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ORIGINAL ARTICLE

# Evaluation of Hemoglobin and Oxyhemoglobin Dynamics Following Trifluralin Administration in Wistar Rats

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**Abstract**

Trifluralin is a widely used dinitroaniline herbicide known for its soil persistence and microtubule-disrupting activity. While its phytotoxic and genotoxic effects are well documented, its acute impact on hematological parameters-particularly oxygen transport biomarkers-remains poorly understood in veterinary models. This study aimed to evaluate the dose- and time-dependent effects of trifluralin on total hemoglobin and oxyhemoglobin concentrations in female Wistar rats following intraperitoneal administration. Rats were administered trifluralin at doses of 250, 500, and 750 mg/kg (24% concentration), and blood samples were collected at 30- and 60-minutes post-injection. Spectrophotometric analysis was used to quantify total hemoglobin and oxyhemoglobin levels. Statistical comparisons between control and treated groups were performed. Trifluralin administration led to dose- and time-dependent elevations in hemoglobin parameters. Significant increases in total hemoglobin were observed at 250 and 500 mg/kg within 30 minutes, with the highest oxyhemoglobin level recorded at 750 mg/kg. At 60 minutes, hemoglobin values in lower dose groups showed partial decline, while oxyhemoglobin remained elevated in the high-dose group. These findings suggest that trifluralin may induce transient hematological stimulation and modulate oxygen transport capacity, depending on dose and exposure duration. Further investigation is needed to clarify the underlying mechanisms and systemic implications.

## 1. Introduction

Trifluralin, a dinitroaniline herbicide chemically known as , , -trifluoro-2,6-dinitro-N, N-dipropyl-p-toluidine, is widely used for pre-emergent weed control in over 40 crop species including cotton, soybean, alfalfa, and various vegetables and fruit trees (Zhang et al., 2023). Commercial formulations such as Treflan and Elancolan are typically prepared as emulsifiable concentrates or granules. Due to its extremely low water solubility (<1 ppm), trifluralin exhibits high soil persistence and strong adsorption to soil particles, limiting its mobility but raising concerns about long-term accumulation in agricultural environments.

The herbicidal action of trifluralin is primarily mediated through inhibition of microtubule polymerization, which disrupts mitotic spindle formation and cell division in root meristems. While its phytotoxic and genotoxic effects are well documented, emerging evidence suggests that trifluralin may also exert systemic toxicity in non-target organisms, including mammals. In rodent models, sublethal exposure has been asso-

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ciated with oxidative stress, hepatocellular damage, and hematological alterations (Fernandes *et al.*, 2013; EFSA, 2009).

In mammals, trifluralin has been associated with oxidative stress, hepatotoxicity, and hematological alterations. Acute oral toxicity studies in rodents report an $LD_{50}$ of approximately 5000 mg/kg, classifying it as moderately toxic. Sublethal exposure may impair erythrocyte integrity and oxygen transport, potentially leading to hypoxia and systemic dysfunction (Fernandes et al. 2013).

Hemoglobin, the principal oxygen-carrying protein in red blood cells, plays a vital role in maintaining tissue oxygenation. It consists of four heme groups bound to globin chains, each capable of reversibly binding oxygen. Oxyhemoglobin, the oxygen-bound form, reflects the functional capacity of hemoglobin and serves as a sensitive biomarker for hypoxic stress. Disruption in hemoglobin synthesis or degradation can result in hematological disorders, including anemia and methemoglobinemia (Rifai, 2017).

Blood constitutes approximately 7–8% of total body weight, with plasma comprising about 55–60% of its volume. Plasma is primarily composed of water, electrolytes, and proteins such as albumin, fibrinogen, and globulins, which play essential roles in maintaining osmotic pressure, immune defense, and coagulation (Cohn and Shaz 2023). Erythrocytes, produced in the bone marrow, are specialized for oxygen transport and contain high concentrations of hemoglobin. Their biconcave shape and membrane flexibility enable efficient passage through narrow capillaries and facilitate optimal gas exchange (Basu and Kulkarni 2014).

