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Modulation of Oxidative Stress Biomarkers by Ascorbic Acid and Creatine Co-Treatment in Male Rabbits

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Abstract

Oxidative stress plays a central role in the pathogenesis of numerous metabolic and degenerative diseases. Antioxidants such as ascorbic acid and creatine (CrS) have been shown to modulate oxidative damage, but limited research has investigated their combined effects in vivo. This study aimed to evaluate the effect of ascorbic acid, creatine, and their co-administration on oxidative stress biomarkers in male rabbits, focusing on catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), and thiobarbituric acid-reactive substances (TBARS). Twenty male rabbits were randomly divided into four groups (n = 5): control, ascorbic acid (20 mg/kg), creatine 5 mg/kg), and a combination of both agents. Treatments were administered orally for 6weeks. Blood samples were analyzed for CAT, SOD, GSH, and TBARS using standard spectrophotometric methods. Data were statistically analyzed using one-way ANOVA followed by Tukey's post hoc test. The co-treatment group showed a significant increase in CAT (1.12 ± 0.043 U/min/ml) and SOD (1.364 ± 0.040 U/ml) activities compared to the control ($p < 0.05$), suggesting enhanced enzymatic antioxidant defense. TBARS levels, an indicator of lipid peroxidation, were significantly reduced in all treated groups, with the lowest level in the ascorbic acid group (3.209 ± 0.051). GSH levels remained stable across all groups without significant variation ($p = 0.882$), indicating preservation of intracellular antioxidant capacity. ANOVA confirmed significant differences for CAT, SOD, and TBARS ($p = 0.000$), while GSH showed no significant change.

Keywords: *Ascorbic Acid; Creatine; Oxidative Stress; Rabbits.*

INTRODUCTION

Oxidative stress is a pathological condition characterized by an imbalance between reactive oxygen species (ROS) production and the capacity of biological systems to detoxify these reactive intermediates or repair the resulting damage [1]. ROS such as superoxide anions, hydrogen peroxide, and hydroxyl radicals are generated as natural byproducts of cellular metabolism, particularly within the mitochondria, and play essential roles in cell signaling and homeostasis[2]. However, excessive ROS accumulation can damage cellular components, including lipids, proteins, and DNA, contributing to various diseases such as cancer, neurodegeneration, cardiovascular disorders, and infertility[3].To counteract oxidative stress, organisms rely on a complex antioxidant defense system comprising both enzymatic components, such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase, as well as non-enzymatic antioxidants like glutathione (GSH), vitamins C and E[4]. The thiobarbituric acid reactive substances (TBARS) assay, which measures malondialdehyde (MDA) levels, is commonly used as a marker of lipid peroxidation and oxidative damage [5]. Ascorbic acid (vitamin C) is a potent water-soluble antioxidant that directly scavenges ROS and restores other antioxidants such as vitamin E to their active forms. It also protects crucial antioxidant enzymes from oxidative inactivation and contributes to the regeneration of glutathione [6]. Numerous animal studies have confirmed its effectiveness in reducing lipid peroxidation and enhancing antioxidant enzyme activities under oxidative stress conditions [7]. Creatine, a naturally occurring nitrogenous compound found primarily in muscle tissue, has traditionally been studied for its role in energy metabolism [8]. However, recent evidence suggests that creatine also possesses antioxidant properties. It can stabilize mitochondrial membranes, enhance ATP buffering, and reduce ROS formation by supporting mitochondrial efficiency [9]. Studies have reported that creatine supplementation mitigates oxidative damage in both muscle and brain tissues [10]. The combined administration of ascorbic acid and creatine may provide a synergistic antioxidant effect by targeting multiple

aspects of oxidative stress [11]. While ascorbic acid directly scavenges free radicals, creatine may enhance cellular bioenergetics and reduce mitochondrial ROS generation [12,13]. Despite their individual benefits, limited studies have examined their combined effect on oxidative stress biomarkers in vivo, particularly in rabbit models. Exploring this combination may offer insights into new therapeutic strategies against oxidative stress-related conditions [14]. Therefore, the present study aims to evaluate the modulatory effect of co-treatment with ascorbic acid and creatine on antioxidant enzyme activities (CAT, SOD), glutathione levels (GSH), and lipid peroxidation (TBARS) in male rabbits. This research could provide a scientific basis for the potential synergistic interaction between these two compounds in enhancing antioxidant defense mechanisms.

