicate that 2,4-D exposure disrupted normal physiological and biochemical functions in the experimental rabbits. The herbicide appears to interfere with membrane permeability, renal filtration, hepatic metabolism, antioxidant defense systems, and cellular ion transport mechanisms. Similar findings have been reported in recent studies demonstrating that 2,4-D induces hepatotoxicity, nephrotoxicity, oxidative stress, apoptosis, inflammation, and metabolic dysfunction in exposed organisms (Martins *et al*., 2024; Demirel *et al*., 2023) [5, 8].

These findings have serious implications for environmental and public health. Continuous application of herbicides in agricultural environments may contaminate soil, water bodies, forage crops, and animal tissues. Residual accumulation in edible animal products may subsequently expose humans to chronic low-dose toxicity through the food chain. Long-term exposure has been associated with liver injury, renal dysfunction, endocrine disruption, oxidative stress, reproductive abnormalities, and increased risk of metabolic diseases (ATSDR, 2023; Martins *et al*., 2024) [8].

Therefore, proper regulation of herbicide application, monitoring of environmental contamination, and surveillance of residue accumulation in food-producing animals are necessary to protect animal welfare and public health. Increased awareness among farmers and agricultural workers is also essential in reducing indiscriminate herbicide use and limiting the transfer of toxic substances through the human food chain.

Conclusion

The biochemical and electrolyte alterations observed in this study indicate that 2,4-D exposure disrupted normal physiological and biochemical functions in the experimental rabbits. The herbicide appears to interfere with membrane permeability, renal filtration, hepatic metabolism, antioxidant defense systems, and cellular ion transport mechanisms. These findings have serious implications for environmental and public health. Continuous application of herbicides in agricultural environments may contaminate soil, water bodies, forage crops, and animal tissues. Residual accumulation in edible animal products may also subsequently expose humans to chronic low-dose toxicity through the food chain.

Therefore, proper regulation of herbicide application, monitoring of environmental contamination, and surveillance of residue accumulation in food-producing animals are necessary to protect animal welfare and public health. Increased awareness among farmers and agricultural workers is also essential in reducing indiscriminate herbicide use and limiting the transfer of toxic substances through the human food chain.

Acknowledgement

The authors are grateful to TETFUND for their financial support to carry out this research. This work was solely sponsored by TETFUND under the Institution Based Research (IBR) scheme during the 2025/2026 intervention year.

Ethical Considerations

All procedures adhered to national and institutional ethical standards for animal research. Humane handling, proper housing, and safe disposal of biological waste were strictly maintained.

Authors' Contributions

All authors contributed substantially to the research design, experimentation, data analysis, and manuscript development.

Competing Interests

The authors declare no conflicts of interest.

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