

Original article

Dose-Dependent Myelosuppression and Hematological Toxicity Induced by Gemcitabine in New Zealand White RabbitsFayrouz Khaled^{*1} , Fadiyah Masoud² ¹Department of Chemistry, Faculty of Science, Omar Al-Mokhtar University, El-Beida, Libya²Department of Biomedical Engineering, Genetic Engineering Division, Libyan Academy for Postgraduate Studies, Jabal Al-Akhdar, LibyaCorresponding email. fayalzobair@yahoo.com**Abstract**

Gemcitabine (GEM) is a widely used cytotoxic chemotherapy agent, yet its dose-limiting toxicity is often linked to severe myelosuppression. This study aimed to investigate the histopathological and hematological effects of two different therapeutic doses of Gemcitabine on the bone marrow of New Zealand White rabbits, serving as a model for systemic toxicity. Rabbits were divided into three groups: a Control group (untreated), a GEM 0.1 mg/kg group, and a high-dose GEM 0.3 mg/kg group. Peripheral blood samples were analyzed for Complete Blood Count (CBC) parameters, and bone marrow smears were prepared for morphological assessment and histopathological examination. Statistical analysis was performed using ANOVA ($p < 0.05$ was considered significant). Results: This study evaluated the hematological and bone marrow effects of gemcitabine administered at 0.1 and 0.3 mg/kg in male rabbits. Bone marrow smears demonstrated a distinct dose-dependent morphological deterioration. The control group showed normal cellularity with balanced hematopoietic lineages, indicating healthy marrow activity. In contrast, rabbits treated with 0.1 mg/kg displayed noticeable hypocellularity and increased extravasated blood, suggesting early stromal and vascular damage. The 0.3 mg/kg group exhibited severe myelosuppression, characterized by markedly depleted hematopoietic cells and a diffuse hemorrhagic background, reflecting profound hypoplasia or near-aplasia of marrow tissue. Hematological results further supported these morphological findings. Total WBC count decreased significantly from $7.1 \pm 0.18 \times 10^3/\mu\text{L}$ in the control group to $5.0 \pm 0.08 \times 10^3/\mu\text{L}$ and $4.4 \pm 0.20 \times 10^3/\mu\text{L}$ in the 0.1 and 0.3 mg/kg groups, respectively ($p = 0.000$). Lymphocytes also declined from $63.6 \pm 2.6\%$ to $62.3 \pm 0.21\%$ and $52.9 \pm 1.37\%$, indicating impaired lymphopoiesis. MID% decreased from $10.2 \pm 0.48\%$ to $7.8 \pm 0.29\%$ and $7.5 \pm 0.02\%$, while granulocytes dropped from $12.3 \pm 0.66\%$ to $10.6 \pm 0.76\%$ and $9.8 \pm 0.5\%$ across the same groups ($p = 0.000$). Red blood cells declined markedly from $6.0 \pm 0.15 \times 10^6/\mu\text{L}$ in controls to $5.0 \pm 0.05 \times 10^6/\mu\text{L}$ and $3.9 \pm 0.26 \times 10^6/\mu\text{L}$, demonstrating significant erythropoietic suppression. Platelet counts showed the sharpest reduction, falling from $435.19 \pm 21.09 \times 10^3/\mu\text{L}$ to $301.86 \pm 7.94 \times 10^3/\mu\text{L}$ and reaching $146.6 \pm 21.01 \times 10^3/\mu\text{L}$, confirming severe thrombocytopenia ($p = 0.000$). Overall, both the quantitative blood results and qualitative marrow morphology clearly demonstrate that gemcitabine induces strong, dose-dependent myelosuppression in rabbits. The higher dose (0.3 mg/kg) produced profound hematopoietic toxicity, highlighting the importance of cautious dose selection and regular hematological monitoring during gemcitabine therapy.

