Histological Effects of Equisetum arvense Extract Against Chloramphenicol-Induced Bone Marrow Toxicity in Rabbits

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***Abstract*— Chloramphenicol-induced bone marrow suppression remains a critical limitation in veterinary therapeutics. This study investigated the histological protective effects of Equisetum arvense extract on bone marrow architecture in rabbits exposed to chloramphenicol. Twenty healthy adult rabbits were divided into four groups: control, chloramphenicol (50 mg/kg), E. arvense (200 mg/kg), and combined treatment. Bone marrow samples were collected after 21 days for histopathological examination. Chloramphenicol induced hypocellularity, depletion of granulocytic and megakaryocytic series, and expansion of adipose spaces. In contrast, E. arvense extract preserved marrow architecture with hypercellularity and megakaryocytic proliferation, while the combined treatment demonstrated partial restoration with evidence of angiogenesis and osteoblastic activity. These results confirm that E. arvense exerts marrow-protective and regenerative effects against chloramphenicol-induced toxicity, likely due to its antioxidant and osteogenic bioactive compounds.**

***Keywords* — Equisetum Extract, Bone Marrow, Chloramphenicol, Rabbits**.

INTRODUCTION

The bone marrow contains hematopoietic stem cells that produce erythrocytes, leukocytes, and platelets, it is essential to hematopoiesis. Immune suppression, compromised tissue oxygenation, and potentially fatal cytopenias can arise from any damage to the integrity of the bone marrow (1,2). Despite being an effective broad-spectrum antibiotic, chloramphenicol has serious side effects, such as idiosyncratic aplastic anemia and reversible bone marrow suppression (3,4). These effects show up histopathologically as increased adiposity in marrow spaces, hypocellularity, and hematopoietic lineage depletion (5).

As protective agents, natural substances with regenerative and antioxidant qualities are being investigated more and more. Rich in flavonoids, phenolic acids, silica, and alkaloids, Equisetum arvense (horsetail) is linked to osteogenesis, collagen synthesis, and tissue regeneration (6).

Its function in increasing trabecular and cortical bone thickness has been demonstrated in animal studies (7) and modulating hematopoietic activity.

Therefore, it is thought that E. arvense can prevent bone marrow damage caused by chloramphenicol by maintaining cellularity and encouraging regenerative pathways.

Chloramphenicol toxicity is characterized by bone marrow suppression.

After long-term chloramphenicol administration, experimental investigations showed hemosiderin-laden macrophages, erythroid hyperplasia, hypocellularity, and megakaryocytic necrosis (5).

Chloramphenicol's toxicity to the mitochondria impairs hematopoietic progenitor protein synthesis, resulting in aplastic alterations (3,4). The medicinal benefits of Equisetum arvense have been investigated in relation to various organ systems (8) demonstrated its diuretic efficacy in humans, while (9) validated its subacute safety profile in rats.

Its osteogenic and regenerative potential is supported by recent data (6) revealed increased osteoblastic activity and bone mineralization, while (7) showed notable increases in cortical and trabecular bone thickness in animal models.

According to these results, E. arvense may stimulate fibroblasts, osteoblasts, and stromal cells to counteract marrow toxicity and create a favorable microenvironment for hematopoiesis.

MATERIALS AND MTHODS

The study was conducted between October 2024 and June 2025 at the Veterinary Medical College of Karbala University. Equisetum arvens 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 arvens 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. Twenty male rabbits in good health, weighing 1.5 to 2.0 kg and aged 4-6 months, were divided into four groups of five each:

1) control(Saline); 2) Chloramphenicol (50 mg/kg orally); 3) Extract of E. arvense (200 mg/kg orally); and 4) Combination treatment (chloramphenicol + extract). An Olympus microscope equipped with a digital imaging system was used to take representative pictures.

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 ± 2°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(10).

