

# "Evaluation of the Protective Role of Ascorbic Acid Against Creatine-Induced Renal Histological Alterations in Male Rabbits"

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**Abstract:** The widespread use of creatine monohydrate as an ergogenic supplement has raised concerns regarding its potential impact on renal structure and function. This study aimed to evaluate the nephrotoxic effects of creatine supplementation in male rabbits and to investigate the possible protective role of ascorbic acid. Twenty adult male rabbits were randomly allocated into four groups (n=5): control, ascorbic acid-treated, creatine-treated, and a combination of creatine and ascorbic acid. Plasma creatinine and urea levels were measured as indicators of renal function, kidney TBARS levels were assessed to evaluate oxidative stress, and histopathological examination was performed using H&E staining. Statistical analysis was conducted using one-way ANOVA with significance set at  $p < 0.05$ . Creatinine levels were significantly reduced in the ascorbic acid group ( $0.58 \pm 0.020$  g/dl) compared with control ( $1.00 \pm 0.074$  g/dl), while the creatine group ( $0.86 \pm 0.035$  g/dl) showed no significant difference from control. The combination group demonstrated intermediate values ( $0.72 \pm 0.045$  g/dl). Urea levels ranged from  $36.10 \pm 1.586$  to  $41.78 \pm 0.748$  mg/dl, with the lowest value observed in the ascorbic acid group. Kidney weight was significantly higher in the ascorbic acid group ( $17.580 \pm 2.768$  g) and significantly lower in the creatine ( $10.946 \pm 0.652$  g) and combination groups ( $10.652 \pm 0.500$  g) compared to control. TBARS levels showed no statistically significant differences among groups, although a numerical decrease was noted in the combination group ( $4.4 \pm 0.45$  nmol/g tissue) compared to control ( $6.00 \pm 0.507$  nmol/g tissue). Histopathological examination revealed normal renal architecture in both the control and ascorbic acid groups. In contrast, the creatine-treated group exhibited marked structural alterations, including damaged renal corpuscles and detachment of proximal tubular epithelial cells from their basement membranes. The combined treatment group showed persistent structural damage similar to the creatine group, indicating no observable histological protection. In conclusion, creatine supplementation induced notable renal structural alterations without marked changes in biochemical oxidative stress markers. Ascorbic acid alone improved certain renal biochemical parameters but did not demonstrate a clear protective effect against creatine-induced histopathological damage under the conditions of this study.

**Keywords:** Creatine monohydrate, Ascorbic acid, Renal function, TBARS, Histopathology, Male rabbits.

## Introduction

In recent years, the use of performance-enhancing supplements has surged globally, with Creatine monohydrate being one of the most widely consumed ergogenic aids among athletes and bodybuilders [1-4]. While it is naturally synthesized in the body and plays a critical role in energy metabolism, chronic or high-dose supplementation has raised concerns regarding its impact on renal physiology [5]. Because the kidneys are the primary organs responsible for filtering creatinine—the metabolic byproduct of creatine they are particularly susceptible to potential structural and functional stress [6-10]. Prolonged exposure to elevated creatine levels can lead to histological alterations within the renal parenchyma, including glomerular congestion, tubular degeneration, and interstitial inflammation [11]. These changes are often driven by oxidative stress, a process where an imbalance between reactive oxygen species (ROS) and the body's antioxidant defenses leads to cellular damage [12]. To counteract these adverse effects, researchers are increasingly investigating the efficacy of exogenous antioxidants. Ascorbic Acid (Vitamin C) is a potent,

water-soluble antioxidant known for its ability to scavenge free radicals and stabilize biological membranes. By neutralizing oxidative byproducts, Ascorbic Acid may offer a protective mechanism against chemically-induced nephrotoxicity [13-15]. This study aims to evaluate the potential nephrotoxic effects of creatine monohydrate on male rabbits a reliable biological model for human renal studies and to investigate whether the administration of Ascorbic Acid can mitigate or prevent the resulting histological damage. Through microscopic examination of renal tissues, this research seeks to provide clarity on the safety margins of creatine and the protective therapeutic potential of Vitamin C.

