

The effect of Equisetum arvense Extract on hematological parameters Against Chloramphenicol Induced Bone Marrow Toxicity in Rabbits

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ABSTRACT

Chloramphenicol is a broad-spectrum antibiotic with well-documented hematotoxic effects, including bone marrow suppression and pancytopenia. This study evaluated the hematological effects of Equisetum arvense (horsetail) extract in rabbits exposed to chloramphenicol. Twenty healthy adult male rabbits were divided into four groups: control, chloramphenicol (50 mg/kg), E. arvense extract (200 mg/kg), and combined treatment (chloramphenicol + extract). Hematological parameters including RBC, Hb, HCT, PLT, WBC, and CRP were assessed after 21 days. Chloramphenicol administration resulted in significant hematological suppression ($p < 0.05$). E. arvense alone maintained near-normal values, while co-administration mitigated chloramphenicol toxicity. These findings suggest that E. arvense possesses hematoprotective potential through antioxidant and anti-inflammatory mechanisms.

Keywords: Equisetum Extract, Bone Marrow, Chloramphenicol, Rabbits.

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INTRODUCTION

Chloramphenicol is a powerful broad spectrum antibiotic that is widely used in veterinary practice, causing effectiveness against both gram-positive and gram-negative organisms. Despite its efficacy, chloramphenicol is limited by severe adverse effects, especially Myelosuppresses and aplastic anemia (1). Such toxicity manifests as pancytopenia, which is characterized by a decrease in red blood cells, white blood cells, and platelets, causing life-threatening complications. As a result, its clinical use in both human and veterinary has a significant decline in favor of safe options (2). In parallel, increasing interest is directed towards the use of medicinal plants as protective agents against drug-inspired poisoning. Equisetum arvense (Field Horsetail) is a perennial plant with a long history of medicinal applications, especially in promoting tissue regeneration and supporting hematopoietic function. Its phytochemical profiles include flavonoids, phenolic acid, alkaloids, and silica, which increase strong antioxidants and anti-inflammatory properties (3). Preclinical studies demonstrate their role in modifying oxidative stress, enhancing hematopoiesis and improving bone mineralization (4). Given these properties, the purpose of

this study is on the hematological profile of rabbits coming in contact with chloramphenicol. E. arvense had to evaluate the protective and restored effects of the ethanol extract. It was investigated that the equisetum arvense Extract will modify chloramphenicol-inspired hematological changes through its antioxidant activity. It will modify chloramphenicol-inspired hematological changes through its antioxidant activity. Several studies have documented the adverse hematological effects of chloramphenicol. According to (5), chloramphenicol hematopoietic ancestors inhibit mitochondrial protein synthesis in cells, leading to bone marrow hypoplasia. (1) stated that chloramphenicol poisoning is both dependent on dosage and unknown, which causes its adverse effects unpredictable. Other reports demonstrated chloramphenicol residues in animal tissue, raising concerns about food security and drug resistance (6). Equisetum Arvense has attracted attention as a protective herbal remedy. reported that E. arvense exhibited no overt toxicological effects even at high doses in rats, supporting its safety for therapeutic applications. (8) demonstrated its diuretic effect equal to hydrochlorothiazide, and accepted its medicinal capacity more. Recent studies confirmed its antioxidant capacity in promoting oxidative stress and

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promoting osteogenesis (3). These findings show that *E. arvense* can compete with chloramphenicol-induced hematotoxicity by supporting hematopoiesis and reducing oxidative damage.

MATERIALS AND METHODS

The study was conducted between October 2024 and June 2025 at the Veterinary Medical College of Karbala University. *Equisetum arvense* L. classification: The plant was purchased from the local market and sent to the University Of Karbala, College of Agriculture, for classification:

Kingdom: plantae

Division: Equisetophyta

Class: Equisetopsida

order: Equisetales

Family: Equisetaceae

Genus: Equisetum L.

Species: Equisetum arvense L.