Materials and Methods

A total of 20 healthy adult male rabbits (Oryctolagus cuniculus), weighing between 1.8 to 2.2 kg**,** were used in this study. The animals were housed under standard laboratory conditions (temperature 22–25°C, 12-hour light/dark cycle) and were provided with acommercial pellet diet and water ad libitum. The rabbits were randomly divided into four groups (n = 5 per group) as follows: Group I (Control)**:** Received no treatment and served as the negative control. Group II (Ascorbic Acid)**:** Administered ascorbic acid at a dose of 20 mg/kg body weight orally, once daily for 6 weeks**.** Group III (Creatine - CrS)**:** Administered creatine monohydrate at a dose of 5 mg/kg body weight orally, once daily for 6 weeks. Group IV (Ascorbic Acid + CrS)**:** Co-treated with both ascorbic acid (20 mg/kg) andcreatine (5mg/kg) orally, once daily for6weeks.All substances were freshly prepared in distilled water before administration. Blood samples were collected from the ear vein of all animals every other week throughout the 6-week experimental period. Blood samples were obtained in the morning before access to feed and water and placed immediately on ice. The blood samples were collected in two tubes, one containing heparin to obtain plasma and the other one without anticoagulants to obtain serum. Glutathione S-transferase (GST; EC 2.5.1.18) activity was determined according to [15]. Catalase (CAT; EC 1.11.1.6) activity was determined using the Luck method involving the decomposition of hydrogen peroxide [16]. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured according to [17]. Plasma thiobarbituric acid-reactive substances (TBARS) were measured by the method of [18]. Statistical Analysis. Data were expressed as mean ± standard error (SE). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test to compare differences between groups. A p-value < 0.05 was considered statistically significant.

Results

The present study assessed the impact of ascorbic acid, creatine (CrS), and their co-administration on key oxidative stress biomarkers in male rabbits. The results provide compelling evidence that these treatments modulate enzymatic antioxidant defenses and lipid peroxidation levels in a statistically significant manner. Catalase is a vital antioxidant enzyme responsible for decomposing hydrogen peroxide into water and oxygen, thereby protecting cells from oxidative damage. In this study, the CAT activity was significantly elevated in the Ascorbic acid + CrS group (1.12 ± 0.043 U/min/ml) compared to the control (0.933 ± 0.050 U/min/ml), as supported by a highly significant ANOVA p-value of 0.000**.** The combination treatment exhibited a synergistic effect, showing greater enhancement than either agent alone. SOD serves as the first line of defense against oxidative stress by converting superoxide radicals into hydrogen peroxide. SOD activity was significantly increased in all treated groups compared to the control, with the CrS (1.356 ± 0.065 U/ml) and Ascorbic acid + CrS (1.364 ± 0.040 U/ml) groups showing the highest values. The strong statistical significance **(**p = 0.000**)** highlights the effectiveness of these interventions in enhancing superoxide scavenging capacity. GSH is a critical non-enzymatic antioxidant that plays a central role in maintaining cellular redox balance. Interestingly, no significant changes were observed in GSH levels across treatment groups (p = 0.882), although slight, non-significant elevations were recorded in the ascorbic acid (6.006 ± 0.205 U/ml) and combination groups (5.97 ± 0.115 U/ml). TBARS is a well-established marker of lipid peroxidation and oxidative tissue damage. The ascorbic acid group exhibited the most pronounced reduction in TBARS levels (3.209 ± 0.051), followed by the combination treatment and the CrS group. The significant reduction in TBARS (p = 0.000) across all treated groups.

Table (1). Average of plasma glutathione (GSH; U/ml), catalase (CAT; U/min/ml), superoxide dismutase (SOD; U/ml), and thiobarbituric acid-reactive substances (TBARS) of male rabbits treated with ascorbic acid and creatine (CrS)and/or their combination (means SE).

Animal Groups	Catalase (CAT; U/min/ml)	Superoxide dismutase (SOD; U/ml)	Glutathione (GSH; U/ml)	Thiobarbituric acid-reactive substances (TBARS)
Control (Mean±SE)	0.933 ± 0.050^{c}	1.115 ± 0.022^{b}	5.845 ± 0.265^{a}	4.577 ± 0.045^{a}
Ascorbic acid (Mean±SE)	$0..985 \pm 0.019^{bc}$	1.273 ± 0.029^{a}	6.006 ± 0.205^{a}	3.209 ± 0.051^{d}
CrS (Mean±SE)	1.056 ± 0.035^{ab}	1.356 ± 0.065^{a}	5.916 ± 0.047^{a}	4.174 ± 0.075^{b}
Ascorbic acid+CrS (Mean±SE)	1.12 ± 0.043^{a}	1.364 ± 0.040^{a}	5.97 ± 0.115^{a}	3.583 ± 0.122^{c}
p-value	0.000	0.000	0.000	0.880

Data are expressed as mean ± SE of 5 rabbit. Within each row, means with different superscript (a, b, c or d) were significantly different at p<0.05. Where means superscripts with the same letters mean that there is no significant difference (p>0.05).