## Materials and Methods

Twenty adult male rabbits were used in this study. The animals were housed in a controlled environment with a 12-hour light/dark cycle and provided with a standard pellet diet and water *ad libitum*. All experimental procedures were conducted in accordance with the institutional guidelines for the care and use of laboratory animals. The rabbits were randomly assigned to four experimental

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groups (n=5 per group): Control Group: Received no treatment (or vehicle). Ascorbic Acid Group: Treated with ascorbic acid. Creatine (CrS) Group: Treated with creatine. Combination Group: Treated with both ascorbic acid and creatine. At the end of the treatment period, rabbits were euthanized, and blood samples were collected to obtain plasma for biochemical analysis. The kidneys were immediately excised and weighed using a sensitive digital balance. Plasma levels of Creatinine (g/dl) and Urea (mg/dl) were determined using commercially available diagnostic kits according to the manufacturer's instructions. These parameters served as indicators of glomerular filtration and renal clearance. To evaluate oxidative stress in the renal tissue, Thiobarbituric Acid Reactive Substances (TBARS) were measured in kidney homogenates. The concentration of TBARS was expressed as nanomoles per gram of tissue (nmol/g tissue), following the method of [16]. Kidney samples were fixed in 10% neutral buffered formalin for 24 hours. The tissues were subsequently dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 5  $\mu\text{m}$  thickness were cut using a microtome and stained with Hematoxylin and Eosin (H&E). The slides were examined under a light microscope to assess structural changes in the renal corpuscles and proximal convoluted tubules. Statistical Analysis: Data were expressed as Mean  $\pm$  Standard Error (SE). Statistical significance was determined using a one-way analysis of variance (ANOVA) followed by a post-hoc test for multiple comparisons. Differences were considered statistically significant at  $p < 0.05$ . Superscript letters (a, b, c) were used in the results to denote significant differences between groups.

