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Activation of cannabinoid type 2 (CB2) receptors promotes the maintenance of redox homeostasis and protects against oxidative distress in the Neotropical freshwater fish matrinxã *Brycon amazonicus* (Characiformes: Bryconidae)

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Recent evidence indicates significant interaction of cannabinoid receptors (CB1 and CB2) with redox mechanisms. This study investigated the effects of the cannabinoid agonists WIN 55,212-2 (CB1+CB2) and HU-308 (CB2) on oxidative biomarkers in the liver and heart of the fish *Brycon amazonicus*. In both the liver and the heart, CB1+CB2 activation led to significant increases in catalase (CAT) and glutathione peroxidase (GPx) activities, accompanied by decreases in glutathione reductase (GR) activity. In contrast, glutathione S-transferase (GST) activity increased in the liver and decreased in the heart following CB1+CB2 activation. In addition, CB1+CB2 agonist had no effect on the GSH/GSSG ratio but increased heart levels of lipoperoxidation (LPO) and hepatic and cardiac protein carbonyl (PC) content. On the other hand, CB2 activation preserved antioxidant enzymatic activities and increased the GSH/GSSG ratio in both tissues. Moreover, the CB2 agonist showed no significant effect on PC levels in either tissue or cardiac LPO levels but decreased hepatic LPO content. In conclusion, activation of CB1+CB2 receptors disrupted the redox balance, leading to oxidative distress and damage, whereas activation of CB2 preserved oxidative eustress. These findings highlight the potential of CB2 receptors to modulate antioxidant defenses and maintain redox homeostasis, critical for improving fish health.

Keywords: Antioxidant defense system, Cannabinoid agonists, Heart, Liver, Oxidative damage.

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Evidências recentes indicam uma interação significativa dos receptores canabinoides (CB1 e CB2) com os mecanismos redox. Este estudo investigou os efeitos dos agonistas canabinoides WIN 55,212-2 (CB1+CB2) e HU-308 (CB2) sobre biomarcadores oxidativos no fígado e coração do peixe *Brycon amazonicus*. Tanto no fígado quanto no coração, a ativação de CB1+CB2 resultou em aumentos significativos nas atividades de catalase (CAT) e glutationa peroxidase (GPx), acompanhados por diminuições na atividade de glutationa redutase (GR). Em contraste, a atividade de glutationa S-transferase (GST) aumentou no fígado e diminuiu no coração após a ativação de CB1+CB2. Além disso, o agonista CB1+CB2 não afetou a razão GSH/GSSG, mas aumentou os níveis de lipoperoxidação (LPO) no coração e o conteúdo de proteína carbonilada (PC) no fígado e coração. Por outro lado, a ativação de CB2 preservou as atividades enzimáticas antioxidantes e aumentou a razão GSH/GSSG em ambos os tecidos. Além disso, o agonista CB2 não teve efeito significativo nos níveis de PC em nenhum tecido ou nos níveis de LPO cardíaco, mas diminuiu o conteúdo de LPO hepático. Em conclusão, a ativação dos receptores CB1+CB2 perturbou o equilíbrio redox, levando a distresse oxidativo e danos, enquanto a ativação de CB2 preservou o eustresse oxidativo. Estes achados destacam o potencial dos receptores CB2 para modular as defesas antioxidantes e manter a homeostase redox, fundamentais para melhorar a saúde dos peixes.

Palavras-chave: Agonistas canabinoides, Coração, Dano oxidativo, Fígado, Sistema de defesa antioxidante.

INTRODUCTION

Oxidative stress is broadly defined as an imbalance arising from the excessive formation of reactive oxygen species (ROS) compared to the capacity of cellular antioxidant defenses (Sies, Jones, 2007; Halliwell, Gutteridge, 2015). In cases of supraphysiological oxidative challenges, a disruption in the redox status occurs, leading to oxidative damage to biomolecules and resulting in a pathological state - a condition referred to as oxidative distress (Sies, 2019, 2020). On the other hand, the term oxidative eustress represents a controlled and beneficial form of oxidative challenge that occurs at a moderate and low-level physiological oxidative stress (Okegbe *et al*., 2012; Niki, 2016; Roach *et al*., 2018; Sies, 2020). It plays a pivotal role in life processes through redox signaling and regulation, whereby ROS acts as secondary messengers to trigger adaptive cellular responses (Sies, 2021; Sies *et al*., 2022). Maintaining a delicate balance between oxidative eustress and oxidative distress is crucial for cellular homeostasis and the overall health of the organism.