4.combined group: administrate 50mg\kg\body weight chloramphenicol orally with equisetum extract 200mg\kg\body weight orally for 21 days. bone marrow was extracted from femoral bones Samples were sectioned, decalcified, embedded in paraffin, fixed in 10% formalin, and stained with hematoxylin and eosin (H&E). Marrow cellularity, lineage distribution, adipocyte expansion, vascular proliferation, fibrosis, and signs of regeneration (osteoblast/osteoclast activity) were all evaluated histologically

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).

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RESULT AND DISCUSSION

Histological examination showed clear group differences. The marrow architecture of the control group was normal, with balanced myeloid, megakaryocytic, and erythroid lineages. The stromal support was intact and adipocytes were distributed physiologically. The chloramphenicol-treated group displayed severe hypocellularity, adipocyte expansion, depletion of granulocytic and megakaryocytic cells, and evidence of necrotic megakaryocytes. Hemosiderin-laden macrophages and immature neutrophils were also observed. Rabbits treated with E.arvense extract exhibited hypercellularity, increased megakaryocytic proliferation, stromal reticular cell activity, perivascular fibroblast proliferation, and angiogenesis. Collagen deposition and osteoblastic activity along bone surfaces were also noted. The combined group demonstrated partial restoration of marrow structure compared to chloramphenicol alone. Cellularity improved with reappearance of erythroid and myeloid precursors, reduced adiposity, and signs of active bone remodeling. Representative micrographs highlighted angiogenesis and osteoblast proliferation in this group.

Figure (1&2). Control group bone marrow showing normal architecture.

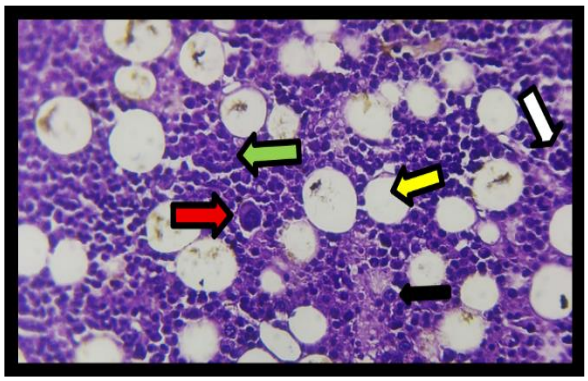

Figure1. Histological section in the bone marrow of control group at 21 days shows adipocytes enclose open spaces that were occupied by lipid droplets bone marrow fragments (yellow arrow) with numbers of neutrophil cells (white arrow),

plasma cells (green arrow), erythroid cells (black arrow) and multinucleated megakaryocyte (red arrow) (H&E stain 400X).

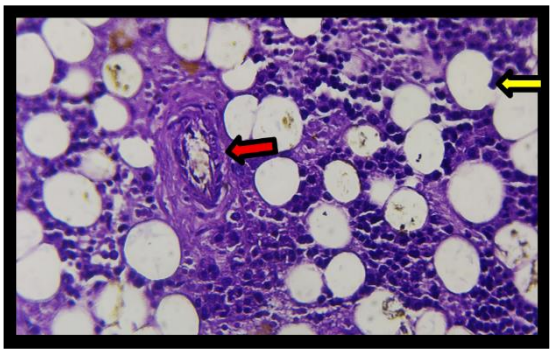

Figure 2. Histological section in the bone marrow of rabbit of control group at 21 days shows bone marrow sinusoids supplied by arteriole (red arrow) spanning throughout the bone marrow with Adventitial cells and their processes (yellow arrow) give support to the hematopoietic cells (H&E stain 400X).

Figure (3,4,5,6&7). Chloramphenicol group showing hypocellularity and expanded adipose spaces.

