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Original article

Differential Effects of Berberine and Clopidogrel on Plasma Lipid Peroxidation and Antioxidant Enzyme Activities in Male Rabbits

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Abstract
Oxidative stress is a critical factor in the progression of cardiovascular diseases, often managed with antiplatelet therapy and natural supplements. This study investigated the effects of Clopidogrel (CLOP), Berberine (BBR), and their combination (CLOP+BBR) on the plasma antioxidant status in male rabbits. Rabbits were divided into four groups: Control, CLOP, BBR, and CLOP+BBR. Following the treatment period, plasma levels of key antioxidant markers, including Catalase (CAT), Superoxide Dismutase (SOD), and Glutathione (GSH), were measured. Additionally, Thiobarbituric Acid-Reactive Substances (TBARS) were quantified as a biomarker for lipid peroxidation. The biochemical analysis revealed that the activity of enzymatic antioxidants (CAT and SOD) and the levels of non-enzymatic GSH remained statistically unchanged ($P > 0.05$) across all treated groups compared to the control. In contrast, a significant reduction ($P < 0.05$) in plasma TBARS levels was observed in the BBR-treated group (3.272 ±0.036) and the combination group (3.820 ±0.038) relative to the control (4.578 ±0.024) and CLOP (4.641 ±0.087) groups. Clopidogrel alone did not exhibit a significant impact on lipid peroxidation or antioxidant enzyme profiles. The findings suggest that while Berberine and its combination with Clopidogrel do not significantly modulate the primary antioxidant enzyme system, they exert a significant reduction in lipid peroxidation. This highlights the potential of Berberine as a protective agent against oxidative membrane damage without interfering with the baseline antioxidant defenses.
Keywords. Berberine, Clopidogrel, Antioxidant Enzymes, Lipid Peroxidation (TBARS), Rabbits.

Introduction

Oxidative stress represents a fundamental biochemical imbalance between the production of reactive oxygen species (ROS) and the capacity of the biological system to detoxify these reactive intermediates [1]. In the context of cardiovascular health, this imbalance leads to lipid peroxidation, protein degradation, and cellular dysfunction. One of the most critical biomarkers for measuring such oxidative damage is the concentration of Thiobarbituric Acid-Reactive Substances (TBARS), which reflects the extent of malondialdehyde (MDA) production during the degradation of polyunsaturated fatty acids [2]. To counteract these oxidative threats, the body relies on a sophisticated defense network comprising enzymatic antioxidants, such as Superoxide Dismutase (SOD) and Catalase (CAT), alongside non-enzymatic molecules like Glutathione (GSH). SOD serves as the first line of defense by catalyzing the dismutation of superoxide radicals into hydrogen peroxide, which is subsequently neutralized by CAT into water and oxygen. Maintaining the equilibrium of these enzymes is crucial for vascular homeostasis [3].

Clopidogrel is a widely prescribed thienopyridine antiplatelet agent used to prevent thromboembolic events. However, emerging evidence suggests that its pharmacological efficacy can be influenced by systemic oxidative status, and in some cases, its administration may not sufficiently address the underlying oxidative stress associated with cardiovascular pathologies. Consequently, there is growing scientific interest in the adjunctive use of natural bioactive compounds [4]. Berberine, a quaternary ammonium salt from the protoberberine group of benzylisoquinoline alkaloids, has garnered significant attention for its diverse pharmacological profile, including anti-inflammatory, lipid-lowering, and antioxidant properties [5]. Unlike synthetic drugs, Berberine often exerts a multifaceted protective effect, potentially stabilizing cellular membranes and reducing lipid peroxidation [6-17].

Despite the widespread use of both agents, the biochemical interactions between Clopidogrel and Berberine, specifically regarding their combined impact on the systemic antioxidant enzyme profile, remain an area that requires further investigation. This study aims to evaluate the effect of Clopidogrel, Berberine, and their co-administration on plasma antioxidant enzyme activities (SOD, CAT, GSH) and lipid peroxidation levels (TBARS) in an animal model, providing insights into their potential synergistic or independent biochemical roles.