Keywords: Gemcitabine, Hematological Toxicity, Bone Marrow Smears, Rabbit Model.**Introduction**

Gemcitabine (2', 2'-difluoro-deoxycytidine, GEM) is a widely utilized nucleoside analog belonging to the antimetabolite class of chemotherapeutic agents [1]. It is approved as a first-line treatment for various solid tumors, including pancreatic, non-small cell lung, breast, and ovarian cancers [2]. Its potent cytotoxic action is achieved after intracellular phosphorylation into its active metabolites, primarily gemcitabine triphosphate (dFdCTP). This active form is incorporated into the elongating DNA strand, leading to a process known as "masked chain termination," which halts DNA synthesis and triggers programmed cell death (apoptosis) [3]. Additionally, the diphosphate metabolite (dFdCDP) inhibits ribonucleotide reductase, further depleting the essential deoxyribonucleotide pools required for DNA replication [4].

The efficacy of Gemcitabine, like many cytotoxic agents, is intrinsically linked to its toxicity towards rapidly dividing cells. Consequently, myelosuppression, the suppression of bone marrow activity, emerges as the principal dose-limiting toxicity (DLT) in clinical practice, frequently necessitating dose reductions or treatment delays [5]. This toxicity is manifested clinically as cytopenias, particularly neutropenia and anemia, which can significantly increase the risk of infection and hemorrhage in patients [6]. Although generally reversible, the severity of myelosuppression is known to be both dose- and schedule-dependent [7,8]. Understanding the exact morphological changes in the hematopoietic tissues that correlate with peripheral blood count alterations is crucial for developing better supportive care strategies. While the hematological effects of Gemcitabine are well-documented in human clinical trials, detailed correlative studies examining the histopathological damage to the bone marrow (morphology) alongside simultaneous changes in the peripheral Complete Blood Count (CBC) in a controlled animal model are essential [9]. Rabbits serve as a suitable preclinical model for studying drug-induced hematological toxicity [10-17].

Therefore, this study aimed to investigate the dose-dependent toxicity of Gemcitabine at two distinct concentrations (0.1 and 0.3 mg/kg) in New Zealand White rabbits. Correlate the observed changes in peripheral blood cell counts (CBC) with the microscopic alterations (hypocellularity and hemorrhage) revealed by bone marrow smears. Provide a clear morphological foundation for the hematological toxicity of Gemcitabine, reinforcing the need for continuous monitoring during clinical use.

Methods

Animals

A total of fifteen healthy New Zealand White rabbits (of similar age and weight, e.g., 2.0-2.5 kg) were used in this experiment. The animals were housed under standard laboratory conditions (temperature $22 \pm 2^\circ\text{C}$, relative humidity 50-60%, and a 12-hour light/dark cycle) and provided with commercial rabbit feed and water *ad libitum*.

Experiments

After a one-week acclimatization period, the rabbits were randomly assigned to three experimental groups (n=5 per group). Gemcitabine (GEM) was reconstituted with sterile normal saline to the required concentrations immediately before administration.

Animals were allocated into three experimental groups to evaluate the effects of gemcitabine in comparison with a control. The control group received sterile saline in an equivalent volume, administered intravenously through the marginal ear vein, thereby serving as the baseline reference for procedural and vehicle-related influences. In the first treatment group (GEM 0.1), gemcitabine was administered at a dose of 0.1 mg per kilogram of body weight, delivered via intravenous injection through the marginal ear vein. The second treatment group (GEM 0.3) received a higher dose of gemcitabine, 0.3 mg per kilogram of body weight, using the same route of administration to ensure methodological consistency. The standardized dosing frequency and uniform route of delivery across all groups allowed for reliable comparison of outcomes and facilitated assessment of dose-dependent effects.

Sample collection

The treatment was administered once a week for 8 weeks. Blood samples were collected from the marginal ear artery or cardiac puncture (terminal procedure) into tubes containing EDTA as an anticoagulant. The samples were immediately analyzed using an automated hematology analyzer calibrated. The following parameters were measured: WBC, LYM, MID, GRAN, and RBCs. Following the terminal blood collection, bone marrow samples were collected from the femur.

Smear Preparation

A small volume of bone marrow aspirate was collected and immediately used to prepare thin smears on clean glass slides. The smears were air-dried. Morphological Assessment-stained smears were examined under a light microscope at a high magnification by a trained pathologist blinded to the treatment groups. The assessment focused on overall cellularity, presence of hematopoietic cell lineages, and evidence of pathology such as hemorrhage and stromal damage.