Equisetum arvense L. extraction

The extraction was performed at the Ministry of Industry and Minerals - Industrial Research and Development Authority - Ibn Al-Bitar Center. The Plant was dried in a well-ventilated room, at room temperature where it was Flipping occasionally to prevent rotting. After drying, it was ground using a Herb Grinder to obtain a powder. 100g of the ground plant was placed in a 2L Conical flask, and 500ml of 70% ethanol was added. The powder was Shaken for 24 hours in the shaker device. Initial filtration was performed Using clean medical Gauze to remove insoluble materials. The extract was Then filtered using a Buechner system and Wattman No. 1 filter paper to Obtain a clear filtrate. The solvent was isolated using a rotary evaporator And dried using a spray Dryer to obtain 5g of the extract, 5% of 100g of Ground plant . The extract was stored in a sealed container. Gas Chromatography/Mass Spectroscopy (GC/MS) was performed In Ministry of Higher Education and Scientific Research - Scientific Research Authority – research and technology center of environment, water And renewable energy. to detect the active ingredients in the extract and it Was found that the alkaloids, flavonoids, tannins and phenolic acid it is Antioxidant agents.

Experimental design

In this study, 20 healthy adult male rabbits (*Oryctolagus cuniculus*), were Acquired at the nearby marketplace, and the rabbits' weights ranged from 1500g to 2000g, and their ages ranged from 4_6 months. This study was Conducted in the animal house of the College of Pharmacy, University of Karbala. The animals were housed in standard laboratory conditions the air Of the room was changed continually by employing ventilation vacuum

(temperature $22 \pm 2^{\circ}\text{C}$, 12-hour light/dark cycle) and provided with Standard rabbit feed and water. Every technique was carried out in Compliance with the institutional ethical standards for the use and care of Animals. The drug chloramphenicol was purchased from the scientific offices and The animals were randomly distributed into four groups, and signs were Placed on the animals. The doses of chloramphenicol and equisetum Extract were measured using a sensitive balance. each group had 5 animals:

1. control group: administrate normal saline orally for 21 days.

2. equisetum extract: administrate 200mg/kg/body weight orally for 21 days.

3. chloramphenicol group: administrate 50mg/kg/body weight orally for 21day(11).

4. combined group: administrate 50mg/kg/body weight chloramphenicol orally with equisetum extract 200mg/kg/body weight orally for 21 days. Blood samples were collected at the end of the 21-day experiment through the cardiac puncture under the ketamine/xylazine anesthesia. Samples were analyzed for WBC, RBC, hemoglobin (HB), hematocrit (HCT), platelets (PLT), and C-Reactive Protein (CRP).

Ethical Approval

All experimental technique was authorized by the College of Veterinary Medicine of Kerbala and complied with the ethical approval number (UOK.VET.AN.2025.123).

Statistical Analysis

Data were analyzed using SAS (2018) software, and results were compared Using the least significant difference (LSD) at the 0.05 probability level.

RESULT AND DISCUSSION

The chloramphenicol administration significantly reduced WBC, RBC, HB, HCT and PLT, increasing CRP compared to the Control Group ($P < 0.05$). In contrast, rabbits treated with *E. arvense* extract maintained values close to normal. The combined group demonstrated partial restoration of hematological parameters compared to chloramphenicol alone.

Table 1 white blood cell analysis

Group	Mean \pm S.E (Significance Letter)
Control	9.80 \pm 0.37 (A)
Equisetum	9.00 \pm 0.31 (A)
Chloramphenicol	4.50 \pm 0.04 (C)
Chloramphenicol + Equisetum	7.60 \pm 0.24 (B)

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Chloramphenicol markedly reduced WBC counts. The combination treatment significantly increased WBC compared to chloramphenicol alone, And there is no difference between The equisetum extract group and the control (Figure 1).

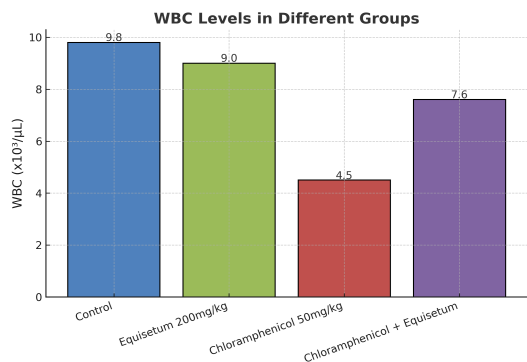


Figure 1 white blood cell analysis in different groups

Table 2 Red blood cell analysis

Group	Mean ± S.E (Significance Letter)
Control	5.52 ± 0.08 (A)
Equisetum	5.30 ± 0.07 (B)
Chloramphenicol	3.40 ± 0.07 (C)
Chloramphenicol + Equisetum	5.18 ± 0.03 (B)

Chloramphenicol significantly decreased RBC counts compared to all other groups. Equisetum treatment alone did not differ significantly from the combination treatment, and both were significantly higher than chloramphenicol alone (Figure 2).

