

Original article

Dose-Dependent Effects of *Costus speciosus* Extract on Growth Performance, Feed Utilization, and Humoral Immune Response in Male Rabbits

Eman M. Abufkeren Abubaker 

Biology Department, Faculty of Education, Omar Al-Mokhtar University, El-Beida, Libya
eman.efkeren@omu.edu.ly

Keywords:

Costus Speciosus; Medicinal Plant; Male Rabbits; Growth Performance; Immunomodulation.

ABSTRACT

Costus speciosus is a medicinal plant containing diverse bioactive constituents with potential biological applications. However, experimental evidence regarding its effects on growth performance and humoral immune responses in rabbits remains limited. This study evaluated the effects of graded doses of *Costus speciosus* extract on growth performance, feed utilization, and humoral immune response in male rabbits. Methods: Male rabbits were allocated to four experimental groups receiving the control treatment or *C. speciosus* extract at doses of 100, 300, and 600 mg/kg. We evaluated growth performance parameters, including final body weight, total body weight gain, daily weight gain, dry matter intake, and feed conversion ratio. We assessed the humoral immune response using anti-sheep red blood cell (SRBC) antibody titers before immunization and on days 7 and 14 after immunization. Initial body weights were comparable among groups. Final body weight increased from 3.01 ± 0.05 kg in controls to 3.18 ± 0.04 , 3.31 ± 0.05 , and 3.32 ± 0.04 kg at 100, 300, and 600 mg/kg, respectively ($P < 0.05$). Total body weight gain increased from 0.86 ± 0.03 kg in controls to 1.04 ± 0.03 , 1.15 ± 0.04 , and 1.25 ± 0.04 kg, while daily weight gain increased from 15.4 ± 0.6 to 18.6 ± 0.6 , 20.5 ± 0.7 , and 22.3 ± 0.7 g/day, respectively ($P < 0.05$). Feed conversion ratio decreased from 4.11 ± 0.09 in controls to 3.49 ± 0.07 , 3.25 ± 0.08 , and 3.04 ± 0.06 , respectively ($P < 0.05$). Anti-SRBC antibody titers on day 7 increased from $6.8 \pm 0.3 \log_2$ in controls to 7.9 ± 0.3 , 8.7 ± 0.3 , and $9.4 \pm 0.3 \log_2$, while day-14 titers increased from 8.1 ± 0.3 to 9.5 ± 0.4 , 10.4 ± 0.3 , and $11.2 \pm 0.3 \log_2$, respectively ($P < 0.05$). *C. speciosus* extract was associated with dose-related improvements in growth performance, feed conversion, and anti-SRBC antibody responses in male rabbits. The findings support further investigation of *C. speciosus* as a botanical candidate for improving productive performance and humoral immune responses in animal production.

Introduction

Rabbit production has attracted considerable attention as an efficient source of high-quality animal protein because rabbits have a relatively short production cycle, rapid growth, high reproductive capacity, and efficient utilization of dietary nutrients [1]. The productive performance of growing rabbits is strongly influenced by dietary quality, feed intake, nutrient utilization, gastrointestinal health, and the ability of animals to maintain an adequate physiological and immune status [2]. Consequently, improving feed efficiency while maintaining optimal health has become an important objective in contemporary rabbit production systems [3]. The increasing interest in natural feed additives has stimulated research into medicinal plants and their extracts as potential alternatives or complementary approaches to conventional feed additives [4]. Plant-derived products contain diverse bioactive compounds, including phenolics, flavonoids, terpenoids, alkaloids, saponins, and steroidal constituents, which may exert antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory activities [5]. These biological properties have attracted considerable interest in animal nutrition because phytochemical additives may influence productive performance, nutrient utilization, intestinal function, and immune responses [6].

