radiation + metformin groups, the level was significantly higher compared to the radiation group at 6 h, 7 d, 14 d, and 28 d after radiation ($p < 0.01$), with the most significant effect in the R + H group ($p < 0.05$ or $p < 0.01$) (Figure 4B). The results showed that the concentrations of testosterone and Inhibin B were significantly altered by microwave radiation, and metformin could dose-dependently inhibit the alterations.

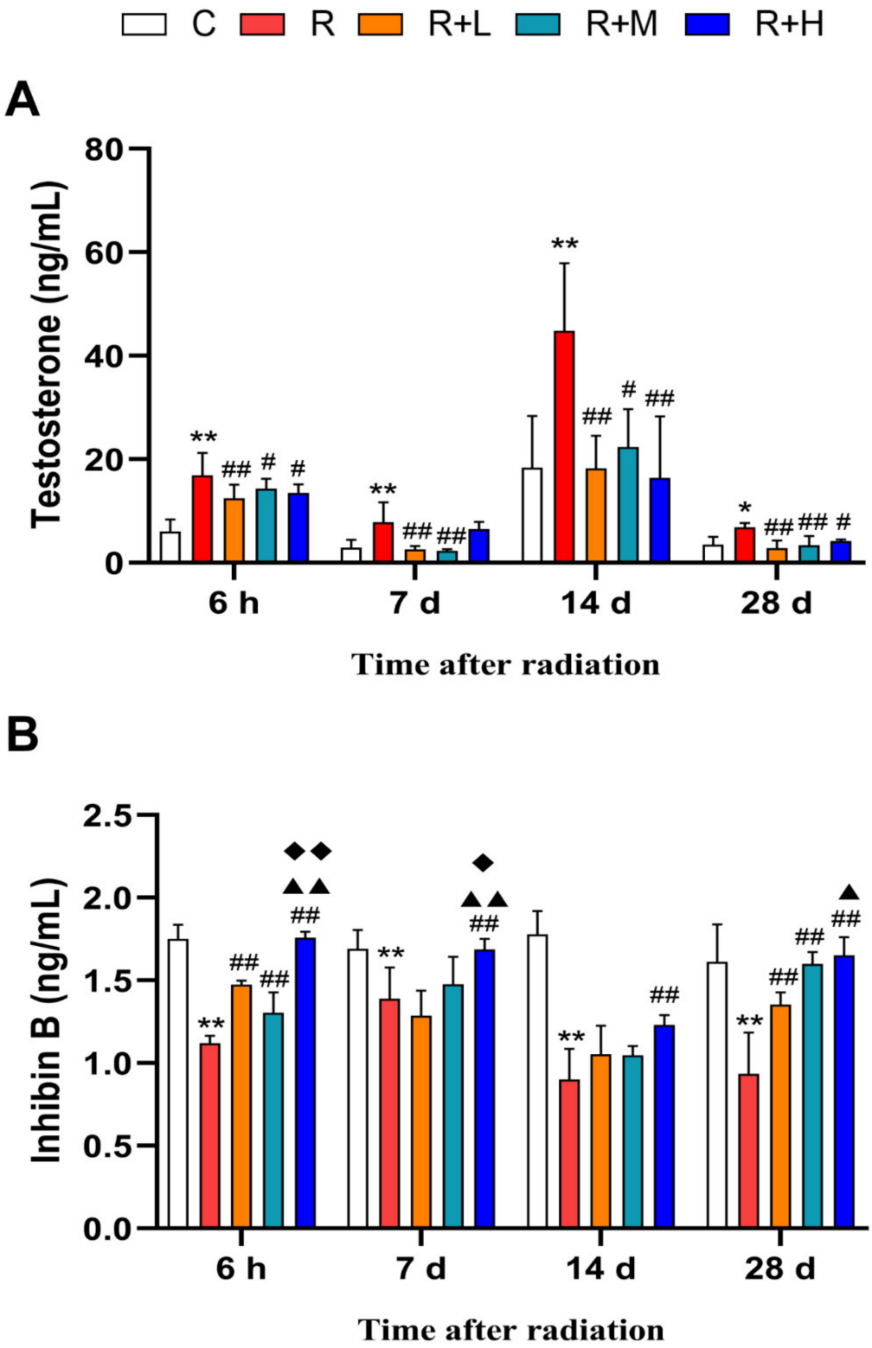


Figure 4. Metformin ameliorated the microwave-radiation-induced alteration of hormones (n = 5 for each group). (**A**) Changes in serum testosterone concentrations. (**B**) Changes in serum Inhibin B concentrations. * shows $p < 0.05$ for the R group compared to the C group, and ** shows $p < 0.01$; # shows $p < 0.05$ for the R + L, R + M, and R + H groups compared to the R group, and ## shows $p < 0.01$; ▲ shows $p < 0.05$ for the R + M and R + H groups compared with the R + L group, and ▲▲ shows $p < 0.01$; and ◆ shows $p < 0.05$ for the R + H group compared to the R + M group, and ◆◆ shows $p < 0.01$.