Statistical Analysis

Data were expressed as the Mean $\pm$ Standard Error (SE). The statistical significance between the different treatment groups was determined using a One-Way Analysis of Variance (ANOVA). Post-hoc analysis (Tukey's HSD test) was used to compare the individual group means. Differences were considered statistically significant at a threshold of $P < 0.05$. All statistical computations were performed using Minitab 17.

Results

As shown in Figure 1, the bone marrow smear from the untreated control rabbit exhibits a normal cellular architecture. The smear is highly cellular, showing a balanced representation of hematopoietic cells from various lineages at different maturation stages, indicating active and healthy hematopoiesis. GEM 0.1 mg/kg Group (Top Right): Treatment with a low concentration of Gemcitabine (0.1 mg/kg) resulted in observable changes. The smear shows signs of hypocellularity (reduced cellularity) compared to the control, suggesting an initial suppressive effect on the hematopoietic tissues. There is an increased presence of extravasated blood (hemorrhage/red areas) and potentially signs of damage to the stromal or vascular components.

GEM 0.3 mg/kg Group (Bottom Center): The high concentration of Gemcitabine (0.3 mg/kg) induced severe alterations. The smear demonstrates a marked decrease in overall cellularity and severe myelosuppression. The field is dominated by a diffuse, often homogenous red background, indicative of extensive hemorrhage and/or a profound reduction in hematopoietic cell populations (aplasia or severe

hypoplasia). This concentration clearly demonstrates a dose-dependent toxic effect of Gemcitabine on the highly proliferative bone marrow tissue.

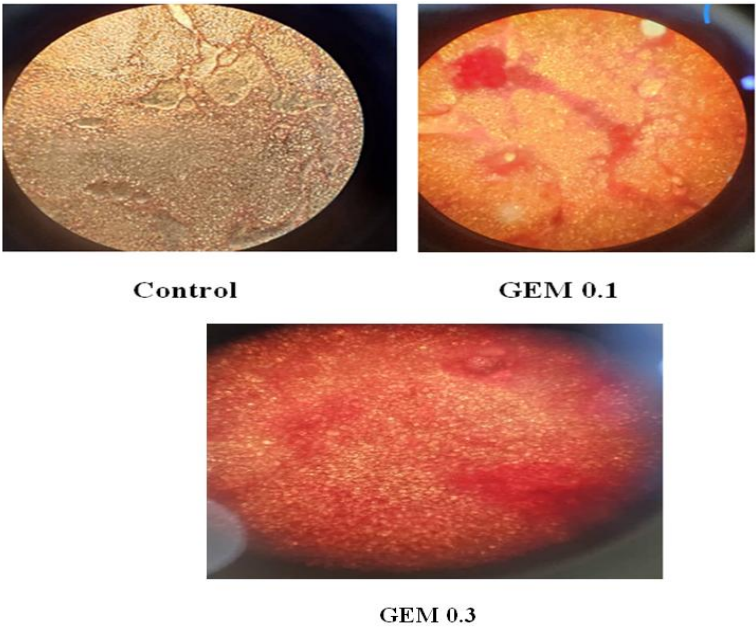


Figure 1. The Effect of Gemcitabine on Rabbit Bone Marrow Morphology: Comparison of 0.1 and 0.3 mg/kg Treatment Groups with Control".

The data presented in (Table 1) demonstrate a clear dose-dependent hematological suppression following gemcitabine administration in rabbits. Total WBC count declined from $7.1 \pm 0.18 \times 10^3/\mu\text{L}$ in the control group to $5.0 \pm 0.08 \times 10^3/\mu\text{L}$ at 0.1 mg/kg and further to $4.4 \pm 0.20 \times 10^3/\mu\text{L}$ at 0.3 mg/kg ($p = 0.000$), indicating pronounced leukopenia associated with bone marrow suppression. Lymphocyte percentage followed a similar pattern, decreasing from $63.6 \pm 2.6\%$ in controls to $62.3 \pm 0.21\%$ at the lower dose and markedly to $52.9 \pm 1.37\%$ at the higher dose ($p = 0.000$). This reduction suggests impaired lymphocyte production or accelerated turnover in response to gemcitabine-induced cytotoxicity. MID% also dropped significantly, declining from $10.2 \pm 0.48\%$ in the control group to $7.8 \pm 0.29\%$ at 0.1 mg/kg and $7.5 \pm 0.02\%$ at 0.3 mg/kg ($p = 0.000$).