The endocannabinoid system includes two G-protein receptors identified as type 1 (CB1) and type 2 (CB2) (Castillo *et al*., 2012; Pisanti *et al*., 2013). The literature supports the positive effects associated with the activation of cannabinoid receptors, particularly CB2, in modulating ROS production under pathological conditions (Paloczi *et al*., 2018; Xin *et al*., 2020; Voicu *et al*., 2023). The imperative advancement of health applications in the field of applied redox biology necessitates the identification and exploration of

mechanisms capable of adjusting the critical balance points between oxidative eustress and distress (Sies *et al*., 2022). In this context, investigating the mechanisms by which the activation of cannabinoid receptors modulates oxidative status holds scientific significance. This is particularly important because a comprehensive understanding of this phenomenon remains incomplete, especially under physiological conditions. Therefore, the regulation of the cannabinoid receptors through the administration of selective agonists emerges as a promising approach for both preventive and therapeutic strategies.

Oxidative distress is a critical factor affecting fish well-being and represents a significant challenge in aquaculture, especially in intensively managed culture systems that frequently elevate levels of stress (Hoseinifar *et al*., 2020; Song *et al*., 2023). Optimizing the efficiency and capacity of antioxidant defenses improves the modulation of immune responses and enhances disease resistance (Marmelo *et al*., 2024). Natural antioxidants (such as carotenoids, polyphenols, and vitamins) and synthetic antioxidants (including propyl gallate, tert-butylhydroquinone, butylated hydroxyanisole, and butylated hydroxytoluene) are commonly used to counteract the harmful effects of oxidative stress, with the goal of improving fish health (Hoseinifar *et al*., 2020; Pereira *et al*., 2022). While the use of synthetic antioxidants is increasingly restricted due to environmental and health safety concerns, the effectiveness of natural antioxidants relies on their structural properties, extraction methods, and stability under varying environmental conditions (*e.g*., light, temperature, and pH), necessitating rigorous risk-benefit analyses to ensure their safety and efficacy (Hoseinifar *et al*., 2020; Pereira *et al*., 2022; Petcu *et al*., 2023)

In our hypothesis, we propose that the activation of the CB2 receptor has the potential to positively modulate the oxidative status, thereby contributing to advantageous adaptations, that mitigate the potential harm caused by ROS in healthy animals. Therefore, this research aimed to explore the impact of activating CB1+CB2 receptors or exclusively CB2 receptors using suitable synthetic agonists on the antioxidant defense system and biomarkers of oxidative distress in key organs such as the liver and heart of *Brycon amazonicus* (Agassiz, 1829), a prominent freshwater fish species farmed in South America. In addition, fish represent a valuable alternative as an experimental model for evaluating innovative pre-clinical research, aligning with the 3Rs concept and offering advantages in terms of cost-effectiveness and ethical considerations.

MATERIAL AND METHODS

Animals and treatment. Juvenile specimens (53.0 ± 3.4 g, mean ± SE) of *Brycon amazonicus*, known as matrinxã, were acquired from the Colpani fish farm (Mococa, SP, Brazil). The fish underwent a 30-day acclimatization period in 1000-liter holding tanks equipped with aerated and filtered water at a temperature of 25 ± 1 °C and exposed to a natural photoperiod (12:12 light: dark cycle). Throughout the acclimatization phase, the fish were provided with *ad libitum* feeding of a commercial ration.