2.4. Metformin Ameliorated the Microwave-Radiation-Induced Reduction in Energy Metabolism Enzymes

Normal reproductive function is inseparable from important enzymes in energy metabolism. LDH, SDH, and α-GLU are sperm-specific energy metabolism enzymes involved in spermatogenesis and energy metabolism. Among these enzymes, LDH is a key enzyme in glycolysis [33] and SDH is an indispensable enzyme in oxidative phosphorylation [34]; they are widely distributed and found in germ cells and are a crucial source for energy metabolism [35]. α-GLU is secreted by the epididymis, which catalyzes glucose catabolism and gives energy to sperm maturation and sperm motility [36,37]. Our results showed that exposure to microwave radiation reduced the content of LDH and SDH enzymes at 6 h, 7 d, 14 d, and 28 d and α-GLU enzyme at 6 h and 7 d after microwave radiation ($p < 0.05$ or $p < 0.01$, respectively), compared with the control group. Compared with the microwave radiation group, the LDH and SDH content increased in the microwave radiation + metformin groups at 6 h, 7 d, 14 d, and 28 d ($p < 0.05$ or $p < 0.01$), and the α-GLU content increased in the microwave radiation + metformin groups at 6 h, 7 d, and 28 d after radiation, especially in the radiation plus the high dose metformin group ($p < 0.05$ or $p < 0.01$) (Figure 5A–C).

2.5. Metformin Alleviated Oxidative Stress Damage Caused by Microwave Radiation

Oxidative stress is one of the major mechanisms of microwave radiation-induced damage to the body. To determine whether the mechanism by which metformin alleviates microwave radiation-induced damage is associated with its antioxidant property, the levels of GSH and MDA and the protein expressions of HO-1 in the testes of rats were determined. Compared to the control group, exposure to microwave radiation caused significant decreases in the GSH/GSSG ratio at 6 h, 7 d, and 14 d and increases in the MDA level at the four points after radiation ($p < 0.05$ or $p < 0.01$, respectively). Compared with the microwave radiation group, the changes induced by microwave radiation were reversed by metformin in a dose-dependent manner ($p < 0.05$ or $p < 0.01$, respectively) (Figure 6A,B). Heme oxygenase-1 (HO-1) is a major factor of antioxidation. We investigated the antioxidative effect of metformin on HO-1 expression. Our results showed that the protein expression of HO-1 was down-regulated in the radiation group at the four points after microwave radiation but increased in microwave radiation + metformin groups ($p < 0.05$ or $p < 0.01$, respectively) (Figure 6C,D). In summary, exposure to microwave radiation could cause oxidative damage to reproductive organs, and metformin could act as an antioxidant to reduce this damage.

2.6. Metformin Mitigated Microwave-Radiation-Induced Mitochondrial Impairments

Mitochondria are a major target of microwave radiation-induced oxidative stress. Mitochondria-related enzymes can reflect mitochondrial function and the degree of oxidative stress damage [38]. Our results showed that the contents of mitochondrial complex IV and ATP synthase at the four points were reduced by microwave radiation ($p < 0.01$). Compared to the microwave radiation group, these deleterious effects were mitigated by metformin in a dose-dependent manner ($p < 0.05$ or $p < 0.01$, respectively) (Figure 7A,B).

2.7. Metformin Mitigated Microwave-Radiation-Induced Apoptosis

The Bax/Bcl-2 ratio (the apoptotic index) reflected the vulnerability of the tissue to apoptosis. In our experiment, Bax/Bcl-2 expression was up-regulated in the radiation group at 6 h, 7 d, and 14 d, and the changes were reversed after the administration of metformin ($p < 0.05$ or $p < 0.01$, respectively) (Figure 8A,B). The expression of cleaved caspase-3 was up-regulated in the radiation group at 6 h, 7 d, 14 d, and 28 d. Compared to the microwave radiation group, the expression of cleaved caspase-3 was down-regulated in microwave radiation + metformin groups ($p < 0.05$ or $p < 0.01$, respectively) (Figure 8A,C). The increase in the Bax/Bcl-2 and cleaved caspase-3 expression suggested that apoptosis could be caused by microwave radiation and that metformin inhibited this phenomenon.