In rabbits, dietary supplementation with herbs and plant extracts has been investigated as a means of improving growth performance and feed utilization, although responses vary according to plant species, extract composition, dose, experimental conditions, and duration of supplementation [7-15]. A recent meta-analysis involving 33 *in vivo* studies and 121 experimental units reported that herbal supplementation was associated with improved feed conversion ratio and nutrient digestibility in farmed rabbits, although effects on final body weight and average daily gain were not consistently observed across all botanical preparations [16-25]. These findings emphasize the importance of evaluating individual plant species and establishing appropriate dose-response relationships rather than assuming uniform effects among different phytochemical additives [26-40].

The immune system is another important determinant of productivity and health in rabbits because adequate immune responsiveness contributes to resistance against infectious and environmental challenges [40]. Plant-derived feed additives have been reported to influence immune-related parameters in livestock, and systematic evidence indicates that phytogetic supplementation can affect both productive performance and immune responses, particularly in monogastric species [41]. In rabbits, natural feed additives have been investigated for their potential to improve immune capacity together with growth performance and feed efficiency [42-47]. Among medicinal plants, *Costus speciosus* (Koen.) Sm., belonging to the family Costaceae, has received considerable scientific interest because of its diverse phytochemical composition and broad range of reported biological activities [48].

Phytochemical investigations of *C. speciosus* have identified steroidal saponins, particularly diosgenin and related compounds, together with flavonoids, terpenoids, phenolic constituents, and other secondary metabolites [49]. Diosgenin is one of the characteristic bioactive constituents associated with *C. speciosus* and has been investigated extensively because of its pharmacological and biological properties [50]. Experimental studies have reported antioxidant and anti-inflammatory activities associated with constituents of *C. speciosus*, suggesting that the plant possesses biologically active compounds capable of interacting with pathways relevant to cellular protection and immune regulation [51]. In particular, diosgenin and other constituents isolated from *C. speciosus* have been investigated in experimental models for their effects on inflammatory mediators and cellular signaling pathways. These findings provide a biological rationale for investigating *C. speciosus* as a potential phytogetic supplement in animal production, although pharmacological activity demonstrated in experimental models should not automatically be equated with a demonstrated nutritional benefit in livestock [52]. The application of medicinal plants in rabbit nutrition requires particular attention to dose because phytogetic compounds may produce different biological responses depending on concentration, preparation method, bioavailability, and duration of administration. Recent systematic evidence indicates that dose optimization is an important consideration when evaluating phytogetic feed additives, with some botanical preparations showing beneficial responses at specific inclusion levels but limited additional effects at higher doses [53]. In particular, the effects of graded doses of *C. speciosus* extract on body weight development, weight gain, dry matter intake, feed conversion ratio, and antibody-mediated responses following immunization require further experimental investigation. The present study was therefore designed to evaluate the effects of 100, 300, and 600 mg/kg *C. speciosus* extract on growth performance, feed utilization, and humoral immune response in male rabbits. The study specifically assessed body weight-related parameters, daily dry matter intake, feed conversion ratio, and anti-sheep red blood cell (SRBC) antibody titers during the early and later phases of the immune response.

Methods

Fresh rhizomes of *Costus speciosus* were collected during the flowering season from a botanical garden and authenticated by a qualified taxonomist. A voucher specimen was deposited in the university herbarium for future reference. The rhizomes were thoroughly washed with distilled water to remove adhering soil and debris, sliced into small pieces, and shade-dried at 40 °C for 7 days. The dried material was subsequently ground into a fine powder using an electric grinder. For extraction, 1 kg of the powdered rhizome material was macerated in 70% ethanol at a ratio of 1:10 (w/v) for 72 h at room temperature with intermittent shaking. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure at 45 °C using a rotary evaporator. The concentrated extract was subsequently lyophilized to obtain a dry crude extract. The extraction yield was 18.6% (w/w). The resulting dark-brown extract was transferred to amber glass containers and stored at 4 °C until administration.