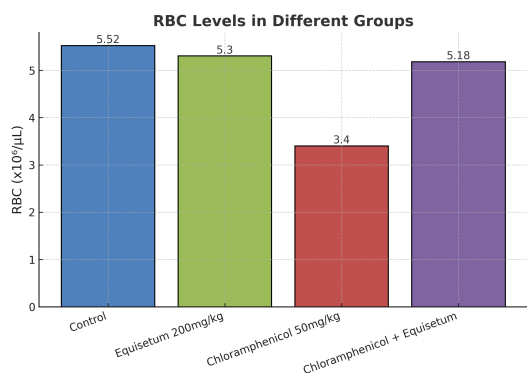


Figure 2 Red blood cell analysis in different groups.

Table 3 Hemoglobin analysis

Group	Mean ± S.E (Significance Letter)
Control	10.94 ± 0.08 (A)
Equisetum	10.78 ± 0.08 (A)
Chloramphenicol	7.72 ± 0.45 (C)

Chloramphenicol + Equisetum	9.20 ± 0.35 (B)
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There was a significant reduction in HGB in the chloramphenicol group. The combination group showed a significant improvement compared to chloramphenicol alone but remained below control values and there is no difference between equisetum group and control group (Figure 3).

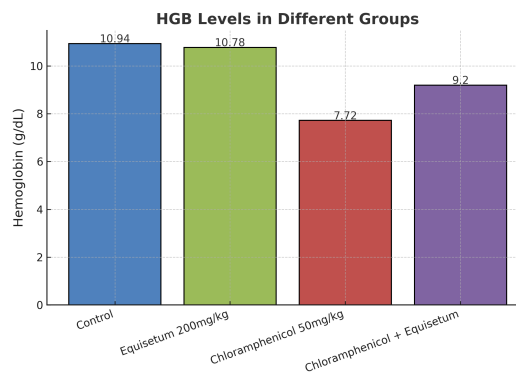


Figure 3 Hemoglobin analysis in different groups.

Table 4 Hematocrit analysis.

Group	Mean ± S.E (Significance Letter)
Control	31.56 ± 0.05 (A)
Equisetum	31.20 ± 0.07 (A)
Chloramphenicol	25.66 ± 0.44 (C)
Chloramphenicol + Equisetum	28.40 ± 0.27 (B)

Chloramphenicol significantly reduced HCT values compared to control and Equisetum groups. The combination treatment significantly improved HCT compared to chloramphenicol alone (Figure 4).

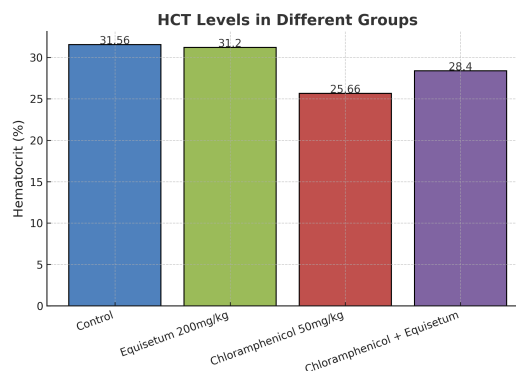


Figure 4 Hematocrit analysis in different groups.

Table 5 C-Reactive protein analysis.

Group	Mean ± S.E (Significance Letter)
Control	3.28 ± 0.08 (D)

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Equisetum	3.62 ± 0.05 (C)
Chloramphenicol	5.34 ± 0.07 (A)
Chloramphenicol + Equisetum	4.70 ± 0.07 (B)

Significant differences were observed. Chloramphenicol caused the highest CRP levels, indicating inflammation. The combination treatment significantly reduced CRP compared to chloramphenicol alone but remained higher than control (Figure 5).

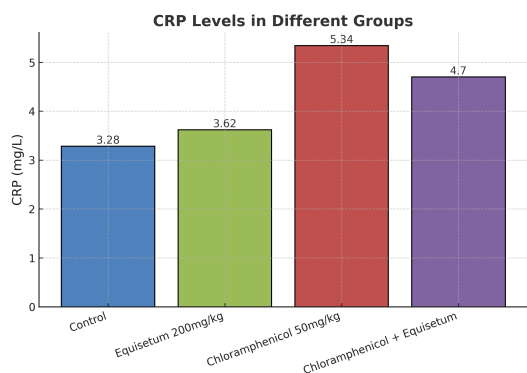


Figure 5C-reactive protein tier analysis in different groups.

Table 6 Platelets analysis.