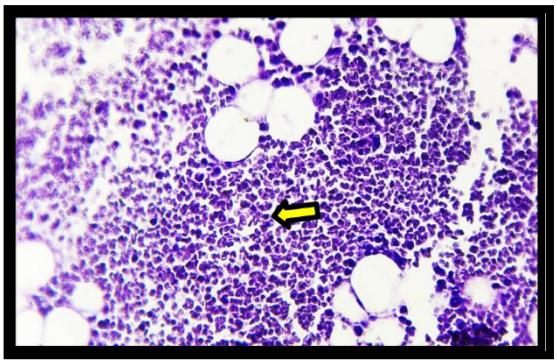

Figure 3. Histological section in the bone marrow of chloramphenicol group at 21 days shows erythroid hyperplasia (yellow arrow) represented by Dilated vascular sinuses filled with erythrocytes are interspersed among adipocytes in the marrow spaces (H&E stain 400X).

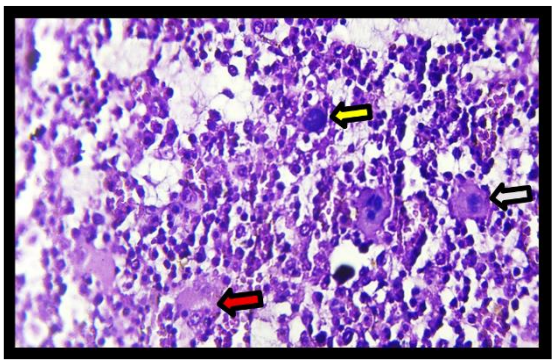

Figure 4. Histological section in the bone marrow of chloramphenicol group at 21 days shows alteration of megakaryocytes represented by An apoptotic megakaryocyte with a condensed nucleus (yellow arrow) & necrosis of megakaryocytes that nuclear karyolitic (red arrow) and pyknosis (white arrow) (H&E stain 400X).

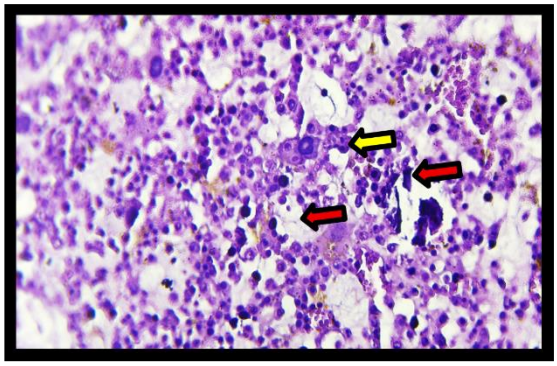

Figure 5. Histological section in the bone marrow of chloramphenicol at 21 days shows immature neutrophils (band neutrophils)(yellow arrow) with lymphopalsmotic inflammatory cells (red arrow) (H&E stain 400X).

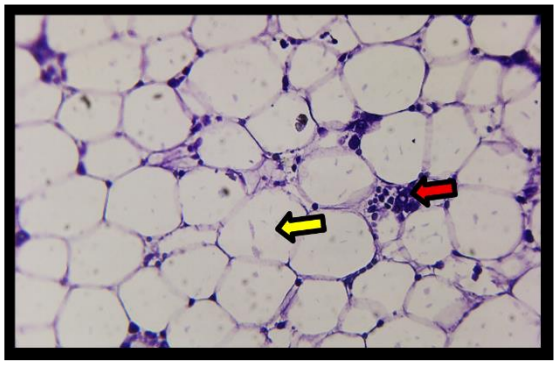

Figure 6. Histological section in the bone marrow of chloramphenicol at 21 days shows hypocellularity characterized by Hypocellular bone marrow with little hematopoiesis (red arrow) and many adipocytes (yellow arrow)(H&E stain 400X).

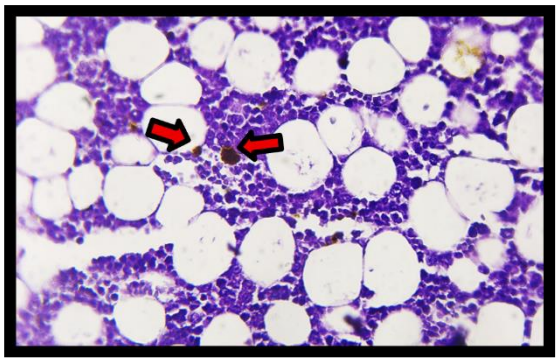

Figure 7. Histological section in the bone marrow of chloramphenicol at 21 days shows golden-brown pigment pigment represented by hemosiderin laden macrophages (red arrow)(H&E stain 400X).