Given the widespread use of trifluralin and its potential to interfere with oxygen transport mechanisms, this study aimed to evaluate dose- and time-dependent changes in total hemoglobin and oxyhemoglobin concentrations in female Wistar rats following intraperitoneal administration. By applying spectrophotometric analysis at two time points, the research provides insight into the acute hematotoxic and hypoxia-inducing potential of trifluralin in a controlled veterinary model.

## 2. Materials and Methods

### *2.1. Experimental Animals*

A total of 18 adult female Wistar rats (mean body weight: 250±4 g) were obtained from the Laboratory Animal House of Urmia University, Faculty of Veterinary Medicine. All procedures involving animals were conducted in accordance with institutional guidelines and approved by the Ethics Committee of Urmia University. Animals were acclimatized for 24 hours under controlled conditions (temperature: 20±2°C; 12-hour light/dark cycle) with unrestricted access to food and water.

### *2.2. Toxin Preparation*

Trifluralin (commercial name: Treflan), a dinitroaniline herbicide, was obtained as a 48% stock solution and diluted to 24% using double-distilled water. The compound is known to disrupt microtubule formation and has been used in rodent toxicity studies (Zhang, et al. 2023).

### *2.3. Hematological Evaluation*

The 18 rats were randomly divided into three experimental groups (n=6 per group), each receiving a different dose of trifluralin (250, 500, or 750 mg/kg body weight) via intraperitoneal injection.

## 3. Blood Sampling Was Performed in three Stages

**1. Baseline Sampling:** Under ether anesthesia, blood was collected from the right retro-orbital sinus using microhematocrit tubes. Approximately 0.7 mL of blood was mixed with potassium EDTA and centrifuged at 2000 rpm for 10 minutes to separate plasma.
**2. 30 Minute Post-Injection Sampling:** Thirty minutes after trifluralin administration, blood was collected from the left retro-orbital sinus under anesthesia. Plasma was separated as described above.
**3. 60 Minute Post-Injection Sampling**: At 60 minutes post-injection, blood was collected via jugular vein incision. One milliliter of blood was mixed with EDTA and centrifuged to obtain plasma.
Plasma samples were analyzed for hemoglobin and oxyhemoglobin concentrations using a UV/Visible spectrophotometer based on Drabkin's method (Drabkin, 1946; Rifai, 2017).

## 4. Statistical Analysis

All statistical analyses were performed using SPSS software version 22.0 (IBM Corp., Armonk, NY, USA). Prior to hypothesis testing, the normality of data distribution was assessed using the Kolmogorov–Smirnov test. Variables with normal distribution were expressed as mean ± standard deviation (SD). Comparisons between groups were performed using one-way ANOVA followed by Tukey's post hoc test. A p-value less than 0.05 was considered statistically significant.

## 5. Results

### *5.1. Hematological and Oxygen-Carrying Parameters*

The effects of intraperitoneal administration of trifluralin (24%) at doses of 250, 500, and 750 mg/kg were evaluated at 30- and 60-minutes post-injection.

Spectrophotometric analysis revealed dose- and time-dependent alterations in total hemoglobin (Total Hb) and oxyhemoglobin (Oxy Hb) concentrations.

**- 250 mg/kg (30 min)**

Total Hb levels increased significantly compared to controls ($p<0.05$), while Oxy Hb levels showed a mild, non-significant elevation (Fig. 1).

**- 250 mg/kg (60 min)**

Total Hb remained slightly elevated, though not statistically significant. Oxy Hb levels declined modestly, without reaching significance (Fig. 2).

**- 500 mg/kg (30 min)**

Total Hb levels increased significantly compared to controls ($p<0.05$). Oxy Hb levels showed a slight, non-significant increase. (Fig. 3).

**- 500 mg/kg (60 min)**

Both Total Hb and Oxy Hb levels showed mild decrease compared to controls, but neither reached statistical significance ($p<0.05$) (Fig. 4).