A comparable trend was observed in granulocytes, which decreased from $12.3 \pm 0.66\%$ in controls to $10.6 \pm 0.76\%$ and $9.8 \pm 0.5\%$ in the low- and high-dose groups, respectively. Red blood cell counts showed substantial reductions, falling from $6.0 \pm 0.15 \times 10^6/\mu\text{L}$ in the control rabbits to $5.0 \pm 0.05 \times 10^6/\mu\text{L}$ at 0.1 mg/kg and reaching $3.9 \pm 0.26 \times 10^6/\mu\text{L}$ at 0.3 mg/kg ($p = 0.000$). This indicates significant inhibition of erythropoiesis. Platelet counts exhibited the most severe suppression, decreasing from $435.19 \pm 21.09 \times 10^3/\mu\text{L}$ in the control group to $301.86 \pm 7.94 \times 10^3/\mu\text{L}$ at the lower dose and dramatically to $146.6 \pm 21.01 \times 10^3/\mu\text{L}$ at the higher dose ($p = 0.000$). This pronounced thrombocytopenia reflects strong inhibitory effects of gemcitabine on megakaryocyte activity. Overall, the numerical values clearly demonstrate that gemcitabine induces significant, dose-dependent reductions across all major hematological parameters, confirming its potent myelosuppressive activity in rabbits.

Table 1. Complete Blood Count Parameters in Rabbits Treated with Gemcitabine (0.1 and 0.3 mg/kg) Compared to the Control Group.

Parameter	Experimental groups			
	Control	Gemcitabine 0.1	Gemcitabine 0.3	P-Value
White blood cells (WBC) $\times 10^3(\mu\text{L})$	7.1±0.18 ^a	5.0± 0.08 ^b	4.4± 0.20 ^b	0.000
Lymphocytes (LYM) %	63.6± 2.6 ^a	62.3± 0.21 ^b	52.9±1.37 ^b	0.000
Middle Cells Cells (MID) %	10.2± 0.48 ^a	7.8± 0.29 ^b	7.5± 0.02 ^b	0.000
Granulocytes (GRAN) %	12.3± 0.66 ^a	10.6± 0.76 ^b	9.8± 0.5 ^b	0.000
Red blood cells "(RBCs) $\times 10^6 (\mu\text{L})$	6.0±0.15 ^a	5.0± 0.05 ^b	3.9± 0.26 ^b	0.000
Platelet $\times 10^3/\mu\text{L}$	435.19 ±21.09 ^a	301.86± 7.94 ^b	146.6± 21.01 ^c	0.000

Values are expressed as means $\pm$ SE; n = 5 for each treatment group. Mean values within a row not sharing a common superscript letter (a, b, c) were significantly different, $p<0.05$.

Discussion

The findings of the present study clearly demonstrate that gemcitabine (GEM) administration in New Zealand White rabbits' results in significant hematological toxicity, characterized by both morphological damage to the bone marrow and a profound reduction in peripheral blood cell counts. The histopathological examination of the bone marrow smears (Figure 1) showed a severe dose-dependent hypocellularity in both treatment groups compared to the normocellular control. This morphological evidence of myelosuppression was substantiated by the Complete Blood Count (CBC) analysis (Table 1), which revealed a statistically significant decrease ($P=0.000$) across all measured parameters, including WBCs, Granulocytes, Lymphocytes, and RBCs. This collective reduction in all major hematopoietic lineages in the peripheral blood is consistent with pancytopenia and directly reflects the profound inhibitory effect of gemcitabine on the hematopoietic tissue [18].