Figure (8,9,1&11). Equisetum group showing hypercellularity and megakaryocytic proliferation.

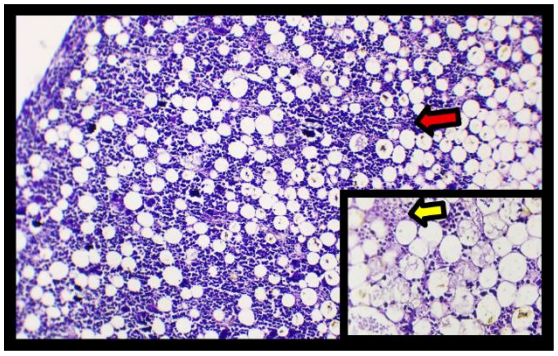

Figure 8. Histological section in the bone marrow of equisetum extract group at 21 days shows Hypercellular bone marrow (red arrow) with cellular vacuolation (yellow arrow) (H&E stain 100+400X).

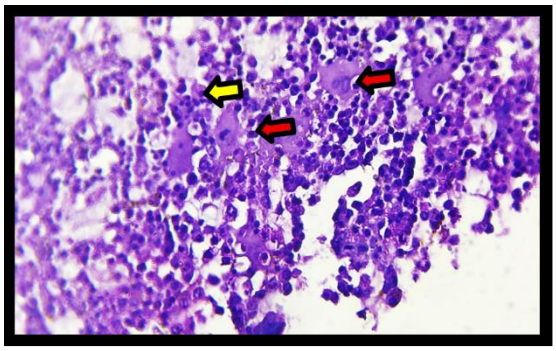

Figure 9. Histological section in the bone marrow of equisetum extract group at 21 days shows megakaryocytes hyperplasia (red arrow) with diffuse proliferation of stromal reticular cells (yellow arrow) (H&E stain 400X).

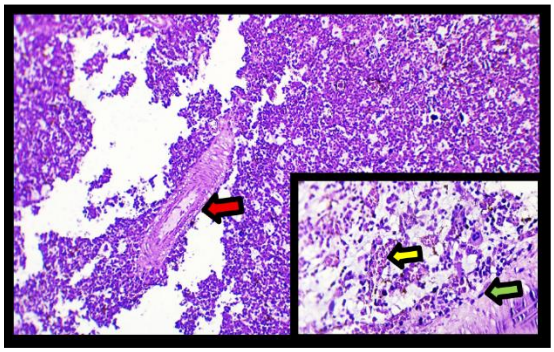

Figure 10. Histological section in the bone marrow of equisetum extract group at 21 days shows Narrowing &Perivascular fibrosis (red arrow) by proliferation of fibroblast (green arrow) around blood vessels with sinusoidal congestion (yellow arrow) (H&E stain 100+400X).

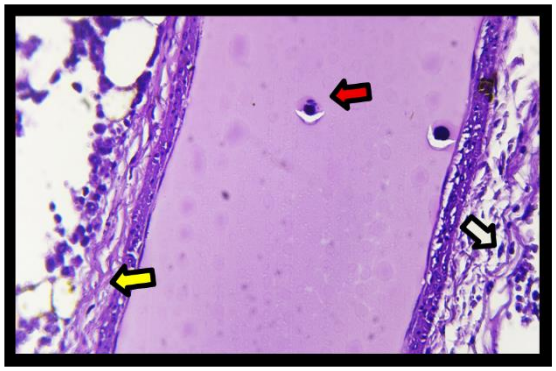

Figure 11. Histological section in the bone marrow of equisetum extract group at 21 days shows collagen (yellow arrow)& osteoblast along bone surface (white arrow) with osteocytes within bone matrix (red arrow) (H&E stain 400X).