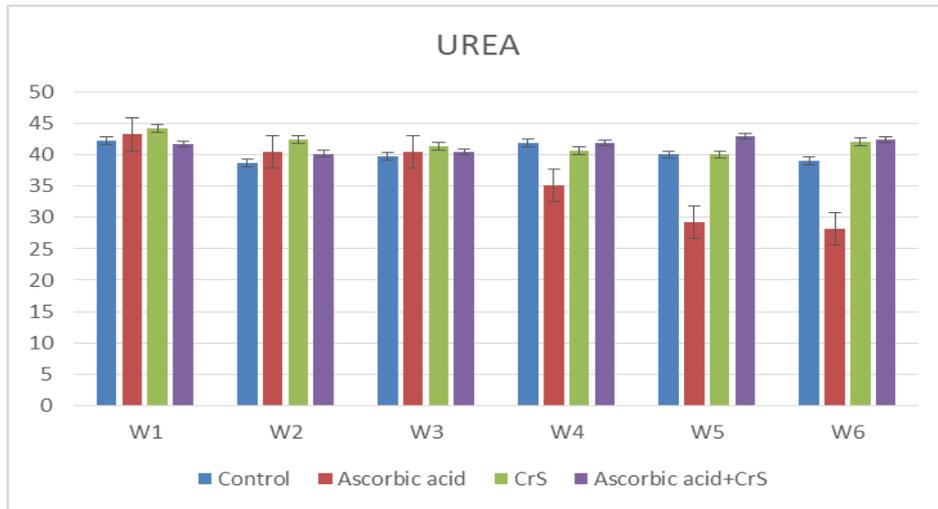
## Results

Table 1 shows statistically significant variations among the experimental groups in several renal parameters. Plasma creatinine levels were significantly reduced in the ascorbic acid group ( $0.58 \pm 0.020$  g/dl, superscript c) compared with the control group ( $1.00 \pm 0.074$  g/dl, a). The creatine group ( $0.86 \pm 0.035$  g/dl, ab) did not differ significantly from control, while the combined treatment group ( $0.72 \pm 0.045$  g/dl, bc) showed intermediate values with partial statistical difference. Plasma urea levels ranged between  $36.10 \pm 1.586$  and  $41.78 \pm 0.748$  mg/dl. The lowest value was recorded in the ascorbic acid group ( $36.10 \pm 1.586$  mg/dl, b), while the creatine group showed the highest level ( $41.78 \pm 0.748$  mg/dl, a). Control ( $40.27 \pm 0.55$  mg/dl, ab) and combination ( $41.58 \pm 1.619$  mg/dl, a) were not markedly different from the highest group. Kidney weight demonstrated significant differences among groups. The ascorbic acid group showed the highest mean value ( $17.580 \pm 2.768$  g, a), whereas the creatine ( $10.946 \pm 0.652$  g, b) and combination groups ( $10.652 \pm 0.500$  g, b) showed significantly lower values. The control group ( $15.200 \pm 0.435$  g, ab) presented intermediate values. Kidney TBARS levels ranged from  $4.4 \pm 0.45$  to  $6.00 \pm 0.507$  nmol/g tissue. All groups shared the same superscript (a), indicating no statistically significant differences ( $p < 0.05$ ), although numerically the lowest value was observed in the combination group ( $4.4 \pm 0.45$  nmol/g tissue) compared with control ( $6.00 \pm 0.507$  nmol/g tissue).

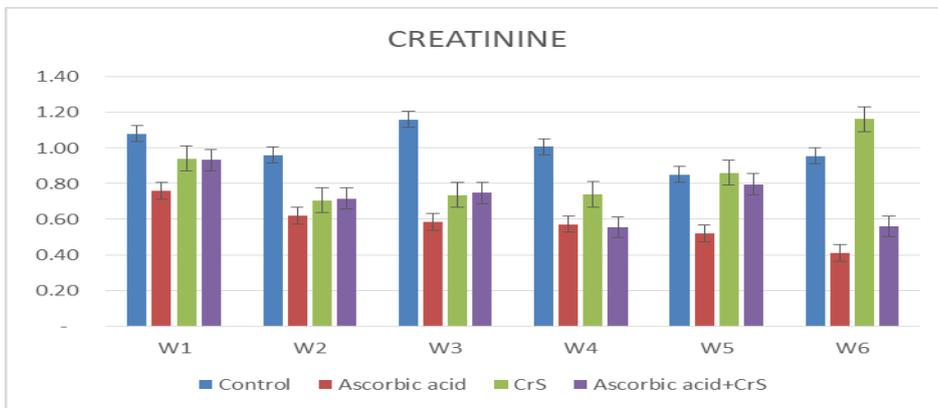
**Table 1.** Plasma renal function test of male rabbits treated with *ascorbic acid*, *Creatine (CrS)*, and their combination.

Parameter	Experimental groups			
	Control	Ascorbic acid	Creatine	Ascorbic acid+ Creatine
Creatinine (g/dl)	$1.00 \pm 0.074^a$	$0.58 \pm 0.020^c$	$0.86 \pm 0.035^{ab}$	$0.72 \pm 0.045^{bc}$
Urea (mg/dl)	$40.27 \pm 0.55^{ab}$	$36.10 \pm 1.586^b$	$41.78 \pm 0.748^a$	$41.58 \pm 1.619^a$
Kidney (g)	$15.200 \pm 0.435^{ab}$	$17.580 \pm 2.768^a$	$10.946 \pm 0.652^b$	$10.652 \pm 0.500^b$
TBARS kidney (nmol/g tissue)	$6.00 \pm 0.507^a$	$5.4 \pm 0.57^a$	$5.00 \pm 0.79^a$	$4.4 \pm 0.45^a$