Forty-eight clinically healthy male New Zealand White rabbits, aged 4–5 months and weighing approximately 2.1–2.5 kg, were obtained from a local commercial rabbit farm. Before commencement of the experiment, all animals were subjected to a 14-day acclimatization period under controlled laboratory conditions. The rabbits were maintained at a temperature of 22 ± 2 °C under a 12-h light/12-h dark cycle and relative humidity of 40–60%. Animals were provided with a standard commercial pelleted diet containing approximately 18% crude protein and 15% crude fiber, with free access to clean drinking water throughout the experimental period. The animals were individually housed in clean, well-ventilated wire-mesh cages. All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals and were approved by the relevant institutional animal ethics committee before initiation of the study.

Following acclimatization, the 48 rabbits were weighed and randomly allocated into four experimental groups of 12 animals each ($n = 12/\text{group}$), with groups balanced according to initial body weight. The experimental groups were as follows: Group I (Control): received distilled water orally. Group II (*C. speciosus* 100 mg/kg): received *C. speciosus* extract at 100 mg/kg body weight/day. Group III (*C. speciosus* 300 mg/kg): received *C. speciosus* extract at 300 mg/kg body weight/day. Group IV (*C. speciosus* 600 mg/kg): received *C. speciosus* extract at 600 mg/kg body weight/day. The extract was freshly suspended in

distilled water and administered once daily by oral gavage for 56 consecutive days. The control animals received an equivalent oral administration of distilled water. The administration volume was adjusted according to individual body weight. Animals were monitored daily throughout the experimental period for general health status, behavioral changes, feed and water consumption, and any signs of morbidity or mortality. Individual body weights were recorded at the beginning of the experiment and subsequently at regular weekly intervals throughout the 56-day treatment period. Final body weight was recorded at the end of the experimental period. Total body weight gain was calculated as: Total body weight gain (kg) = Final body weight – Initial body weight.

Daily weight gain was calculated from the total body weight gain and experimental duration: Daily weight gain (g/day) = [Final body weight – Initial body weight] × 1000 / 56. Daily feed intake was determined by providing a known quantity of feed to each rabbit and recording the residual feed after the designated feeding period. Daily dry matter intake was calculated based on the dry matter content of the supplied commercial diet. Feed conversion ratio (FCR) was calculated as: FCR = Total dry matter intake (g) / Total body weight gain (g). Lower FCR values were interpreted as indicating improved feed utilization. The humoral immune response was evaluated using the sheep red blood cell (SRBC) hemagglutination assay. All rabbits were immunized intramuscularly with 1 mL of a 10% suspension of SRBCs prepared in sterile physiological saline on day 42 of the experimental treatment. Blood samples were collected from the marginal ear vein immediately before immunization (day 0) and subsequently on days 7 and 14 post-immunization. Blood samples were allowed to clot at room temperature and were then centrifuged to obtain serum. The separated sera were stored at –20 °C until analysis. Anti-SRBC antibody titers were determined using a micro-hemagglutination assay. Briefly, serial two-fold dilutions of serum were prepared in microtiter plates and incubated with an equal volume of standardized SRBC suspension.

Following incubation, the plates were examined for visible hemagglutination. The antibody titer was expressed as the reciprocal of the highest serum dilution showing complete agglutination and was converted to a log₂ value for statistical analysis. Antibody titers obtained on day 7 were considered representative of the early antibody response, whereas titers measured on day 14 represented the later antibody response. At each scheduled sampling point, blood samples were collected using standard aseptic procedures. Individual animal identification was maintained throughout the experimental period to permit repeated measurements of body weight, feed intake, and immune-response variables. The primary outcome variables were final body weight, total body weight gain, daily weight gain, daily dry matter intake, feed conversion ratio, and anti-SRBC antibody titers on days 0, 7, and 14 following immunization.

All quantitative data were expressed as mean ± standard error of the mean (SEM), with 12 rabbits per experimental group (n = 12). Growth performance and feed utilization variables were compared among the four experimental groups using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison post hoc test when significant differences were detected. For parameters measured repeatedly over time, including body weight and anti-SRBC antibody titers, an appropriate repeated-measures analysis was performed to account for measurements obtained from the same animals at different time points. Where applicable, treatment group and sampling time were considered as fixed factors. Statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant at $P < 0.05$.