Table 2. Analysis of variance for the effect of ascorbic acid and creatine (CrS) and/or their combination on plasma catalase (CAT) of male rabbits

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Groups	3	0.8100	0.27000	7.28	0.000
Error	116	4.3041	0.03710		
Total	119	5.1140			

Table 3. Analysis of variance for the effect of ascorbic acid and creatine (CrS) and/or their combination on plasma superoxide dismutase (SOD) of male rabbits

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Groups	3	1.197	0.3984	11.46	0.000
Error	116	4.039	0.03482		
Total	119	5.236			

Table 4. Analysis of variance for the effect of ascorbic acid and creatine (CrS) and/or their combination on plasma glutathione (GSH) of male rabbits

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Groups	3	0.2514	0.8380	0.22	0.882
Error	116	43.9929	0.37925		
Total	119	44.2443			

Table 5. Analysis of variance for the effect of ascorbic acid and creatine (CrS) and/or their combination on plasma thiobarbituric acid-reactive substances (TBARS) of male rabbits

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Groups	3	34.55	11.5158	51.95	0.000
Error	116	25.71	0.2217		
Total	119	60.26			

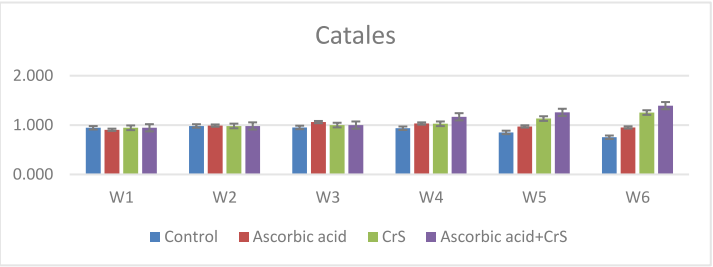

Figure 1. Changes of Catalase (CAT) treatment of male rabbits with ascorbic acid, creatine (CrS), and their combination

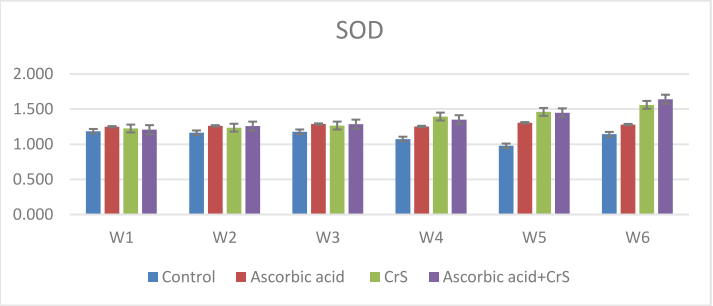

Figure 2. Changes of superoxide dismutase (SOD) treatment of male rabbits with ascorbic acid, creatine (CrS), and their combination

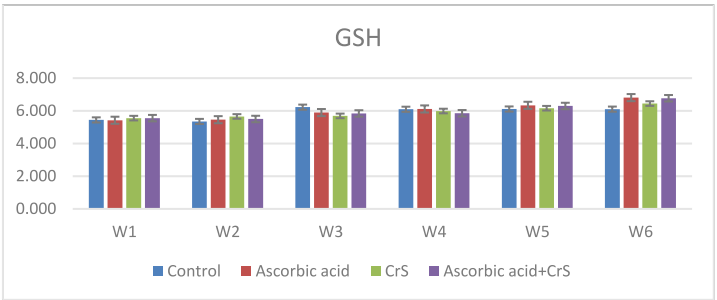

Figure 3. Changes of glutathione (GSH) treatment of male rabbits with ascorbic acid, creatine (CrS), and their combination

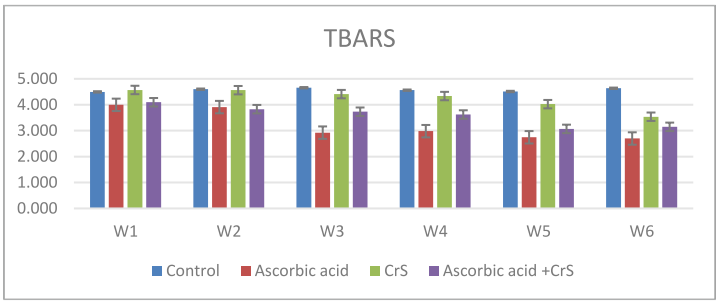

Figure 4. Changes of thiobarbituric acid-reactive substances (TBARS) treatment of male rabbits with ascorbic acid, creatine (CrS), and their combination