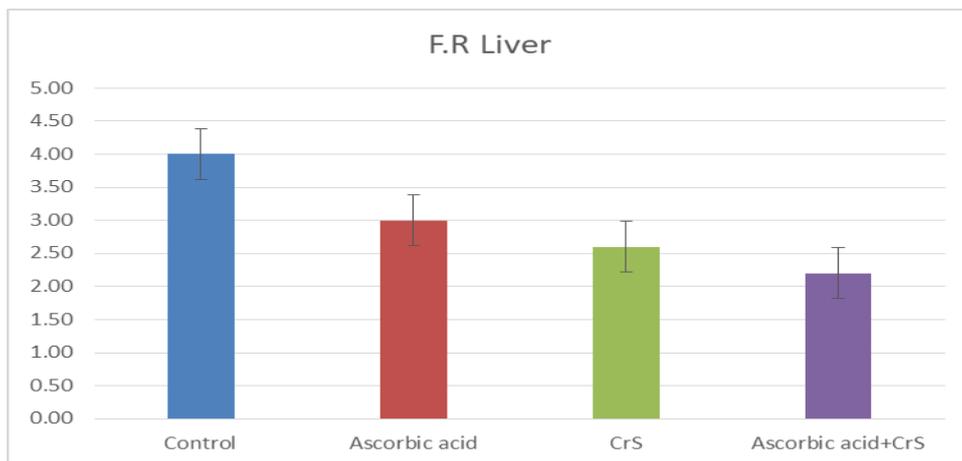
The means  $\pm$  SE for each treatment group are provided; n = 5. When mean values within a row did not share a common superscript letter (a, b, or c), significant differences ( $p < 0.05$ ) were observed.



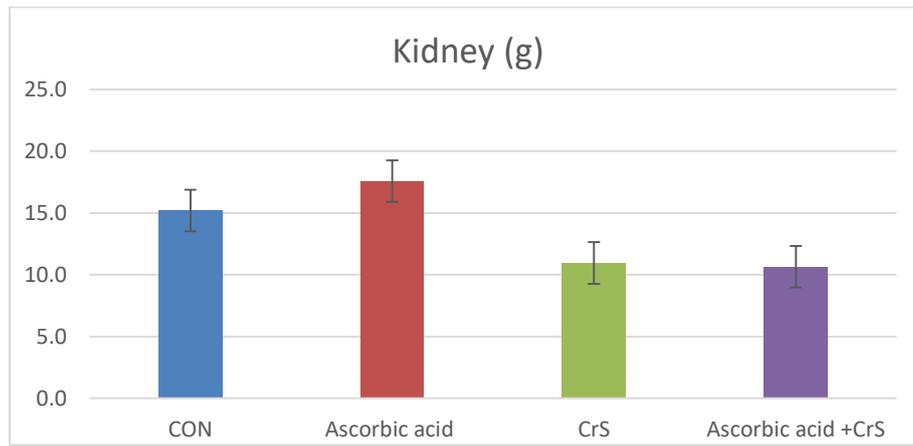
**Figure 1.** Effects of *ascorbic acid*, *CrS*, and their combination on urea levels in male rabbits.



**Figure 2.** Alterations Creatinine levels after male rabbits were treated with *ascorbic acid*, *CrS*, their combination.