Materials and Methods

A total of 20 adult male rabbits (*Oryctolagus cuniculus*), weighing 2170 ±150 g, were used. Animals were acclimated for one week under controlled environmental conditions (25 ±2°C, 12-hour light/dark cycle) with free access to a standard pellet diet and water. All procedures adhered to the ethical guidelines for animal care and use. Rabbits were randomly assigned into four groups (n=5each): Control (CON): Received distilled

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water (vehicle). Clopidogrel (CLOP): Received an oral dose of 75 mg/kg body weight.Berberine (BBR): Received an oral dose of 2 mg/kg body weight. Combined (CLOP+BBR): Received both Clopidogrel (75 mg/kg) and Berberine (2 mg/kg). The treatments were administered orally for four consecutive weeks. At the end of the experimental period, blood samples were collected from the ear vein into heparinized tubes. Plasma was separated by centrifugation at 3000 rpm for 15 minutes and stored at appropriate conditions for subsequent biochemical assays.

The plasma antioxidant profile and lipid peroxidation markers were quantified spectrophotometrically: Catalase (CAT): Determined by monitoring the decomposition of hydrogen peroxide at 240 nm [18]. Superoxide Dismutase (SOD): Measured by the inhibition of phenazine methosulfate-mediated reduction of nitroblue tetrazolium (NBT) at 560 nm [19]. Reduced Glutathione (GSH): Estimated using Ellman's reagent (DTNB) at 412 nm [20]. Lipid Peroxidation (TBARS): Quantified by measuring malondialdehyde (MDA) levels through the reaction with thiobarbituric acid, forming a pink chromogen measured at 532 nm [21].

Statistical Analysis Data were analyzed using one-way Analysis of Variance (ANOVA). Results are expressed as Mean ±Standard Error (SE). Significant differences between groups were determined using post-hoc tests, with a significance threshold set at $P < 0.05$.

Results

The plasma antioxidant profiles presented in Table 4 and Figures 13–16 show that catalase, superoxide dismutase, and glutathione levels remained statistically similar across all groups, while TBARS exhibited clear significant differences. Catalase activity ranged from 0.923 ± 0.026 U/min/ml in the CLOP group to 0.9954 ± 0.020 U/min/ml in the control group, with all groups sharing the same superscript letter, indicating no significant differences among treatments. A similar pattern was observed for superoxide dismutase, where values varied from 1.187 ± 0.014 U/ml in the CLOP group to 1.280 ± 0.024 U/ml in the BBR group, with no statistically significant changes detected. Plasma glutathione levels also remained consistent across treatments.

GSH values ranged between 5.686 ± 0.054 U/ml in the CLOP+BBR group and 5.821 ± 0.037 U/ml in the BBR group, and all groups showed the same superscript classification, confirming the absence of significant differences. In contrast, TBARS levels showed a clear distinction between groups. The BBR group recorded the lowest value (3.272 ± 0.036), followed by the CLOP+BBR group (3.820 ± 0.038), and both differed significantly from the control (4.578 ± 0.024) and CLOP (4.641 ± 0.087) groups, as indicated by the use of two superscript letters (a, b). The control and CLOP groups showed similar TBARS levels, with no significant variation between them. Overall, the results indicate that CAT, SOD, and GSH remained unchanged across treatments, whereas TBARS levels showed a significant reduction in the BBR and CLOP+BBR groups, demonstrating the only statistically meaningful shift within the antioxidant panel.

Table 1. Plasma Levels of GSH, CAT, SOD, and TBARS in Male Rabbits Treated with Clopidogrel, Berberine, and Their Combination.