Figure (12,13,14&15). Combined group showing partial restoration with angiogenesis and osteoblastic activity.

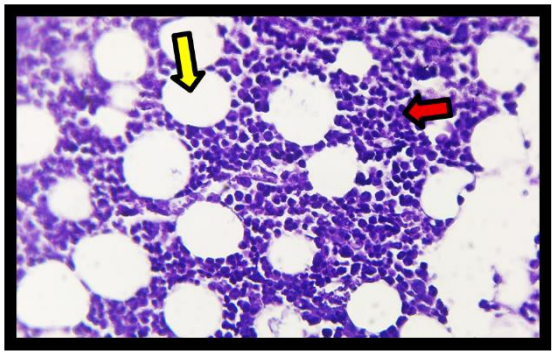

Figure 12. Histological section in the bone marrow of combined group at 21 days shows hypercellularity characterized by Hypercellular bone marrow with hematopoiesis (red arrow)and many adipocytes(yellow arrow) (H&E stain 400X).

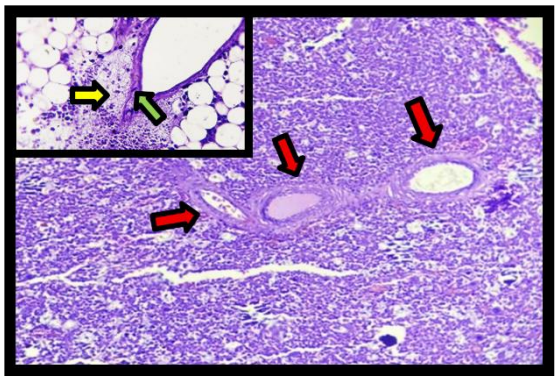

Figure 13. Histological section in the bone marrow of combined group at 21 days shows tissue regeneration process by a fibrin network formation (yellow arrow) that promoting angiogenesis (blood vessel formation red arrow) with perivascular fibroblast infiltration (green arrow)(H&E stain100+ 400X).

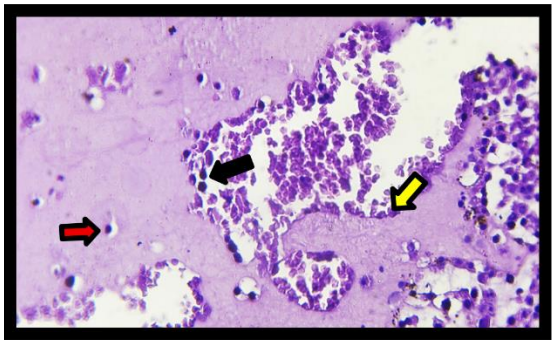

Figure 14. Histological section in the bone marrow of combined group at 21 days shows proliferation of osteoblast that cuboidal on the bone surface (red arrow) with some osteoclast (black arrow) &osteocytes in side lacuna (yellow arrow) (H&E stain 400X).

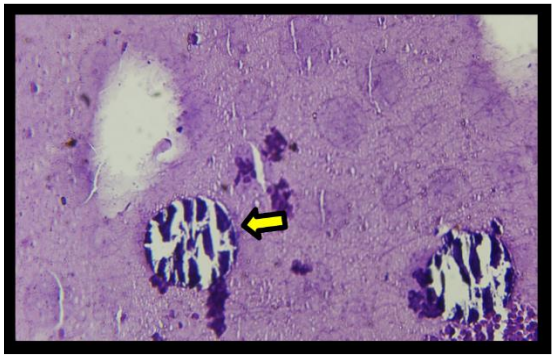

Figure 15. Histological section in the bone marrow of combined group at 21 days shows bone remodeling by deposition of calcium salts within haversian canal that stained bluish color (yellow arrow) H&E stain 400X).