**- 750 mg/kg (30 min)**

A pronounced and statistically significant elevation in both Total Hb and Oxy Hb was observed ($p<0.05$), indicating a strong hematological response. (Fig. 5).

**- 750 mg/kg (60 min)**

Total Hb returned to near-control levels, while Oxy Hb remained significantly elevated ($p<0.05$), suggesting sustained oxygen transport stimulation (Fig. 6).

**- Cumulative Comparison**

At 30 minutes post-injection, total hemoglobin levels increased significantly across all treated groups. The highest concentration was observed at 500 mg/kg, followed by 250 and 750 mg/kg. This pattern suggests a non-linear, dose-dependent hematological response, with peak stimulation occurring at the intermediate dose (Fig. 7).

Oxyhemoglobin levels also rose at 30 minutes in all groups, with the most pronounced and statistically significant increase recorded at 750 mg/kg. The 250 and 500 mg/kg groups showed mild elevations that did not reach statistical significance. At 60 minutes, oxyhemoglobin levels declined in lower dose groups, while the 750 mg/kg group maintained elevated levels (Fig. 7).

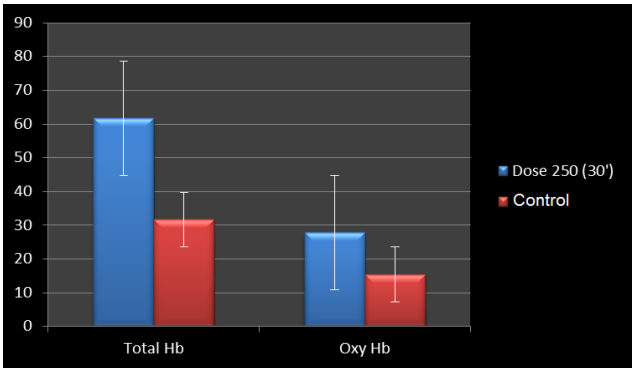