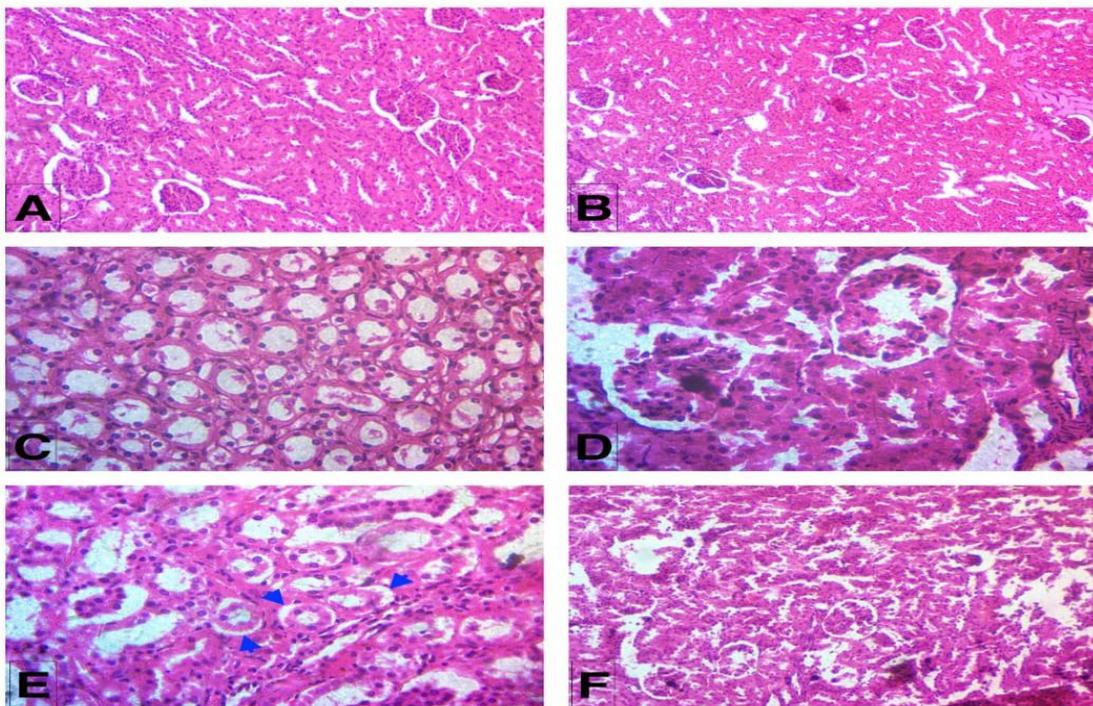
**Figure 3.** Changes in TBARS in the kidney when male rabbits are treated with *ascorbic acid*, *CrS*, and their combination.



**Figure 4.** Changes weight of kidney treatment of male rabbits with ascorbic acid, CrS, and their combination

The following alterations were revealed by histopathological analysis of rabbit kidney tissue from the several groups under study: Control group: Figure 1/A shows normal kidney tissue free of pathological alterations based on histological analysis. 2. Ascorbic acid group: The histological analysis revealed no pathological alterations and normal renal tissue (Figure 5/B). 3. Creatine group: The cuboidal cells lining the proximal convoluted

tubules were completely separated from their basement membranes, and the histological analysis revealed damaged renal corpuscles (Figure 5/C). 4. Creatine + ascorbic acid group: The histological analysis revealed no discernible protective benefit of ascorbic acid against the destruction impact of creatine, as the slides from this group still display the destructed effects of creatine.



**Figure 5.** Histological kidney sections stained with H&E: (A) Normal kidney in control group ( $\times 20$ ); (B–C) Normal renal tubules ( $\times 40$ ) and corpuscles ( $\times 20$ ) in ascorbic acid group; (D–E) Damaged renal corpuscles and detachment of proximal tubule cells in creatine group ( $\times 40$ ); (F) No protective effect observed in creatine + ascorbic acid group ( $\times 20$ ).

## Discussion

The present study evaluated the effects of creatine monohydrate and ascorbic acid, individually and in combination, on renal biochemical markers, oxidative stress status, and histological integrity in male rabbits. The findings demonstrate distinct biochemical and structural responses among the experimental

groups. Plasma creatinine levels were significantly reduced in the ascorbic acid group compared with the control group, indicating improved renal functional status. In contrast, creatine administration did not produce a statistically significant elevation in creatinine relative to control, suggesting that under the present experimental conditions, creatine supplementation did not