The fish were then allocated into three experimental groups: Control group (Ct; n = 15) - these specimens were administered an intraperitoneal (i.p.) injection of 0.5 ml of a vehicle solution (2.5% DMSO in sterile saline, Solbrig *et al*., 2013); 2) CB1+CB2-treated group (WIN; n= 15) - the fish received an i.p. injection of 1 $mg.kg^{-1}$ of the CB1+CB2 non-selective agonist WIN 55 212-2 (> 98% purity, Cayman Chemical), dissolved in

vehicle solution; and 3) CB2-treated group (HU; n = 15) - the fish received an i.p. injection of 1 $mg.kg^{-1}$ of a CB2 receptor-selective agonist, HU 308 (> 98% purity, Cayman Chemical) dissolved in vehicle. Previous studies have demonstrated that the selected dosage exhibits anti-inflammatory effects (González *et al*., 2011; Wang *et al*., 2012). After 24 h, the fish were euthanized using an anesthetic overdose of benzocaine at a concentration of 0.3%. The hearts and livers were meticulously removed and rinsed with cold saline, then promptly frozen in liquid nitrogen and stored at -80°C until analyses were conducted.

Oxidative biomarkers. The liver and heart samples were homogenized (1:5 w/v) in a 0.1 M sodium-potassium phosphate buffer (pH 7.0) containing 1 mM phenylmethylsulfonyl fluoride (PMSF), then centrifuged at 10,000 g for 30 minutes at 4°C. The supernatants were utilized for the analyses described below. All readings were performed in duplicate at 25°C using either a UV-Vis spectrophotometer (BEL Engineering, Italy) or a microplate reader (Spectramax i3, Molecular Devices, USA).

Superoxide dismutase (SOD) activity was assessed following the method described by Flohé, Ötting (1984). The assay measures the rate of reduction of cytochrome c by superoxide anions at 550 nm. One unit of SOD corresponded to the enzyme amount that caused 50 % maximum inhibition of the cytochrome c reduction.

Catalase (CAT) activity was determinated by measuring the degradation of hydrogen peroxide (H_2O_2) at 240 nm according to the procedures described by Aebi (1974). One unit of CAT was defined as the quantity of enzyme required to release half of the peroxide oxygen within 100 seconds at 25°C (Bergmeyer unit - BU).

Glutathione peroxidase (GPx) activity was estimated in a reaction coupled with glutathione reductase (GR) and monitored by the decrease in absorbance of reduced NADPH at 340 nm (Nakamura *et al*., 1974). One unit of GPx was defined as the amount of enzyme needed to oxidize 1 µmol of NADPH per min.

Glutathione reductase (GR) activity was quantified in homogenates by monitoring the oxidation of NADPH at 340 nm in the presence of oxidized glutathione (GSSG) (Carlberg, Mannervik, 1985). One unit of GR corresponded to the enzyme amount that oxidized 1 µmol of NADPH per min.

Glutathione S-transferase (GST) activity was determined using 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) (Habig *et al*., 1974). The rate of production of S-2, 4-dinitrophenyl glutathione was detected at 340 nm. One unit of GST corresponded to enzyme level responsible for the formation of 1 µmol of product per min.

The levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) were determined using the 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB)-GSSG reductase recycling assay as described by Anderson (1985). Homogenates were treated with 5% sulfosalicylic acid and centrifuged at 8,000 g for 5 minutes at 4°C. The production of thionitrobenzoic acid (TNB) was monitored at 412 nm to assess the levels of total glutathione (GSH plus GSSG). Specifically, GSSG levels were measured after treating homogenates with vinyl pyridine to sequester GSH (Cunha Bastos *et al*., 2007). Quantification of GSH and GSSG was performed in µmol per gram of tissue using standard curves. The GSH/GSSG ratio was defined by the formula: GSH/GSSG = [(GSH - 2*GSSG) / GSSG].

Lipoperoxidation (LPO) was assayed using the FOX-ferrous oxidation-xylenol orange method following the procedures reported by Jiang *et al*. (1992). This method involves the oxidation of Fe^{2+} by hydroperoxides in acidic samples, with the reaction catalyzed by orange xylenol, a Fe^{3+} complexing agent. The resulting complex was quantified at a wavelength of 560 nm. Lipid peroxidation (LPO) levels were then calculated using a standard curve of cumene hydroperoxide and expressed in nmol per mg of protein.