Gemcitabine, as a deoxycytidine analog, is a potent S-phase-specific inhibitor, primarily targeting cells undergoing active DNA synthesis [19]. The bone marrow is one of the most highly proliferative tissues in the body, making it exceptionally vulnerable to the cytotoxic action of GEM. Our results align perfectly with this known mechanism: the hypocellularity observed in the marrow is the direct consequence of GEM's ability to destroy progenitor cells and disrupt the differentiation and maturation of rapidly dividing hematopoietic cells [20]. Furthermore, the dose-dependent nature of the toxicity is evident, as the high concentration (0.3 mg/kg) induced notably lower cell counts and more severe morphological damage compared to the 0.1 mg/kg dose, confirming the established pharmacokinetic characteristics of the drug [21-30]. The presence of hemorrhage is critical, as it suggests a compromise of marrow integrity, possibly exacerbated by drug-induced thrombocytopenia (though platelet count was not directly measured; it is often co-reduced) [31-53].

Conclusion

In summary, this study utilizes a correlative approach to demonstrate that the hematological toxicity of Gemcitabine in the rabbit model is the direct consequence of dose-dependent, severe myelosuppression. The clear visual evidence of hypocellularity and hemorrhage in the bone marrow smears provides a crucial morphological link to the observed peripheral cytopenias. These findings reinforce the clinical understanding that myelosuppression is the primary dose-limiting toxicity of Gemcitabine and highlight the necessity for continuous hematological monitoring during treatment to mitigate the associated risks of severe neutropenia and anemia.

References

1. Pourquier P, Gioffre C, Kohlhagen G, Urasaki Y, Goldwasser F, Hertel LW, Pommier Y. Gemcitabine (2',2'-difluoro-2'-deoxycytidine), an antimetabolite that poisons topoisomerase I. *Clin Cancer Res*. 2002;8(8):2499-2504.
2. Barton-Burke M. Gemcitabine: a pharmacologic and clinical overview. *Cancer Nurs*. 1999;22(2):176-183.
3. Lin JC, Oludare A, Jung H. Connecting dots between nucleotide biosynthesis and DNA lesion repair/bypass in cancer. *Biosci Rep*. 2024;44(9):BSR20231382.
4. Mini E, Nobili S, Caciagli B, Landini I, Mazzei T. Cellular pharmacology of gemcitabine. *Ann Oncol*. 2006;17 Suppl 5:v7-v12.
5. Iwata K, Aizawa K, Sakai S, Jingami S, Fukunaga E, Yoshida M, Saito H. The relationship between treatment time of gemcitabine and development of hematologic toxicity in cancer patients. *Biol Pharm Bull*. 2011;34(11):1765-1768.
6. Toschi L, Finocchiaro G, Bartolini S, Gioia V, Cappuzzo F. Role of gemcitabine in cancer therapy. *Future Oncol*. 2005;1(1):7-17.
7. Garcia-Gil M, Turri B, Gabriele M, Pucci L, Agnarelli A, Lai M, Marracci S. Protopine/gemcitabine combination induces cytotoxic or cytoprotective effects in cell type-specific and dose-dependent manner on human cancer and normal cells. *Pharmaceuticals*. 2021;14(2):90.
8. Chandira M, Jayakar B, Bhowmik D, Kumar KS. Formulation development and evaluation of gemcitabine hydrochloride dry powder for intravenous infusion. *Int J Pharm Technol*. 2010;2(1):23-36.
9. Sanchez de Medina F, Gamez MJ, Jimenez I, Jimenez J, Osuna JI, Zarzuelo A. Hypoglycemic activity of juniper berries. *Planta Med*. 1994 Jun;60(3):197-200.
10. Trease GE, Evans WC. *Pharmacognosy*. 16th ed. Philadelphia: W.B. Saunders; 2009.
11. Dunn JS, McLetchie NG. Experimental alloxan diabetes in the rat. *Lancet*. 1943;242(6265):384-7.
12. Kunas SL, Sari DC, Kartikasari S, Purnomosari E. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *MOJ Bioequiv Availab*. 2017;4(2):53.
13. Sachan AK, Rao CV, Sharma S, Singh AB. Extraction and evaluation of hypoglycemic and wound healing potential of hydro-ethanolic extract of *Alhagi pseudalhagi* wild. *Am J Ethnomed*. 2016;3(4):15-25.
14. Barton-Burke M. Gemcitabine: a pharmacologic and clinical overview. *Cancer Nurs*. 1999;22(2):176-83.
15. Doroshow DB, Herbst RS. Treatment of advanced non-small cell lung cancer in 2018. *JAMA Oncol*. 2018;4(4):569-70.
16. Lin JC, Oludare A, Jung H. Connecting dots between nucleotide biosynthesis and DNA lesion repair/bypass in cancer. *Biosci Rep*. 2024;44(9):BSR20231382.