**Fig. 1.** Comparison of Total Hb and Oxy Hb between control and 250 mg/kg (30 min).

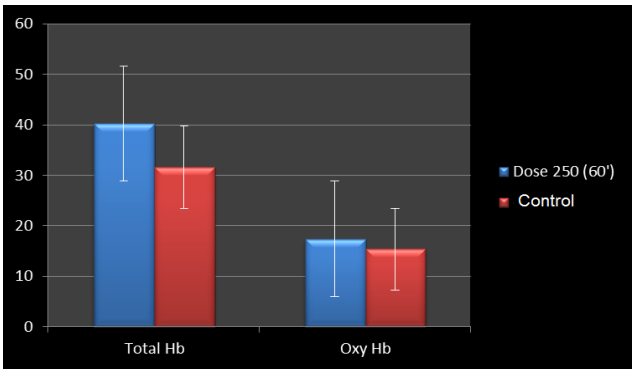


**Fig. 2.** Comparison of Total Hb and Oxy Hb between control and 250 mg/kg (60 min).

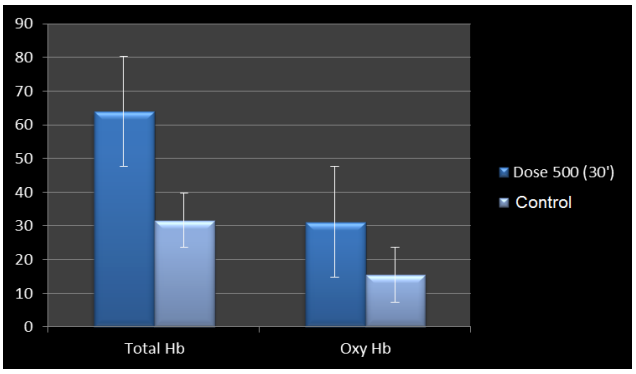


**Fig. 3.** Comparison of Total Hb and Oxy Hb between control and 500 mg/kg (30 min).

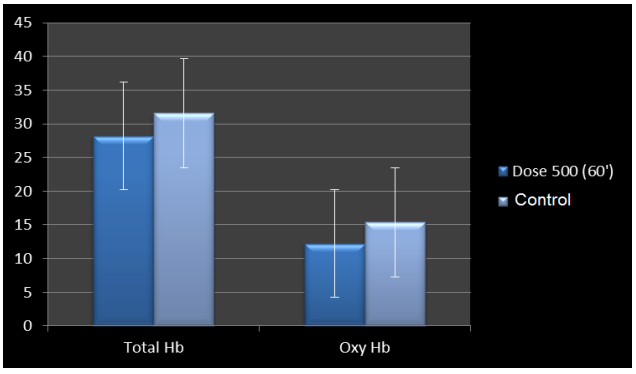


**Fig. 4.** Comparison of Total Hb and Oxy Hb between control and 500 mg/kg (60 min).

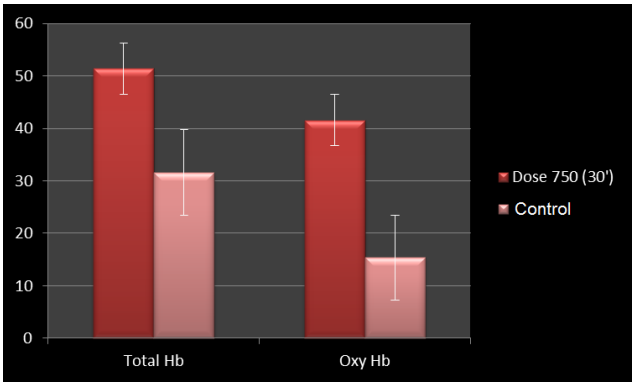


**Fig. 5.** Comparison of Total Hb and Oxy Hb between control and 750 mg/kg (30 min).

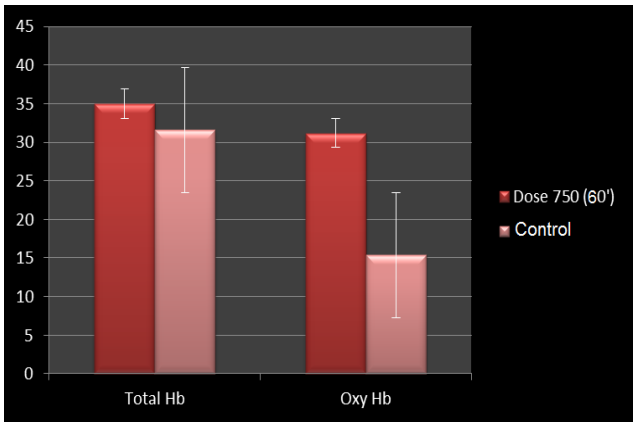


**Fig. 6.** Comparison of Total Hb and Oxy Hb between control and 750 mg/kg (60 min).

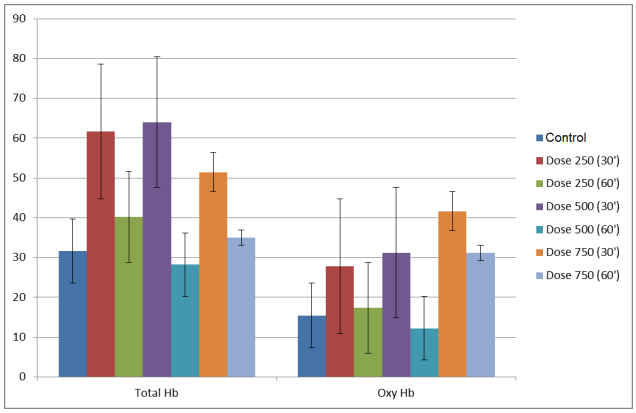


**Fig. 7.** Overall comparison of Total Hb and Oxy Hb across all treatment groups and time points.

## 6. Discussion

The present study investigated the acute hematological effects of trifluralin, a widely used dinitroaniline herbicide, in female Wistar rats. By quantifying total hemoglobin and oxyhemoglobin levels at two time points following intraperitoneal administration of three different doses, the results revealed a biphasic, dose- and time-dependent pattern of hematological response.