markedly impair glomerular filtration [17]. The intermediate creatinine value observed in the combined treatment group suggests that co-administration of ascorbic acid did not substantially modify the creatine-associated biochemical profile. Plasma urea levels remained relatively stable across groups, with only a modest decrease observed in the ascorbic acid-treated animals[18-20]. The absence of significant urea elevation in the creatine group indicates that creatine supplementation did not induce pronounced disturbances in nitrogen metabolism or renal clearance capacity. These biochemical findings suggest that functional impairment, if present, was not severe enough to be reflected in circulating urea concentrations [21-25]. Kidney weight showed significant variation among groups. The increase observed in the ascorbic acid group and the reduction in both the creatine and combination groups may reflect treatment-related physiological or structural changes. However, kidney weight alone does not provide definitive evidence of either hypertrophy or atrophy without correlation to histological findings[26-30]. Regarding oxidative stress, TBARS levels in renal tissue did not differ significantly among groups, although a numerical decrease was observed in the combined treatment group. The lack of statistical significance may indicate that lipid peroxidation was not markedly altered under the applied doses or duration of treatment, or that the sample size limited the detection of subtle differences[31-40]. Despite the relatively modest biochemical alterations, histopathological analysis revealed clear structural damage in the creatine-treated group. The observed detachment of proximal tubular epithelial cells from the basement membrane and damage to renal corpuscles indicate structural disruption at both tubular and glomerular levels. Interestingly, co-administration of ascorbic acid did not prevent these histological alterations, as similar structural damage was observed in the combination group [41]. This discrepancy between biochemical stability and histological injury suggests that early structural changes may precede detectable alterations in conventional plasma renal markers [42]. Overall, the findings indicate that creatine supplementation induced notable renal structural alterations without significant changes in oxidative stress markers or marked elevations in plasma urea. Ascorbic acid alone was associated with improved creatinine levels but did not confer evident histological protection when administered concurrently with creatine. These results highlight the importance of integrating biochemical and histopathological assessments when evaluating renal safety[34-46].

**Conclusion:** Creatine supplementation induced noticeable renal histological alterations in male rabbits despite relatively stable biochemical and oxidative stress markers. Ascorbic acid improved plasma creatinine levels but did not demonstrate a protective effect against creatine-induced structural renal damage when administered concurrently.

## References

- Salem, A. M., Khaled, F. A., Mohamed, H. E. A., & Husayn, A. S. (2026). The Protective Role of Melatonin against Sodium Arsenite-Induced Hepato-Toxicity in Male Rabbits: Biochemical and Histopathological Study. *World Journal of Applied Medical Sciences*, 3(1), 1-6.
- Saad, E., Ibrahim, M., Khaled, F., & Ali, M. (2021). A comparative study between effects of some antioxidants on levels of hormones in male rabbits. *AlQalam Journal of Medical and Applied Sciences*, 60-68.
- Al-Ailla, A. K., & Khaled, F. A. (2019). Effect of ginger extract on antioxidant enzymes and free radicals in rabbits. *J Biotechnol Biochem*, 5(1), 37-40.
- Khaled, F. A., & Qataf, R. A. (2022). Comparative study of curcumin and garlic as antioxidants in male rabbits on biochemical parameters. *Libyan J Basic Sci*, 19(2), 1-19.
- Khaled, F. A., & Saad, G. I. (2025). Evaluation of the protective effects of cinnamon on liver and kidney function in rabbits exposed to paracetamol toxicity. *Applied Sciences Research Periodicals*, 3(04), 37-46.
- Khaled, F. A., & Qataf, R. A. (2021). Study the enhance role of Garlic and Curcumin on Hematolglcal parameters in male rabbits. *International Journal of Pharmacy & Life Sciences*, 12(8).
- Silverman, M. N., Pearce, B. D., Biron, C. A., & Miller, A. H. (2005). Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. *Viral immunology*, 18(1), 41-78.
- Omar, O. A. E., Eman, G. A., & Khaled, F. A. (2021). Biochemical consider on the defensive role of Ginseng in male rabbits. *International Journal of Pharmacy & Life Sciences*, 12(3).
- AA, S. A., & Marwa, J. S. (2021). Biochemical study on the role of curcumin in male rabbits. *Annals of Pharma Research*, 9(3).
- Atalhi, F. M. (2022). Paracetamol induces decrease in antioxidant enzymes and TBARS in male rabbits. *Saudi J Biomed Res*, 7(6), 182-6.
- Atalhi, F. M. (2022). Phenolic compounds of Graviola enhance lipid profile in male rabbits. *Int J Pharm Res*, 14(2), 1-7.
- Riahi-Zanjani, B., Delirrad, M., Fazeli-Bakhtiyari, R., Sadeghi, M., Zare-Zardini, H., Jafari, A., & Ghorani-Azam, A. (2022). Hematological consequences of valproic acid in pediatric patients: a systematic review with a mechanistic approach. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)*, 21(4), 316-325.
- Ali, M. S., Khaled, F. A., & Saloumah, H. S. (2021). Annona muricata L. suppresses stannous chloride effects through changing hematological parameters in male New Zealand white rabbits. *Journal of Complementary and Alternative Medical Research*, 251-262.
- Aldeeb, O. H., Ibridan, B. A., Khaled, F. A., & Shah, A. (2022). Harmful impact of dimethoate and chlorpyrifos on hematological parameters in male rabbits. *South Asian Res J Bio Appl Biosci*, 4(1), 11-7.
- Mokhtar, I., Khaled, F. A., Abdel-Aziz, F., Hanaa, A., & Kamel, I. (2021). Ginger suppresses DEHP testicular toxicity through hormonal regulation in rabbits. *Asian Pac J Reprod*, 10(3), 121-8.
- Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, 95(2), 351-358.
- Khaled, F. A., & Ahmed, A. I. (2025). Experimental study on the effect of Ziziphus spina-christi leaves on blood plasma hormones in male rabbits. *World Journal of Applied Medical Sciences*, 2(9), 1-5.
- Amharib, A. M., Khaled, F. A., & Younis, F. M. (2021). Protective role of vitamin E against chlorpyrifos toxicity on semen quality and testosterone in rabbits. *AlQalam J Med Appl Sci*, 4(4), 18-26.