Discussion

The present findings demonstrate that both ascorbic acid and creatine, individually and in combination, significantly modulate oxidative stress biomarkers in male rabbits. This modulation was evident through increased enzymatic antioxidant activities and reduced lipid peroxidation, highlighting the potential of these agents as protective interventions against oxidative stress. The significant elevation of catalase (CAT) activity in the ascorbic acid + CrS group compared to the control group suggests a synergistic antioxidant interaction [19]. Catalase plays a critical role in cellular defense by catalyzing the decomposition of hydrogen peroxide, a harmful reactive oxygen species (ROS), into water and oxygen [20]. The observed increase is consistent with studies reporting that ascorbic acid enhances catalase gene expression and activity via modulation of the Nrf2 signaling pathway [21]. Similarly, creatine supplementation has been shown to upregulate CAT activity and reduce oxidative injury in animal models, likely by improving mitochondrial bioenergetics and scavenging free radicals [22]. Superoxide dismutase (SOD), which catalyzes the dismutation of superoxide radicals into hydrogen peroxide, was significantly elevated in all treated groups, with the highest activity observed in the ascorbic acid groups ($p = 0.000$). This supports the hypothesis that both treatments enhance cellular defense against superoxide radicals, the primary ROS generated during oxidative stress [23]. Previous studies have confirmed the capacity of creatine to improve SOD activity, possibly through mitochondrial stabilization and reduced oxidative load [24]. Likewise, ascorbic acid is a potent electron donor that regenerates SOD and prevents its oxidative inactivation [25]. In contrast, glutathione (GSH) levels did not show significant variation among the treatment groups, though slight non-significant elevations were noted in the ascorbic acid and combination groups. GSH is a non-enzymatic thiol antioxidant that maintains redox homeostasis and detoxifies ROS via glutathione peroxidase activity [26]. The lack of statistical significance may reflect either the limited sample size or compensatory antioxidant responses involving enzymatic systems such as SOD and CAT [27]. A similar outcome was reported by [28], where short-term antioxidant supplementation in rabbits resulted in minimal changes in plasma GSH levels despite marked improvements in enzymatic antioxidants. The TBARS assay, which measures malondialdehyde (MDA) as an index of lipid peroxidation, revealed a pronounced decrease in all treated groups.

The lowest TBARS level was seen in the ascorbic acid group, followed by the combination group, indicating a strong lipid-protective effect [29]. This reduction can be attributed to the free radical scavenging activity of ascorbic acid, which interrupts lipid peroxidation chain reactions[19]. Creatine's lipid membrane-stabilizing effects have also been documented in oxidative models of ischemia and exercise, and may account for the observed reduction in TBARS in CrS-treated rabbits [30]. In conclusion, Ascorbic acid and creatine, particularly in combination, enhance antioxidant enzyme activities and reduce lipid peroxidation in male rabbits. These findings support the potential synergistic use of these agents in mitigating oxidative stress-related disorders.

REFERENCES

1. Schieber, M., & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current biology*, *24*(10), R453-R462.

2. de Almeida, A. J. P. O., de Oliveira, J. C. P. L., da Silva Pontes, L. V., de Souza Júnior, J. F., Gonçalves, T. A. F., Dantas, S. H., ... & de Medeiros, I. A. (2022). ROS: basic concepts, sources, cellular signaling, and its implications in aging pathways. *Oxidative medicine and cellular longevity*, *2022*(1), 1225578.

3. Juan, C. A., Pérez de la Lastra, J. M., Plou, F. J., & Pérez-Lebeña, E. (2021). The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *International journal of molecular sciences*, *22*(9), 4642.

4. Jomova, K., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2024). Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Archives of toxicology*, *98*(5), 1323-1367.

5. Ghani, M. A., Barril, C., Bedgood Jr, D. R., & Prenzler, P. D. (2017). Measurement of antioxidant activity with the thiobarbituric acid reactive substances assay. *Food chemistry*, *230*, 195-207.

6. Gęgotek, A., & Skrzydlewska, E. (2022). Antioxidative and anti-inflammatory activity of ascorbic acid. *Antioxidants*, *11*(10), 1993.

7. Casado, A., Encarnación López-Fernández, M., Concepción Casado, M., & de La Torre, R. (2008). Lipid peroxidation and antioxidant enzyme activities in vascular and Alzheimer dementias. *Neurochemical Research*, *33*(3), 450-458.

8. Juneja, K., Bhuchakra, H. P., Sadhukhan, S., Mehta, I., Niharika, A., Thareja, S., ... & Sahu, S. (2024). Creatine Supplementation in Depression: A Review of Mechanisms, Efficacy, Clinical Outcomes, and Future Directions. *Cureus*, *16*(10).