Protein carbonyl (PC) content was determined following the protocol outlined by Reznick, Packer (1994) using 2,4-dinitrophenylhydrazine (DNPH) and guanidine hydrochloride. The amount of carbonyl content was measured at 370 nm using the molar extinction coefficient of ε_{370} = 22 mM^{-1} cm^{-1} and expressed in nmol per mg of protein. Protein content in tissue homogenates was quantified at 595 nm using the Bradford method (Kruger, 1994), with bovine serum albumin as the standard.

Statistical analysis. Data are displayed as mean ± standard error (SE). Sample normality was verified employing the Kolmogorov-Smirnov method, and variance homogeneity was confirmed with Levene's test. To identify significant differences between the experimental groups (Ct, WIN, and HU), a one-way analysis of variance (ANOVA) was conducted, followed by Tukey's post hoc test for multiple comparisons (GraphPad Prism v. 8.0, GraphPad Software, USA). Differences between means were accepted as significant at $p < 0.05$.

RESULTS

The administration of the non-selective cannabinoid agonist (CB1+CB2), represented by the WIN group, caused a significant 29% decrease in hepatic SOD activity when compared to controls (Fig. 1A). Concurrently, the activities of CAT and GPx increased by 28% and 70%, respectively (Figs. 1B, C), while GST activity showed a significant decrease of 35%, coupled with a 30% reduction in GR activity (Figs. 1D, E, respectively). On the other hand, the HU group (CB2 activation) did not exhibit any significant changes in antioxidant enzyme activities compared to the Ct group.

Regarding cardiac tissue, the WIN group demonstrated a significant enhancement in SOD (103%), CAT (73%), GPx (150%), and GST (54%) activities (Figs. 2A, B, C, D, respectively), along with a concomitant decrease in GR (44%) activity (Fig. 2E) compared to the Ct group. In contrast, the HU group showed no significant alterations in antioxidant enzymatic activities (Fig. 2) compared to the Ct group.

In the context of the non-enzymatic GSH system, the WIN group demonstrated a significant decline in hepatic GSH levels (37%) while showing no alterations in ventricular GSH content compared to the Ct group (Figs. 3A and D, respectively). Meanwhile, the HU group exhibited no alterations in hepatic GSH content but showed a significant increase in ventricular GSH levels (115%) compared to the Ct group (Figs. 3A and D, respectively). Regarding GSSG contents, the CB1+CB2 agonist induced a significant 37% increase in ventricular GSSG levels, with no substantial change in hepatic tissue (Figs. 3B and E, respectively). In contrast, the HU group displayed a reduction in both hepatic (33%) and cardiac (53%) GSSG contents compared to the Ct group (Figs. 3B and E, respectively). The GSH:GSSG ratio remained unchanged

in the hepatic and cardiac tissues of the WIN group. Nevertheless, this ratio showed a substantial increase in the liver (112%) and heart (606%) of the HU group compared to controls (see Figs. 3 C and F, respectively).