19. Khaled, F. A., Ali, M. S., Qowaidar, S. R., & Farge, R. (2017). Zingiber officinale Syrup Reduces Bacterial Load in Study Cases Infected with Helicobacter pylori in Northeast-Libya. *Journal name unknown*. [year].
20. Khaled, F., Hussien, S., Mahmoud, R., & Belhamad, N. (2025). Effects of Libyan Balanites aegyptiaca on steroid hormones in male rabbits. *Khalij-Libya J Dent Med Res*, 9(2), 208-12.
21. Khaled, F., & Mohammed, S. (2025). Investigating the impact of creatine and vitamin C supplementation on insulin sensitivity and glycated hemoglobin levels in male rabbits. *Razi Medical Journal*, 106-111.
22. Khaled F, Ali M. Role of vitamin E in reducing tramadol-induced TBARS in male rabbits. *Attahadi Med J*. 2025;16-9.
23. Yousef, M., Hassan, H., Mohammed, A., Kamel, K., & Khaled, F. (2012, May). The protective role of ginger against Di-(2-ethylhexyl) phthalate-induced reproductive toxicity and oxidative stress in male rabbits. In *Endocrine abstracts* (Vol. 29). Bioscientifica.
24. Yousef, M. I., Awad, T. I., Elhag, F. A., & Khaled, F. A. (2007). 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*, 235(3), 194-202.
25. Khaled, F. A., Yousef, M. I., & Kamel, K. I. (2016). The protective role of propolis against the reproductive toxicity of mono-sodium glutamine in male rabbits. *IJCS*, 4(2), 4-9.
26. El-Speiy, M. E., Khaled, F. A., & El-Hanoun, A. M. (2017). Effect of ginger supplementation on reproductive performance of male rabbits. *Glob Sci J Biol*, 2(2), 26-31.
27. Elgazwi, S. M., Khaled, F. A., & Alsanous, M. F. (2021). Study the protective effect of ginger against the toxicity of dimethoate on hormones in rabbits. *Asian Journal of Research in Biochemistry*, 8(3), 24-33.
28. Khaled, F., Shoaib, A., & Attia, M. (2021). Hepatoprotective effect of ginger induced experimentally by dimethoate and liver injury in adult male rabbits. *AlQalam Journal of Medical and Applied Sciences*, 24-30.
29. Mohammed, N., Hassan, H., Ali, A., Khaled, F., & Mohamed, S. (2022). Histopathological alterations in liver of male rabbits exposed to deltamethrin and the ameliorative effect of folic acid. *AlQalam Journal of Medical and Applied Sciences*, 454-460.
30. Khaled, F. A., Ali, M. S., & Radad, H. S. (2019). Influence of ascorbic acid supplementation on hematological parameters and free radical in adult male rabbits. *Saudi J Biomed Res*, 4(5), 244-7.
31. Khaled, F. A., Younus, A. A., & Sale, R. M. (2021). Hematological parameters and blood smear effects of tramadol on male rabbits. *J Med Sci*, 20(2), 1-6.
32. Khaled, F., Ali, R., & Asbaaq, M. (2025). Effect of Folic Acid and Ascorbic Acid Supplementation on Hematological Parameters of Male Rabbits. *Attahadi Medical Journal*, 35-38.