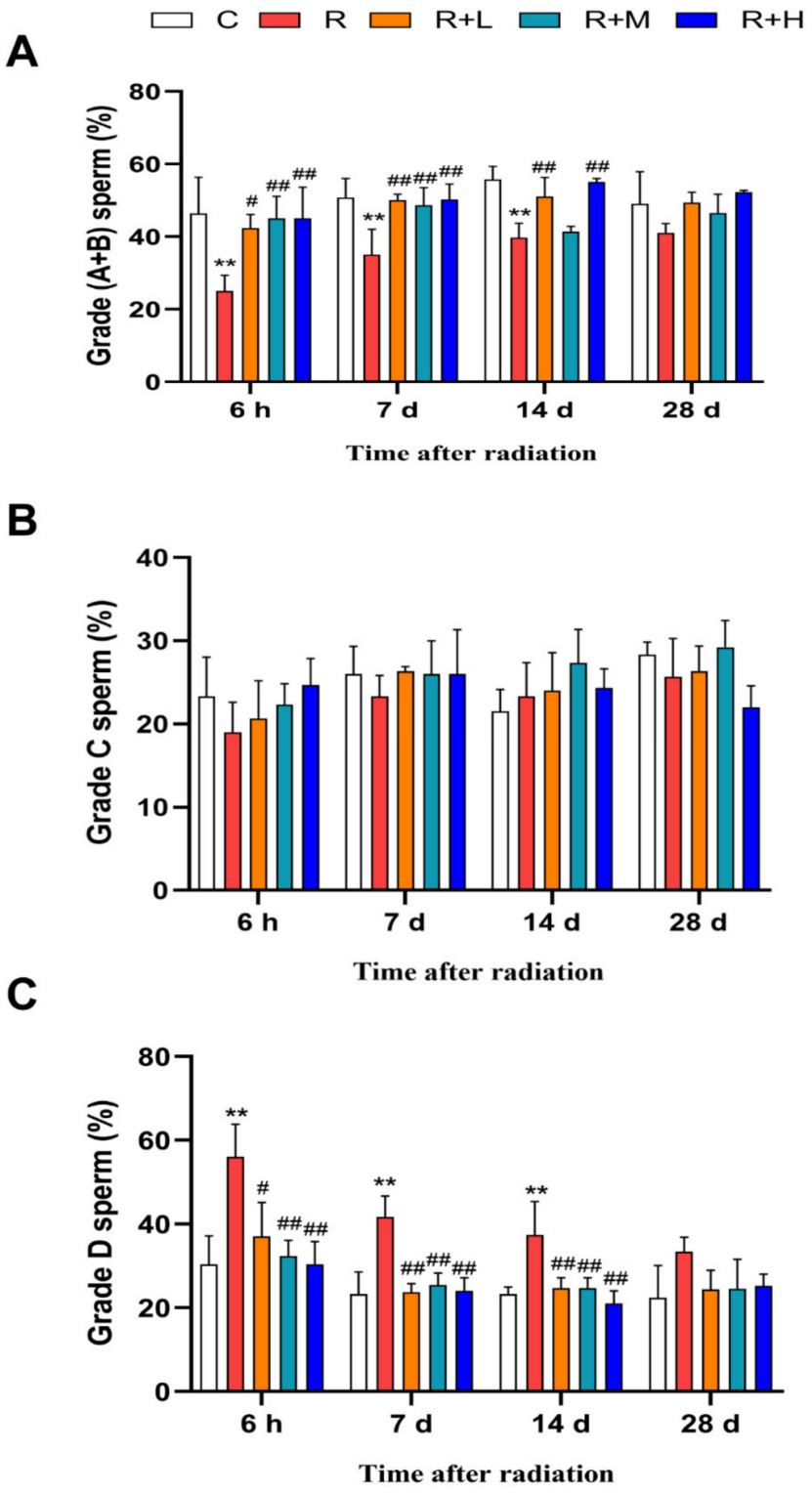


Figure 5. Metformin ameliorated the reduction in energy metabolism enzymes induced by microwave radiation (n = 5 for each group). (**A**) Changes in LDH content. (**B**) Changes in SDH content. (**C**) Changes in α-GLU content. * shows $p < 0.05$ for the R group compared to the C group, and ** shows $p < 0.01$; # shows $p < 0.05$ for the R + L, R + M and R + H groups compared to the R group, and ## shows $p < 0.01$; ▲ shows $p < 0.05$ for the R + M and R + H groups compared with the R + L group, ▲▲ shows $p < 0.01$; ◆ shows $p < 0.05$ for the R+ H group compared to the R + M group.