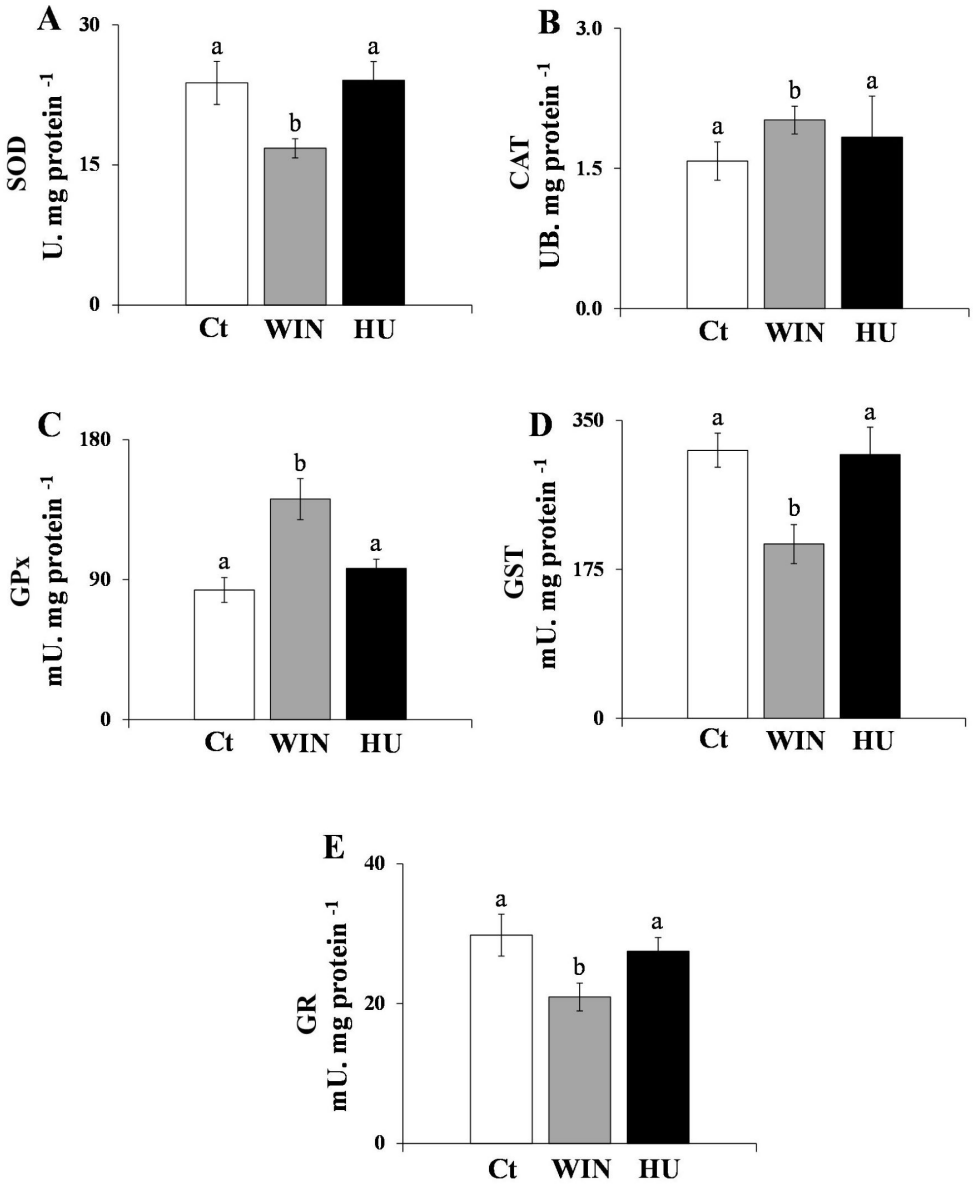

FIGURE 1 | Enzymatic activities of superoxide dismutase (SOD) (**A**), catalase (CAT) (**B**), glutathione peroxidase (GPx) (**C**), glutathione-S-transferase (GST) (**D**), and glutathione reductase (GR) (**E**) in the liver of *Brycon amazonicus* from control (Ct, n = 15), CB1+CB2-treated group (WIN, n = 15), and CB2-treated group (HU, n = 15). Mean values ± SE. Different letters indicate significant differences between groups ($p < 0.05$).

Oxidative stress analysis revealed that the WIN group did not exhibit changes in hepatic LPO levels compared to controls (Fig. 4A). However, the PC content showed a significant increase (51%) (Fig. 4B). In the cardiac tissue, both LPO and PC levels increased by 70% and 99%, respectively (Figs. 4C, D, respectively). Conversely, in comparison to the Ct group, HU treatment resulted in a significant decrease in hepatic LPO levels (33%) (Fig. 4C), with no significant alterations in hepatic PC content (Fig. 4A) or in cardiac LPO and PC levels (Figs. 4C, D, respectively).