Animal Groups (Mean±SE)	Catalase (CAT; U/min/ml)	Superoxide dismutase (SOD; U/ml)	Glutathione (GSH; U/ml)	Thiobarbituric acid-reactive substances (TBARS)
Control	0.995 ± 0.020[a]	1.190 ± 0.013[a]	5.779± 0.134[a]	4.578 ± 0.024[a]
CLOP	0.923 ±0.026[a]	1.187 ± 0.014[a]	5.723 ± 0.077[a]	4.641± 0.087[a]
BBR	0. 988 ± 0.030[a]	1.280 ± 0.024[a]	5.821 ± 0.037[a]	3.272 ± 0.036[b]
CLOP+BBR	0.963 ± 0.027[a]	1.274 ± 0.026[a]	5.686± 0.054[a]	3.820 ± 0.038[b]

Values are mean ± SE (n = 5). Different superscript letters within rows indicate significant differences (p< 0.05).

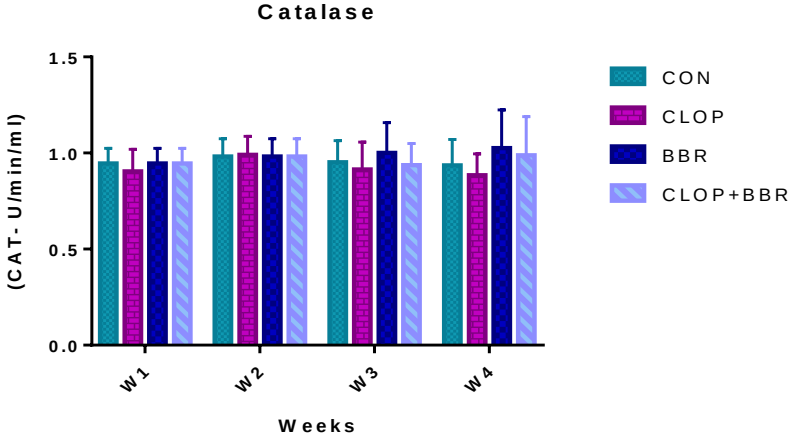

Figure 1. Catalase activity in the plasma of male rabbits treated with Clopidogrel, Berberine, and

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their combination.

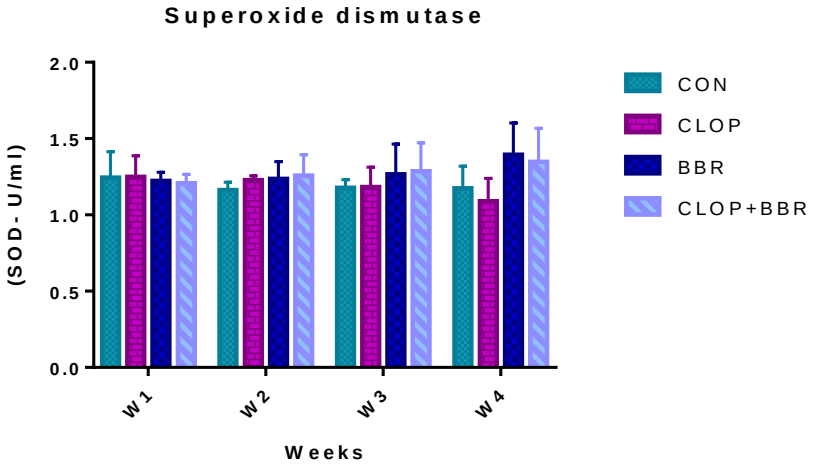

Figure 2. Superoxide dismutase (SOD) activity in the plasma of male rabbits treated with Clopidogrel, Berberine, and their combination.