Results

The results demonstrated a clear dose-related improvement in growth performance following administration of *Costus speciosus* extract. Initial body weights were comparable among the experimental groups, ranging from 2.13 ± 0.03 to 2.16 ± 0.04 kg. Final body weight increased from 3.01 ± 0.05 kg in the control group to 3.18 ± 0.04 , 3.31 ± 0.05 , and 3.32 ± 0.04 kg in rabbits receiving 100, 300, and 600 mg/kg, respectively, with significant differences compared with the control group ($P < 0.05$). Similarly, total body weight gain increased significantly from 0.86 ± 0.03 kg in controls to 1.04 ± 0.03 , 1.15 ± 0.04 , and 1.25 ± 0.04 kg at 100, 300, and 600 mg/kg, respectively ($P < 0.05$). Daily weight gain followed the same pattern, increasing from 15.4 ± 0.6 g/day in the control group to 18.6 ± 0.6 , 20.5 ± 0.7 , and 22.3 ± 0.7 g/day at the respective extract doses ($P < 0.05$). Daily dry matter intake showed a gradual increase across the groups, from 63.2 ± 1.4 g/day in controls to 65.0 ± 1.3 , 66.7 ± 1.5 , and 67.8 ± 1.4 g/day at 100, 300, and 600 mg/kg, respectively. In contrast, feed conversion ratio decreased progressively from 4.11 ± 0.09 in the control group to 3.49 ± 0.07 , 3.25 ± 0.08 , and 3.04 ± 0.06 , respectively, with all treated groups showing significant differences from the control ($P < 0.05$). Overall, the growth-related parameters showed progressively greater responses across the tested doses.

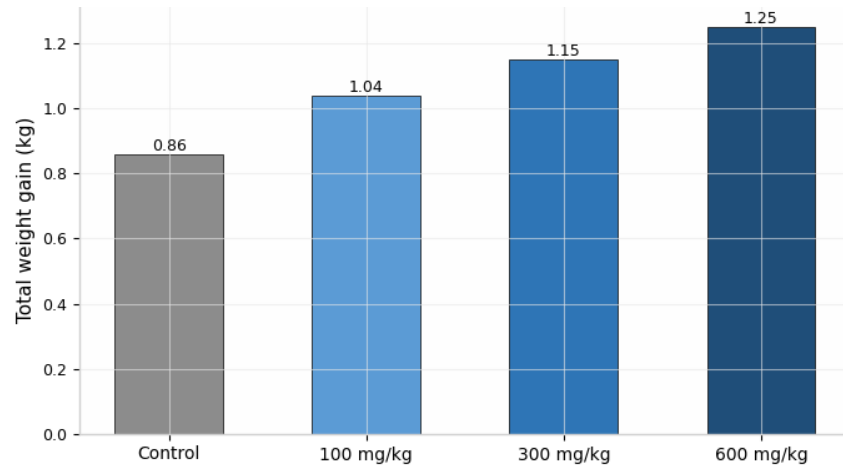


Figure 1. Effect of *C. speciosus* extract on total body weight gain of male rabbits (mean $\pm$ SEM). * $P < 0.05$ vs. control.

Table 1. Effect of *C. speciosus* extract on growth performance in male rabbits (mean $\pm$ SEM, $n = 12$).

Parameter	Control	100 mg/kg	300 mg/kg	600 mg/kg
Initial body weight (kg)	2.15 $\pm$ 0.03	2.14 $\pm$ 0.03	2.16 $\pm$ 0.04	2.13 $\pm$ 0.03
Final body weight (kg)	3.01 $\pm$ 0.05	3.18 $\pm$ 0.04*	3.31 $\pm$ 0.05*	3.32 $\pm$ 0.04*
Total weight gain (kg)	0.86 $\pm$ 0.03	1.04 $\pm$ 0.03*	1.15 $\pm$ 0.04*	1.25 $\pm$ 0.04*
Daily weight gain (g)	15.4 $\pm$ 0.6	18.6 $\pm$ 0.6*	20.5 $\pm$ 0.7*	22.3 $\pm$ 0.7*
Daily DM intake (g)	63.2 $\pm$ 1.4	65.0 $\pm$ 1.3	66.7 $\pm$ 1.5	67.8 $\pm$ 1.4
Feed conversion ratio	4.11 $\pm$ 0.09	3.49 $\pm$ 0.07*	3.25 $\pm$ 0.08*	3.04 $\pm$ 0.06*