17. Awoyomi OF, Gorospe CM, Das B, Mishra P, Sharma S, Diachenko O, et al. RRM2B deficiency causes dATP and dGTP depletion through enhanced degradation and slower synthesis. *Proc Natl Acad Sci U S A*. 2025;122(16):e2503531122.
18. Noble S, Goa KL. Gemcitabine: a review of its pharmacology and clinical potential in non-small cell lung cancer and pancreatic cancer. *Drugs*. 1997;54(3):447-72.
19. Rejeski K, Subklewe M, Aljurf M, Bachy E, Balduzzi A, Barba P, et al. Immune effector cell-associated hematotoxicity: EHA/EBMT consensus grading and best practice recommendations. *Blood*. 2023;142(10):865-77.
20. Khaled FA, Ali MS, Radad HS. Influence of ascorbic acid supplementation on hematological parameters and free radical in adult male rabbits. *Saudi J Biomed Res*. 2019;4(5):244-7.
21. Khaled FA, Younus AA, Sale RM. Hematological parameters and blood smear effects of tramadol on male rabbits. 2021.
22. Bleve A, Consonni FM, Porta C, Garlatti V, Sica A. Evolution and targeting of myeloid suppressor cells in cancer: a translational perspective. *Cancers (Basel)*. 2022;14(3):510.
23. Yousef M, Hassan H, Mohammed A, Kamel K, Khaled F. The protective role of ginger against DEHP-induced reproductive toxicity and oxidative stress in male rabbits. *Endocr Abstr*. 2012;29.
24. Yousef MI, Awad TI, Elhag FA, Khaled FA. Study of the protective effect of ascorbic acid against the toxicity of stannous chloride on oxidative damage, antioxidant enzymes and biochemical parameters in rabbits. *Toxicology*. 2007;235(3):194-202.
25. Khaled FA, Yousef MI, Kamel KI. The protective role of propolis against the reproductive toxicity of monosodium glutamate in male rabbits. *Int J Chem Stud*. 2016;4(2):4-9.
26. El-Speiy ME, Khaled FA, El-Hanoun AM. Effect of ginger supplementation on reproductive performance of male rabbits. *Glob Sci J Biol*. 2017;2(2):26-31.
27. Elgazwi SM, Khaled FA, Alsanous MF. Study the protective effect of ginger against the toxicity of dimethoate on hormones in rabbits. *Asian J Res Biochem*. 2021;8(3):24-33.
28. Khaled FA, Shoaib A, Attia M. Hepatoprotective effect of ginger induced experimentally by dimethoate and liver injury in adult male rabbits. *AlQalam J Med Appl Sci*. 2021;24-30.
29. Mohammed N, Hassan H, Ali A, Khaled F, Mohamed S. Histopathological alterations in liver of male rabbits exposed to deltamethrin and the ameliorative effect of folic acid. *AlQalam J Med Appl Sci*. 2022;454-60.
30. Iwata K, Aizawa K, Sakai S, Jingami S, Fukunaga E, Yoshida M, et al. Relationship between treatment time of gemcitabine and development of hematologic toxicity in cancer patients. *Biol Pharm Bull*. 2011;34(11):1765-8.
31. Zhou X, Ao X, Jia Z, Li Y, Kuang S, Du C, et al. Non-coding RNA in cancer drug resistance: underlying mechanisms and clinical applications. *Front Oncol*. 2022;12:951864.
32. Valadares MC, Bincoletto C, Oliveira SC, de Melo A, Saad ST, Queiroz ML. Bone marrow progenitor cells from chemically exposed workers display an intrinsic ability for autonomous proliferation. *Immunopharmacol Immunotoxicol*. 2005;27(1):137-45.
33. Dai S, Wang C, Zhao X, Ma C, Fu K, Liu Y, et al. Cucurbitacin B: A review of its pharmacology, toxicity, and pharmacokinetics. *Pharmacol Res*. 2023;187:106587.