Group	Mean ± S.E (Significance Letter)
Control	250.00 ± 7.08 (A)
Equisetum	237.60 ± 5.22 (A)
Chloramphenicol	124.00 ± 6.94 (C)
Chloramphenicol + Equisetum	213.00 ± 7.68 (B)

There was a significant difference ($p \leq 0.05$) among groups. Chloramphenicol markedly reduced PLT compared to control and Equisetum groups, while the combination treatment significantly improved PLT compared to chloramphenicol alone but remained lower than control (Figure 6).

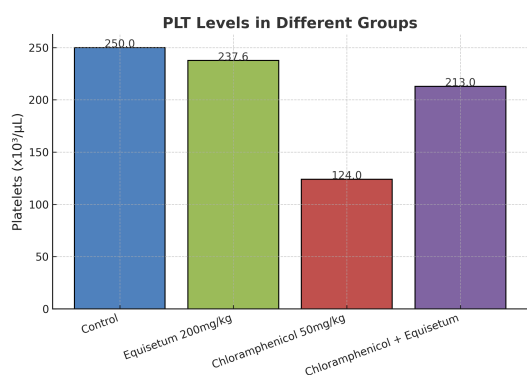


Figure 6 Platelets analysis in different groups.

Table 7 The effect of treatments on body weight

Treatment	Means ± S.E	
	Body weight(kg) Before	Body weight(kg) After
Control	1.74± 0.09 A	1.84± 0.06 A
Equisetum extract	1.78± 0.08 A	1.96± 0.09 A
Chloramphenicol	1.74± 0.09 A	1.54± 0.09 B
Equisetum + chloramphenicol	1.78± 0.05 A	1.84± 0.10 A
L.S.D 0.05	0.2508	0.2698

Discussion

The present study confirmed the hematotoxicity of chloramphenicol, as evidenced by reductions in RBC, Hb, HCT, PLT, and WBC, along with elevated CRP. These results align with previous findings that chloramphenicol induces aplastic anemia through mitochondrial dysfunction (5). Elevated CRP further reflects systemic inflammation and tissue injury (9). Treatment with *E. arvense* extract preserved hematological values, supporting its protective role. The ethanolic extract of *Equisetum arvense* is characterized by a High content of antioxidant compounds, mainly flavonoids such as Quercetin, kaempferol, luteolin, and apigenin, along with phenolic acids Including caffeic, ferulic, gallic, and chlorogenic acids. In addition, the Extract contains vitamin C and silica, which contribute to its overall Antioxidant profile. These bioactive compounds exert their protective Effects by scavenging reactive oxygen and nitrogen species (ROS/RNS), Stabilizing erythrocyte membranes, and reducing lipid peroxidation and DNA oxidative damage. Recent investigations have demonstrated that *E. Arvense* extract exhibits strong antioxidant and anti-inflammatory Activities, thereby supporting its potential role in hematopoietic protection And cellular homeostasis. chloramphenicol induce oxidative stress in the Bone marrow, which triggers the activation of NF-κB signaling and Subsequent overproduction of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IFN-γ. This cytokine storm exacerbates hematopoietic Suppression and tissue injury. In contrast, the ethanolic extract of *Equisetum arvense* exerts potent anti-inflammatory effects by inhibiting NF-κB and MAPK pathways, scavenging reactive oxygen species, and Upregulating endogenous antioxidant defenses. Consequently, the extract Significantly downregulates chloramphenicol-induced cytokine expression, Thereby mitigating inflammation and preserving bone marrow cellular Integrity., and promoting hematopoiesis (3). In the combined treatment group, hematological indices were partially restored, indicating that *E. arvense* mitigated but did not fully reverse chloramphenicol-induced cytopenia. This observation is consistent with reports that plant-derived

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antioxidants can reduce drug-induced marrow toxicity (10). These findings support the hypothesis that *E. arvense* exerts hematoprotective effects by enhancing antioxidant defense and modulating inflammatory pathways.

CONCLUSION

Equisetum arvense extract demonstrated hematoprotective effects against chloramphenicol-induced toxicity in rabbits.

It preserved RBC, Hb, HCT, PLT, and WBC levels while reducing CRP, suggesting a role in mitigating myelosuppression and systemic inflammation. These results highlight the potential of *E. arvense* as an adjunctive therapy in veterinary medicine to counteract antibiotic-induced hematotoxicity.

Acknowledgements

N/A

Conflict of Interest

The authors declare no conflict of interest.

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