The anti-SRBC antibody titers were comparable before immunization, with values of 2.2 ± 0.2 , 2.3 ± 0.2 , 2.2 ± 0.2 , and 2.3 ± 0.2 log₂ in the control, 100, 300, and 600 mg/kg groups, respectively. Following immunization, antibody titers increased in all groups, with significantly higher responses observed in rabbits receiving *C. speciosus* extract ($P < 0.05$). On day 7, corresponding to the IgM response, antibody titers increased from 6.8 ± 0.3 log₂ in the control group to 7.9 ± 0.3 , 8.7 ± 0.3 , and 9.4 ± 0.3 log₂ in the 100, 300, and 600 mg/kg groups, respectively. On day 14, corresponding to the IgG response, titers increased from 8.1 ± 0.3 log₂ in controls to 9.5 ± 0.4 , 10.4 ± 0.3 , and 11.2 ± 0.3 log₂ at 100, 300, and 600 mg/kg, respectively ($P < 0.05$). The observed antibody responses therefore demonstrated a consistent increase with increasing extract dose at both sampling times, with the highest values recorded at 600 mg/kg.

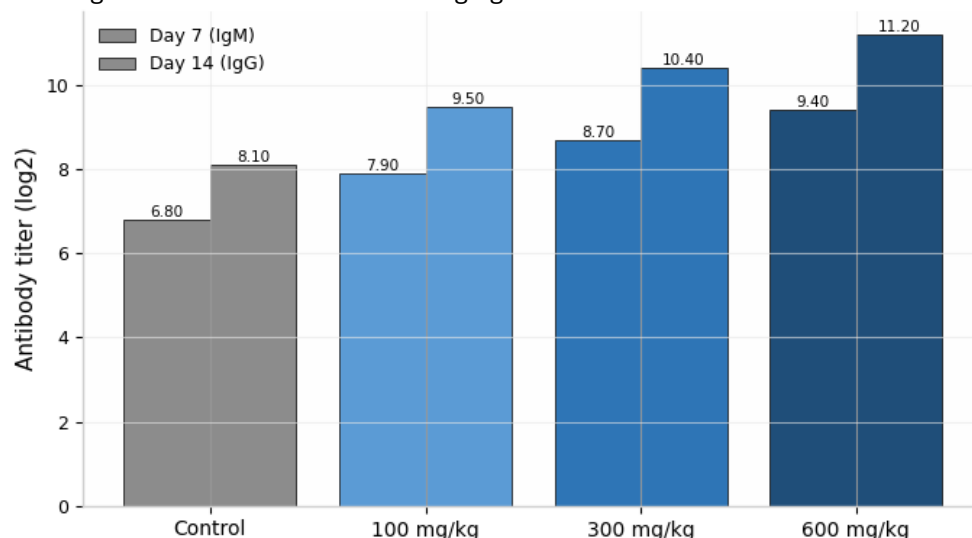


Figure 2. Anti-SRBC antibody titers (log₂) on days 7 and 14 post-immunization (mean $\pm$ SEM). * $P < 0.05$. vs. control.

Table 2. Effect of *C. speciosus* extract on anti-SRBC antibody titers (log2, mean $\pm$ SEM, n = 12).