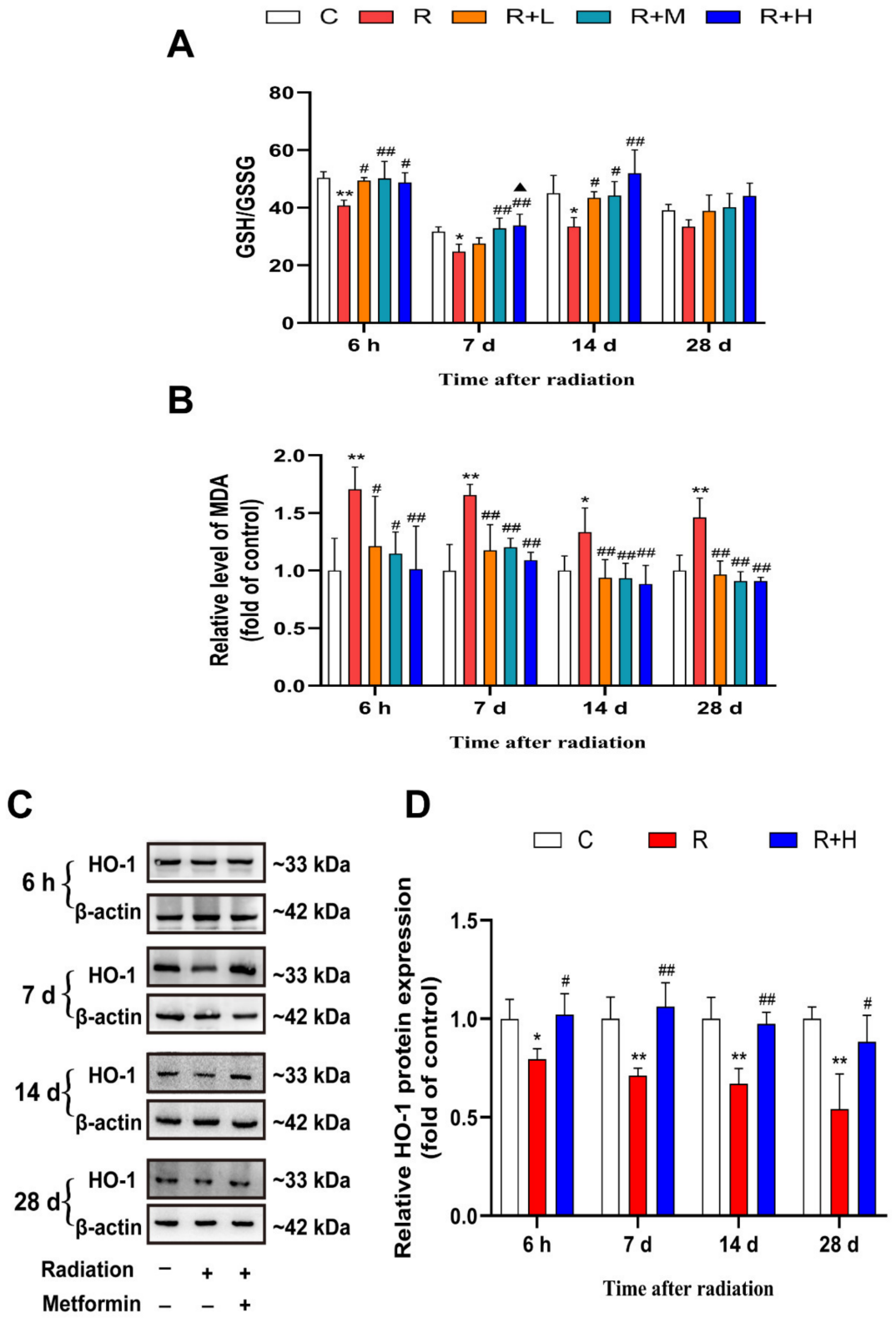


Figure 6. Metformin alleviated oxidative stress damage induced by microwave radiation (n = 5 for each group). (**A**) Changes in the GSH/GSSG ratio. (**B**) Changes in MDA content. (**C**) Changes in HO-1 expression. (**D**) Analysis of HO-1 expression. * shows $p < 0.05$ for the R group compared to the C group, and ** shows $p < 0.01$; # shows $p < 0.05$ for the R + L, R + M and R + H groups compared to the R group, and ## shows $p < 0.01$; ▲ shows $p < 0.05$ for the R + M and R + H groups compared with the R + L group.