At 30 minutes post-injection, total hemoglobin levels increased significantly in all treated groups (250, 500, and 750 mg/kg), with the highest elevation observed at 500 mg/kg. This early rise may reflect hemoconcentration, splenic contraction, or redistribution of erythrocytes in response to chemical stress. Such compensatory mechanisms have been described in acute toxicological models and may serve to preserve oxygen transport under transient challenge (de Kort *et al.*, 2020; Rifai, 2017).

Oxyhemoglobin levels also rose at 30 minutes, with the most pronounced and statistically significant increase occurring at 750 mg/kg. This suggests a dose-dependent enhancement in oxygen-binding capacity, potentially reflecting early physiological adaptation. However, the response was not sustained: by 60 minutes, hemoglobin parameters in the lower dose groups declined toward baseline, while oxyhemoglobin at 750 mg/kg showed a significant reduction, indicating persistent hypoxic stress or oxidative disruption.

Previous studies have primarily focused on the genotoxic and hepatotoxic effects of trifluralin (Fernandes et al., 2013; Zhang et al., 2023), with limited attention to its hematological impact. The current findings expand this understanding by demonstrating acute modulation of hemoglobin dynamics, including transient stimulation and delayed suppression. The decline in oxyhemoglobin at high dose and later time point aligns with documented cases of methemoglobinemia and red blood cell deformation following exposure to nitroaromatic compounds (Iolascon *et al.*, 2021).

From a mechanistic perspective, hemoglobin undergoes continuous redox cycling, and its autoxidation generates reactive oxygen species such as superoxide and hydrogen peroxide, which can damage erythrocyte membranes and impair oxygen delivery (Daraghmeh & Karaman, 2024; Rifkind et al., 2003). Disruption of this balance may lead to oxidative stress, membrane instability, and impaired tissue oxygenation. The sustained suppression of oxyhemoglobin at 750 mg/kg supports this hypothesis and warrants further investigation into antioxidant defense systems and erythrocyte integrity (Rifkind *et al.*, 2015).

The use of Wistar rats provides translational relevance to veterinary toxicology, given their well-characterized hematological baselines and physiological similarity to larger mammals (Patel et al., 2024). Spectrophotometric analysis based on Drabkin's method enabled precise quantification of hemoglobin derivatives, offering a robust framework for assessing pesticide-induced hematotoxicity (Drabkin, 1946; Chinwuba *et al.*, 2024).

This study represents a novel contribution by quantifying oxyhemoglobin dynamics following acute trifluralin exposure. The results highlight the herbicide's potential to induce both transient stimulation and delayed hypoxia, establishing a foundation for future research into its systemic effects. These findings may inform regulatory guidelines and veterinary risk assessment protocols, particularly in contexts of frequent or unavoidable pesticide exposure.

Further studies are needed to explore subacute and chronic exposure scenarios, dose thresholds for irreversible damage, and the role of antioxidant supplementation or membrane stabilization strategies. Comparative investigations across species and developmental stages could enhance translational relevance and guide safer pesticide practices in both veterinary and agricultural settings.

## 7. Conclusion

Acute exposure to trifluralin significantly alters hemoglobin and oxyhemoglobin concentrations in female Wistar rats, indicating a rapid modulation of oxygen transport mechanisms. The dose- and time-dependent elevations observed-especially the peak in total hemoglobin at 500 mg/kg and oxyhemoglobin at 750 mg/kg within 30 minutes-suggest transient hematological stimulation followed by dose-sensitive shifts. By applying spectrophotometric analysis, this research provides novel quantitative insight into the systemic effects of a widely used herbicide.

These findings highlight the importance of incorporating hemoglobin-based biomarkers into pesticide toxicity assessments and underscore the need for further investigation into compensatory responses, oxidative stress, and long-term consequences. The study establishes a foundation for future research in veterinary toxicology and environmental health, particularly regarding erythrocyte dynamics and oxygen transport regulation.

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