Sampling time	Control	100 mg/kg	300 mg/kg	600 mg/kg
Day 0 (pre-immunization)	2.2 $\pm$ 0.2	2.3 $\pm$ 0.2	2.2 $\pm$ 0.2	2.3 $\pm$ 0.2
Day 7 (IgM response)	6.8 $\pm$ 0.3	7.9 $\pm$ 0.3*	8.7 $\pm$ 0.3*	9.4 $\pm$ 0.3*
Day 14 (IgG response)	8.1 $\pm$ 0.3	9.5 $\pm$ 0.4*	10.4 $\pm$ 0.3*	11.2 $\pm$ 0.3*

Discussion

The present study demonstrated a clear dose-related improvement in growth performance and feed utilization in male rabbits receiving *Costus speciosus* extract. Treated rabbits showed higher final body weight, total and daily weight gain, together with progressively improved feed conversion ratio. These findings are consistent with evidence that phytogetic feed additives can enhance productive performance and feed efficiency in rabbits, although responses may vary according to plant species, phytochemical composition, dose, and extraction method [54]. The improved feed conversion observed with increasing extract dose is particularly relevant because it indicates more efficient utilization of dietary nutrients. The response may be associated with the diverse phytochemical constituents of *C. speciosus*, including steroidal saponins, diosgenin-related compounds, flavonoids, phenolics, and terpenoids, which have been reported to possess several biological activities [55]. However, the specific mechanisms responsible for the observed growth response cannot be confirmed because nutrient digestibility, intestinal microbiota, and metabolic pathways were not evaluated. The extract also significantly enhanced the humoral immune response following SRBC immunization.

Anti-SRBC antibody titers were comparable among groups before immunization but increased significantly in treated rabbits on days 7 and 14, with the highest responses recorded at 600 mg/kg. This pattern indicates a dose-related enhancement of antigen-specific antibody production and agrees with previous evidence that phytogetic additives can modulate immune responses in livestock [56]. The immunomodulatory effects of *C. speciosus* may be related to its steroidal and phenolic constituents, particularly diosgenin and flavonoids, which have been associated with modulation of inflammatory and cellular signaling pathways [57]. Nevertheless, these mechanisms remain hypothetical in the present study because cytokines, immune-cell populations, and antioxidant biomarkers were not measured. Overall, the concurrent improvement in growth performance, feed efficiency, and humoral immune response suggests that *C. speciosus* extract has potential as a botanical feed additive in rabbit production and warrants further investigation.

Conclusion

Oral supplementation with *Costus speciosus* extract produced dose-related improvements in growth performance, feed utilization, and anti-SRBC antibody responses in male rabbits. The 600 mg/kg dose showed the highest values for body weight gain and humoral antibody response and the lowest feed conversion ratio under the conditions of the present study. These findings suggest that *C. speciosus* represents a promising botanical candidate for further investigation as a natural feed additive in rabbit production. Future studies should characterize its active phytochemicals and clarify the nutritional, antioxidant, intestinal, and molecular mechanisms underlying its productive and immunomodulatory effects.

Conflict of interest.

Nil

References

- Goswami N, Solomon Ahamba I, Kinkpe L, Mujtaba Shah A, Xiangyang Y, Song B, et al. Enhancing rabbit farming efficiency with integrated genomics and nutritional strategies. *Front Anim Sci.* 2025;5:1514923.
- Elbaz AM, Farrag B, Mesalam NM, Basuony HA, Badran AM, Abdel-Moneim AME. Growth performance, digestive function, thyroid activity, and immunity of growing rabbits fed olive cake with or without *Saccharomyces cerevisiae* or citric acid. *Trop Anim Health Prod.* 2023;55(6):376.
- Gidenne T, Garreau H, Drouilhet L, Aubert C, Maertens L. Improving feed efficiency in rabbit production: a review on nutritional, technico-economical, genetic and environmental aspects. *Anim Feed Sci Technol.* 2017;225:109-122.
- Alem WT. Effect of herbal extracts in animal nutrition as feed additives. *Heliyon.* 2024;10(3).
- Sharma BP, Jeline R, Vimala VC, Poonam DS, Sanjeet K, Sugimani M, et al. Bioactive compounds of medicinal plants: structure, function, and bioactivity. *J Biodivers Conserv.* 2025;9(4):34-50.
- Nantapo CW, Marume U. Strategic technologies to improve phytogetic feed additive efficacy in pigs and poultry. *Anim Nutr.* 2025;23:286-303.
- 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.