9. Clarke, H., Kim, D. H., Meza, C. A., Ormsbee, M. J., & Hickner, R. C. (2020). The evolving applications of creatine supplementation: could creatine improve vascular health?. *Nutrients*, *12*(9), 2834.

10. Arazi, H., Eghbali, E., & Suzuki, K. (2021). Creatine supplementation, physical exercise and oxidative stress markers: a review of the mechanisms and effectiveness. *Nutrients*, *13*(3), 869.

11. Deminice, R., Rosa, F. T., Franco, G. S., Jordao, A. A., & de Freitas, E. C. (2013). Effects of creatine supplementation on oxidative stress and inflammatory markers after repeated-sprint exercise in humans. *Nutrition*, *29*(9), 1127-1132.

12. Njus, D., Kelley, P. M., Tu, Y. J., & Schlegel, H. B. (2020). Ascorbic acid: The chemistry underlying its antioxidant properties. *Free Radical Biology and Medicine*, *159*, 37-43.

13. Adhihetty, P. J., & Beal, M. F. (2008). Creatine and its potential therapeutic value for targeting cellular energy impairment in neurodegenerative diseases. *Neuromolecular medicine*, *10*(4), 275-290.

14. Kushwah, N., Bora, K., Maurya, M., Pavlovich, M. C., & Chen, J. (2023). Oxidative stress and antioxidants in age-related macular degeneration. *Antioxidants*, *12*(7), 1379.

15. Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. *Journal of biological Chemistry*, *249*(22), 7130-7139.

16. Tariq, A. M. (2020). The toxic effect of di-(2-ethylhexyl) phthalate on testes antioxidant and enzyme activities in adult male rabbits. *International Journal of Pharmacognosy and Chemistry*, 55-62.

17. Al-Ailla, A. K., & Khaled, F. A. (2019). The Effect of Ginger extract (Zinigber officinale Roscoe) on Antioxidant Enzymes and free Radical in Rabbits. *Journal of Biotechnology and Biochemistry*, *5*(1), 37-40.

18. Tappel, A. L., & Zalkin, H. (1959). Inhibition of lipide peroxidation in mitochondria by vitamin E. *Archives of Biochemistry and Biophysics*, *80*(2), 333-336.

19. 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.

20. Nandi, A., Yan, L. J., Jana, C. K., & Das, N. (2019). Role of catalase in oxidative stress-and age-associated degenerative diseases. *Oxidative medicine and cellular longevity*, *2019*(1), 9613090.

21. Moretti, M., Budni, J., Dos Santos, D. B., Antunes, A., Daufenbach, J. F., Manosso, L. M., ... & Rodrigues, A. L. S. (2013). Protective effects of ascorbic acid on behavior and oxidative status of restraint-stressed mice. *Journal of Molecular Neuroscience*, *49*(1), 68-79.

22. Xu, J., Xing, T., Li, J., Zhang, L., & Gao, F. (2023). Efficacy of creatine nitrate supplementation on redox status and mitochondrial function in pectoralis major muscle of preslaughter transported broilers. *Animal Biotechnology*, *34*(8), 3988-3999.

23. Jomova, K., Raptova, R., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. *Archives of toxicology*, *97*(10), 2499-2574.

24. Arazi, H., Eghbali, E., & Suzuki, K. (2021). Creatine supplementation, physical exercise and oxidative stress markers: a review of the mechanisms and effectiveness. *Nutrients*, *13*(3), 869.

25. Gęgotek, A., & Skrzydlewska, E. (2022). Antioxidative and anti-inflammatory activity of ascorbic acid. *Antioxidants*, *11*(10), 1993.

26. Forman, H. J., Zhang, H., & Rinna, A. (2009). Glutathione: overview of its protective roles, measurement, and biosynthesis. *Molecular aspects of medicine*, *30*(1-2), 1-12.

27. Payan-Carreira, R., Santos, C., & Santos, D. (2025). Antioxidant enzyme dynamics suggest the absence of oxidative stress in the canine endometrium across the estrous cycle. *Animal Reproduction Science*, *275*, 107819.

28. Michailidis, Y., Karagounis, L. G., Terzis, G., Jamurtas, A. Z., Spengos, K., Tsoukas, D., ... & Fatouros, I. G. (2013). Thiol-based antioxidant supplementation alters human skeletal muscle signaling and attenuates its inflammatory response and recovery after intense eccentric exercise. *The American journal of clinical nutrition*, *98*(1), 233-245.

29. 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), 244-47.