33. Khaled, F., Ali, R., & Aboulqassim, T. (2025). Strawberry juice reduces renal and hematotoxic effects of carbon tetrachloride in rabbits. *AlQalam J Med Appl Sci*, 8(2), 731-8.
34. Khaled, F., & Masoud, F. (2025). Dose-dependent myelosuppression and hematological toxicity induced by gemcitabine in New Zealand white rabbits. *AlQalam Journal of Medical and Applied Sciences*, 2650-2655.
35. Salih, A., Amharib, A., Khaled, F., & Alsba, A. (2025). The impact of metformin therapy on liver and kidney function in type 2 diabetic patients: a comparative study between men and women in Al-Bayda City. *Libyan Medical Journal*, 164-168.
36. Alfageih, L., Amharib, A., Khaled, F., & Auad, F. (2024). Function of alpha-lipoic acid in rabbits as an antioxidant and to lower lipid levels. *AlQalam Journal of Medical and Applied Sciences*, 615-621.
37. Shoaib, A., Husayn, A., Khaled, F., & Abdulhamid, M. (2025). Hematological and biochemical differences in vaccinated vs unvaccinated COVID-19 cases in Libya. *Libyan Med J*, 12(1), 218-21.
38. Citrine, E. M., Yunas, A., & Khaled, F. (2025). The effects of chromium and tin present in the environment and seawater on biochemical parameters in male rabbits. *AlQalam Journal of Medical and Applied Sciences*, 91-95.
39. Khaled, F. A., Saleh, A. M., & Saleh, A. H. (2023). Toxic effect of Chloropyrpehos, Deltamethrinand Dimethoate on biochemical parameters in male rabbits. *Journal of Medical Sciences*, 18(3), 1-6.
40. Othman, A. (2025). Hepatoprotective Effect of Jojoba (Simmondsia chinensis) Against Valproic Acid-Induced Hematological Alterations in Male Rabbits. *AlQalam Journal of Medical and Applied Sciences*, 2548-2551.
41. Hammad, A., & Khaled, F. (2026). Inorganic mineral dynamics in two medicinal plants and their influence on body weight gain in experimental rabbits. *AlQalam Journal of Medical and Applied Sciences*, 5-10.
42. Salem, A. M., Khaled, F. A., Mohamed, H. E. A., & Husayn, A. S. (2026). The Protective Role of Melatonin against Sodium Arsenite-Induced Hepato-Toxicity in Male Rabbits: Biochemical and Histopathological Study. *World Journal of Applied Medical Sciences*, 3(1), 1-6.
43. Mohammed, N., Hassan, H., Ali, A., & Khaled, F. (2026). 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 Medical Journal*, 42-47.
44. Hammad, A. A., Khaled, F. A., & Hussien, S. M. (2026). GC-MS Characterization of Ziziphus spina-christi Essential Oil and its Antioxidant Efficacy in Rabbit Models. *World Journal of Applied Medical Sciences*, 3(1), 14-18.
45. Hammad, A. A., & Khaled, F. A. " GC-MS Characterization of Phenolic Compounds in Ginger (Zingiber officinale) and Their Impact on Serum Cortisol and Reproductive Hormones in Rabbits.
46. Abdurabbih, E., & Khaled, F. A. " Correlation between Ziziphus Secondary Metabolites and Amino Acid Homeostasis: Effects on Growth and Renal Biomarkers in a Rabbit Model.