8. 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.
9. 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.
10. Khaled FA, Qataf RA. Enhanced role of garlic and curcumin on hematological parameters in male rabbits. *Int J Pharm Life Sci.* 2021;12(8).
11. Omar OAE, Eman GA, Khaled FA. Biochemical consideration on the defensive role of ginseng in male rabbits. *Int J Pharm Life Sci.* 2021;12(3).
12. AA SA, Marwa JS. Biochemical study on the role of curcumin in male rabbits. *Ann Pharm Res.* 2021;9(3).
13. Atalhi FM. Paracetamol induces decrease in antioxidant enzymes and TBARS in male rabbits. 2022.
14. Atalhi FM. Phenolic compounds of *Graviola* enhance lipid profile in male rabbits. 2022.
15. 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):316-325.
16. Ali MS, Khaled FA, Saloumah HS. *Annona muricata* suppresses stannous chloride effects by modulating hematological parameters in rabbits. *J Complement Altern Med Res.* 2021;251-262.
17. 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):11-17.
18. Mokhtar I, Khaled FA, Abdel-Aziz F, Hanaa A, Kamel I. Ginger suppresses DEHP testicular toxicity through hormonal regulation in rabbits. 2021.
19. Khaled FA, Ahmed AI. Effect of *Ziziphus spina-christi* leaves on plasma hormones in male rabbits. *World.* 2025;2(9).
20. Amharib AM, Khaled FA, Younis FM. Protective role of vitamin E against chlorpyrifos toxicity on semen quality and testosterone in rabbits. 2021.
21. Khaled FA, Ali MS, Qowaider SR, Farge R. *Zingiber officinale* syrup reduces bacterial load in *Helicobacter pylori* cases in Libya. 2021.
22. 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.* 2025:208-212.
23. Khaled F, Mohammed S. Impact of creatine and vitamin C on insulin sensitivity and HbA1c in male rabbits. *Razi Med J.* 2025:106-111.
24. Khaled F, Ali M. Role of vitamin E in reducing tramadol-induced TBARS in male rabbits. *Attahadi Med J.* 2025:16-19.
25. 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.
26. 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.
27. 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.
28. 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.
29. 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.
30. 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.
31. 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.* 2022:454-460.
32. 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):244-247.
33. Khaled FA, Younus AA, Sale RM. Hematological parameters and blood smear effects of tramadol on male rabbits. 2021.
34. Khaled F, Ali R, Asbaaq M. Effect of folic acid and ascorbic acid on hematological parameters of male rabbits. *Attahadi Med J.* 2025:35-38.
35. Khaled F, Ali R, Aboulqassim T. Strawberry juice reduces renal and hematotoxic effects of carbon tetrachloride in rabbits. *AlQalam J Med Appl Sci.* 2025:731-738.
36. Khaled F, Masoud F. Dose-dependent myelosuppression and hematological toxicity induced by gemcitabine in New Zealand white rabbits. *AlQalam J Med Appl Sci.* 2025:2650-2655.
37. Salih A, Amharib A, Khaled F, Alsba A. Metformin therapy impact on liver and kidney function in diabetic patients in Libya. *Libyan Med J.* 2025:164-168.
38. Alfageih L, Amharib A, Khaled F, Auad F. Function of alpha-lipoic acid as antioxidant and lipid-lowering agent in rabbits. *AlQalam J Med Appl Sci.* 2024:615-621.
39. Shoaib A, Husayn A, Khaled F, Abdulhamid M. Hematological and biochemical differences in vaccinated vs unvaccinated COVID-19 cases in Libya. *Libyan Med J.* 2025:218-221.