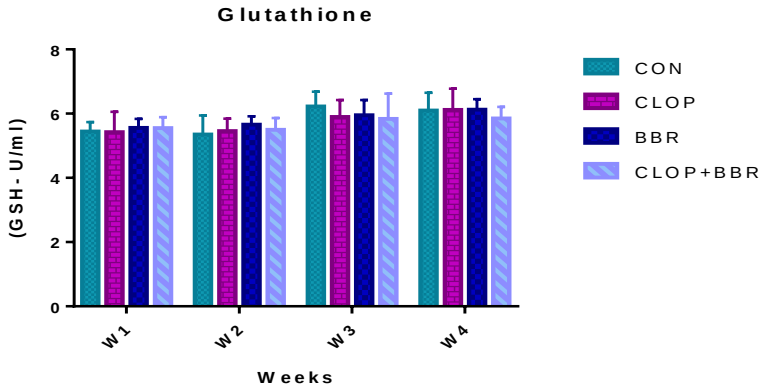

Figure 3. Glutathione (GSH) levels in plasma of male rabbits treated with Clopidogrel, Berberine, and their combination.

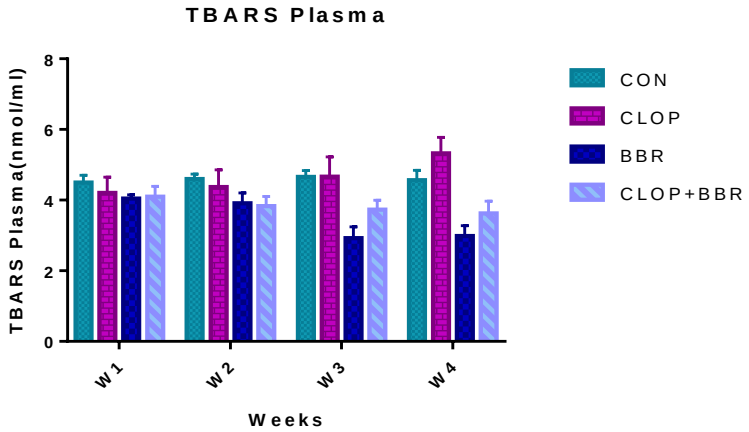

Figure 4. Plasma TBARS concentrations in male rabbits treated with Clopidogrel, Berberine, and their combination.

Discussion

The evaluation of plasma antioxidant enzymes in male rabbits treated with Clopidogrel, Berberine, and their combination revealed a selective response within the antioxidant defense system. While catalase, superoxide dismutase, and glutathione levels remained relatively stable across all experimental groups, lipid peroxidation, as reflected by TBARS levels, showed clear and significant variation among treatments. The absence of significant changes in catalase and superoxide dismutase activities suggests that the administered treatments did not markedly disturb the primary enzymatic antioxidant defenses [22-25].

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These enzymes represent the first line of protection against reactive oxygen species, and their stability indicates that oxidative stress was not severe enough to trigger compensatory upregulation or depletion. Similarly, the unchanged glutathione levels across groups imply preservation of intracellular redox balance and adequate antioxidant capacity under the experimental conditions [26-35]. In contrast, TBARS levels demonstrated a pronounced reduction in animals treated with Berberine, either alone or in combination with Clopidogrel.

Since TBARS is a well-established marker of lipid peroxidation, this decrease indicates a clear attenuation of oxidative damage to membrane lipids [36-44]. This finding strongly supports the antioxidant potential of Berberine, which has been widely reported to suppress lipid peroxidation through free radical scavenging and inhibition of oxidative chain reactions [45-49]. Interestingly, Clopidogrel treatment alone did not significantly alter TBARS levels compared to the control group, suggesting that the drug did not induce overt systemic oxidative stress at the dose and duration used. However, the reduction of TBARS in the combined treatment group indicates that Berberine retained its protective effect even in the presence of Clopidogrel, highlighting its ability to counteract subtle oxidative challenges associated with pharmacological stress [50].

Conclusion

The study concludes that Berberine, alone or with Clopidogrel, significantly reduces lipid peroxidation (TBARS) without altering primary antioxidant enzymes (CAT, SOD, and GSH). This suggests that Berberine's protective role is mediated through direct inhibition of membrane damage rather than enzymatic upregulation. These findings highlight Berberine's potential as a safe antioxidant adjunct to Clopidogrel therapy.

Conflict of interest. Nil

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