34. Saad E, Ibrahim M, Khaled F, Ali M. Comparative study between effects of some antioxidants on levels of hormones in male rabbits. *AlQalam J Med Appl Sci*. 2021;60-8.
35. Al-Ailla AK, Khaled FA. Effect of ginger extract on antioxidant enzymes and free radicals in rabbits. *J Biotechnol Biochem*. 2019;5(1):37-40.
36. Khaled FA, Qataf RA. Comparative study of curcumin and garlic as antioxidants in male rabbits on biochemical parameters. *Libyan J Basic Sci*. 2022;19(2):1-19.
37. Khaled FA, Saad GI. Evaluation of the protective effects of cinnamon on liver and kidney function in rabbits exposed to paracetamol toxicity. *Appl Sci Res Period*. 2025;3(4):37-46.
38. Khaled FA, Qataf RA. Enhanced role of garlic and curcumin on hematological parameters in male rabbits. *Int J Pharm Life Sci*. 2021;12(8).
39. Omar OAE, Eman GA, Khaled FA. Biochemical consider on the defensive role of ginseng in male rabbits. *Int J Pharm Life Sci*. 2021;12(3).
40. AA SA, Marwa JS. Biochemical study on the role of curcumin in male rabbits. *Ann Pharm Res*. 2021;9(3).
41. Atalhi FM. Paracetamol induces decrease in antioxidant enzymes and TBARS in male rabbits. 2022.
42. Atalhi FM. Phenolic compounds of Graviola enhance lipid profile in male rabbits. 2022.
43. Riahi-Zanjani B, Delirrad M, Fazeli-Bakhtiyari R, Sadeghi M, Zare-Zardini H, Jafari A, et al. Hematological consequences of valproic acid in pediatric patients: a systematic review. *CNS Neurol Disord Drug Targets*. 2022;21(4):316-25.
44. Ali MS, Khaled FA, Saloumah HS. Annona muricata suppresses stannous chloride effects by modulating hematological parameters in rabbits. *J Complement Altern Med Res*. 2021;251-62.
45. Aldeeb OH, Ibridan BA, Khaled FA, Shah A. Harmful impact of dimethoate and chlorpyrifos on hematological parameters in male rabbits. *South Asian Res J Bio Appl Biosci*. 2022;4(1):11-7.
46. Mokhtar I, Khaled FA, Abdel-Aziz F, Hanaa A, Kamel I. Ginger suppresses DEHP testicular toxicity through hormonal regulation in rabbits. 2021.
47. Khaled FA, Ahmed AI. Effect of Ziziphus spina-christi leaves on plasma hormones in male rabbits. *World*. 2025;2(9).
48. Amharib AM, Khaled FA, Younis FM. Protective role of vitamin E against chlorpyrifos toxicity on semen quality and testosterone in rabbits. 2021.
49. Khaled FA, Ali MS, Qowaider SR, Farge R. Zingiber officinale syrup reduces bacterial load in Helicobacter pylori cases in Libya. 2021.
50. Khaled F, Hussien S, Mahmoud R, Belhamad N. Effects of Libyan Balanites aegyptiaca on steroid hormones in male rabbits. *Khalij-Libya J Dent Med Res*. 2025;208-12.

51. Khaled F, Mohammed S. Impact of creatine and vitamin C on insulin sensitivity and HbA1c in male rabbits. *Razi Med J*. 2025;106-11.
52. Khaled F, Ali M. Role of vitamin E in reducing tramadol-induced TBARS in male rabbits. *Attahadi Med J*. 2025;16-9.
53. Iamsaard S, Sukhorum W, Arun S, Phunchago N, Uabundit N, Boonruangsri P, et al. Valproic acid induces histologic changes and decreases androgen receptor levels in rats. *Int J Reprod Biomed*. 2017;15(4):217-25.
54. Chakrabarty S, Jigdel K, Mukherjee P, Paul T, Drakpa D, Gupta J. Bioactivities of jojoba oil beyond skincare. *J Med Food*. 2024;27(7):579-88.
55. Belhadj S, Hentati O, Hamdaoui G, Fakhreddine K, Maillard E, Dal S, et al. Jojoba seed extracts reduce hyperglycemia-induced oxidative stress in beta cells. *Nutrients*. 2018;10(3):384.