40. Citrine EM, Yunas A, Khaled F. Effects of chromium and tin on biochemical parameters in male rabbits. *AlQalam J Med Appl Sci.* 2025:91-95.
41. Khaled FA, Saleh AM, Saleh AH. Toxic effect of chlorpyrifos, deltamethrin and dimethoate on biochemical parameters in male rabbits. *J Med Sci.* 2023;18(3):1-6.
42. Othman A. Hepatoprotective effect of jojoba against valproic acid-induced hematological alterations in male rabbits. *AlQalam J Med Appl Sci.* 2025:2548-2551.
43. Hammad A, Khaled FA. Inorganic mineral dynamics in two medicinal plants and their influence on body weight gain in experimental rabbits. *AlQalam J Med Appl Sci.* 2026:5-10.
44. Salem AM, Khaled FA, Mohamed HEA, Husayn AS. The protective role of melatonin against sodium arsenite-induced hepato-toxicity in male rabbits: biochemical and histopathological study. *World J Appl Med Sci.* 2026;3(1):1-6.
45. Hammad AA, Khaled FA, Hussien SM. GC-MS characterization of *Ziziphus spina-christi* essential oil and its antioxidant efficacy in rabbit models. *World J Appl Med Sci.* 2026;3(1):14-18.
46. Salem AM, Khaled FA, Mohamed HEA, Husayn AS. The protective role of melatonin against sodium arsenite-induced hepato-toxicity in male rabbits: biochemical and histopathological study. *World J Appl Med Sci.* 2026;3(1):1-6.
47. Mohammed N, Hassan H, Ali A, Khaled F. Toxic effects of deltamethrin on the histological structure of the urogenital system in adult male rabbits and the protective role of folic acid antioxidants. *Libyan Med J.* 2026:42-47.
48. Hammad AA, Khaled FA, Hussien SM. GC-MS characterization of *Ziziphus spina-christi* essential oil and its antioxidant efficacy in rabbit models. *World J Appl Med Sci.* 2026;3(1):14-18.
49. Mir MA, Sawhney SS, Jassal MMS. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. *Wudpecker J Pharm Pharmacol.* 2013;2(1):1-5.
50. Adarkar R, Viswambaran VL, Dessai A, Lobo R, Tippavajhala VK, Pai V, et al. Diosgenin-mediated NF- κ B and MAPK pathway modulation for osteoarthritis treatment: molecular mechanisms, bioavailability enhancement, and therapeutic applications. *Inflammopharmacology.* 2026;34(2):833-851.
51. Al-Attas AA, El-Shaer NS, Mohamed GA, Ibrahim SR, Esmat A. Anti-inflammatory sesquiterpenes from *Costus speciosus* rhizomes. *J Ethnopharmacol.* 2015;176:365-374.
52. Selim S, Al Jaouni S. Anti-inflammatory, antioxidant and antiangiogenic activities of diosgenin isolated from traditional medicinal plant, *Costus speciosus* (Koen ex. Retz.) Sm. *Nat Prod Res.* 2016;30(16):1830-1833.
53. Sohrab S, Mishra P, Mishra SK. Phytochemical competence and pharmacological perspectives of an endangered boon—*Costus speciosus* (Koen.) Sm.: a comprehensive review. *Bull Natl Res Cent.* 2021;45(1):209.
54. Abdelsalam M, Fathi M. Improving productivity in rabbits by using some natural feed additives under hot environmental conditions—a review. *Anim Biosci.* 2023;36(4):540.
55. Francis G, Makkar HPS, Becker K. Dietary supplementation with a *Quillaja* saponin mixture improves growth performance and metabolic efficiency in common carp (*Cyprinus carpio* L.). *Aquaculture.* 2002;203(3-4):311-320.
56. Purchiaroni F, Tortora A, Gabrielli M, Bertucci F, Gigante G, Ianiro G, et al. The role of intestinal microbiota and the immune system. *Eur Rev Med Pharmacol Sci.* 2013;17(3):323.
57. Shediwah FMH, Naji KM, Gumaih HS, Alhadi FA, Al-Hammami AL, D'Souza MR. Antioxidant and antihyperlipidemic activity of *Costus speciosus* against atherogenic diet-induced hyperlipidemia in rabbits. *J Integr Med.* 2019;17(3):181-191.