Discussion

The histopathological findings confirm the myelotoxic effects of chloramphenicol, consistent with reports of bone marrow suppression through mitochondrial disruption (5). The presence of hypocellularity, megakaryocytic necrosis, and adipocyte infiltration mirrors established patterns of aplastic changes (3). Equisetum arvense treatment demonstrated clear marrow-stimulating effects. Hypercellularity and megakaryocytic proliferation observed in this study are in line with its reported osteogenic and regenerative roles (6). The extract's antioxidant compounds likely reduced oxidative stress, while its silica and flavonoids enhanced collagen synthesis and osteoblastic activity (7). These mechanisms may explain the observed fibroblast proliferation, angiogenesis, and bone remodeling in the E. arvense group. In the combined treatment group, partial restoration of marrow architecture was observed, indicating that E. arvense mitigated chloramphenicol-induced damage.

Although not fully protective, the improvements highlight the therapeutic potential of E. arvense as a supportive agent in drug-induced myelosuppression. Future studies should investigate molecular mechanisms, optimal dosing, and translational applicability in other animal models. 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.

CONCLUSION

Equisetum arvense extract demonstrated significant histological protective effects on rabbit bone marrow exposed to chloramphenicol. It preserved marrow cellularity, promoted hematopoietic activity, stimulated stromal and osteogenic responses, and mitigated adipocyte infiltration. These findings underscore its potential as a natural adjuvant in managing drug-induced bone marrow toxicity in veterinary practice.

Acknowledgements
N/A

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1) Travlos, G.S. (2006) 'Normal Structure, Function, and Histology of the Bone Marrow', Toxicologic Pathology, 34(5), pp. 548–565. Available at: https://doi.org/10.1080/01926230600939856.
2) El-Alfy, N.Z.I. et al. (2024) 'Potential protection by vitamin D against DNA fragmentation and bone marrow cytotoxicity induced by chloramphenicol', Toxicology Reports, 13(November). Available at: https://doi.org/10.1016/j.toxrep.2024.101828.
3) Tsirogianni, A. et al. (2023) 'Chloramphenicol Derivatization in Its Primary Hydroxyl Group with Basic Amino Acids Leads to New Pharmacophores with High Antimicrobial Activity', Antibiotics, 12(5). Available at: https://doi.org/10.3390/antibiotics12050832.
4) Mehlhorn, A., Rahimi, P. and Joseph, Y. (2018) 'Aptamer-based biosensors for antibiotic detection: A

review', Biosensors, 8(2). Available at: https://doi.org/10.3390/bios8020054.

5) Weiss, D. J., & Wardrop, K.J. (2022) 'bone marrow normal distribution'.
6) Luanda, A., Ripanda, A. and Makangara, J.J. (2023) 'Therapeutic potential of Equisetum arvense L. for management of medical conditions', Phytomedicine Plus, 3(2), p. 100444. Available at: https://doi.org/10.1016/j.phyplu.2023.100444.
7) Ibrahem, E.S., Mohamed, G.H. and El-Gazar, A.F. (2022) 'Therapeutic Effect of Equisetum arvense L. on Bone and Scale Biomarkers in Female Rats with Induced Osteoporosis', Egyptian Journal of Chemistry, 65(12), pp. 457–466. Available at: https://doi.org/10.21608/EJCHEM.2022.127817.5699
8) Al-Snafi, P.D.A.E. (2017) 'Pharmacology and therapeutic potential of Euphorbia hirta (Syn: Euphorbia pilulifera)- A review', IOSR Journal of Pharmacy (IOSRPHR), 07(03), pp. 07–20. Available at: https://doi.org/10.9790/3013-0703010720.
9) Carneiro, D.M. et al. (2021) 'Equisetum arvense: New Evidences Supports Medical use in Daily Clinic', Pharmacognosy Reviews, 13(26), pp. 50–58. Available at: https://doi.org/10.5530/phrev.2019.2.4.
10) Ngoula, F. et al. (2020) 'Effect of Chloramphenicol on Rabbit Doe Fertility and Prenatal Development', Animal Production, 21(1), p. 1. Available at: https://doi.org/10.20884/1.jap.2019.21.1.678.