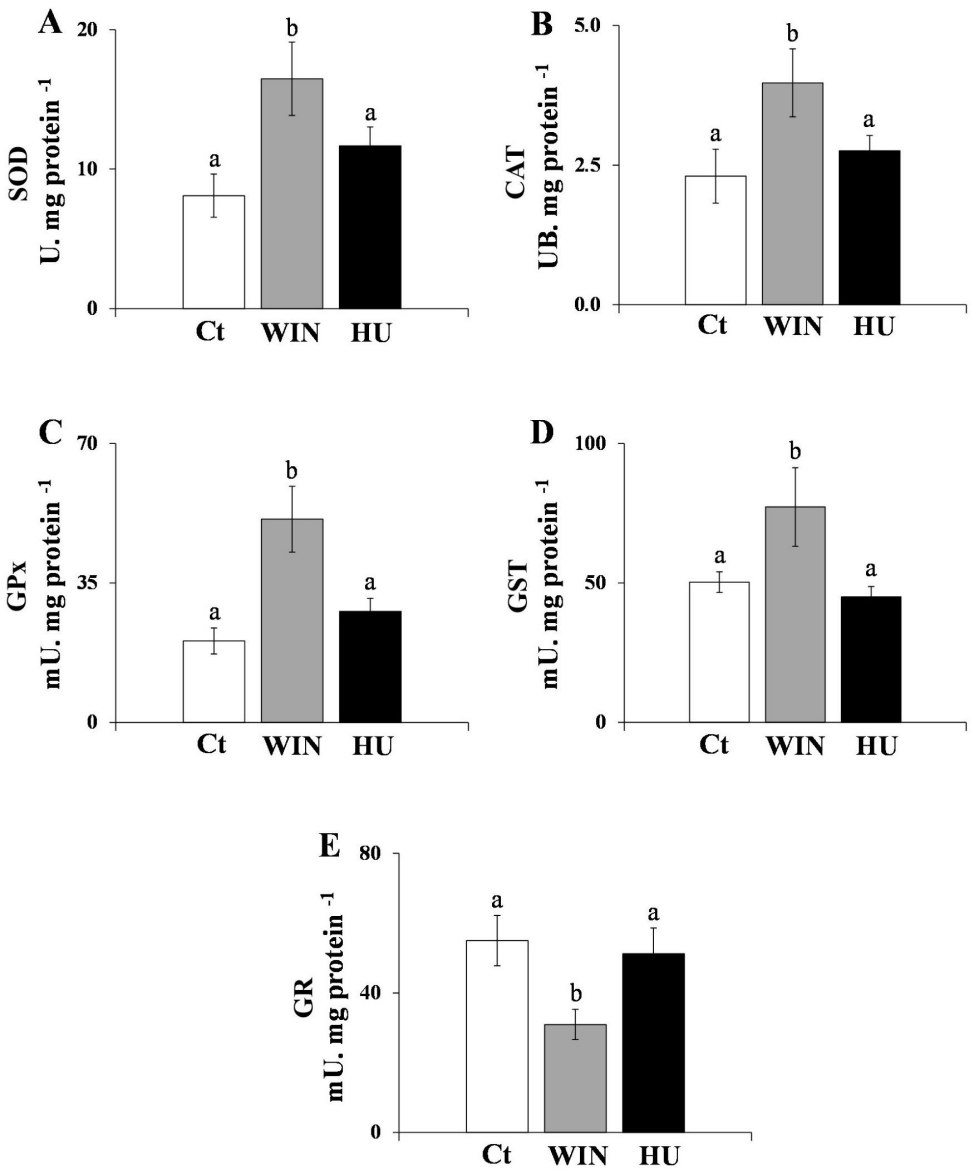

FIGURE 2 | Enzymatic activities of superoxide dismutase (SOD) (**A**), catalase (CAT) (**B**), glutathione peroxidase (GPx) (**C**), glutathione-S-transferase (GST) (**D**), and glutathione reductase (GR) (**E**) in the heart of *Brycon amazonicus* from control (Ct, n = 15), CB1+CB2-treated group (WIN, n = 15), and CB2-treated group (HU, n = 15). Mean values ± SE. Different letters indicate significant differences between groups ($p < 0.05$).

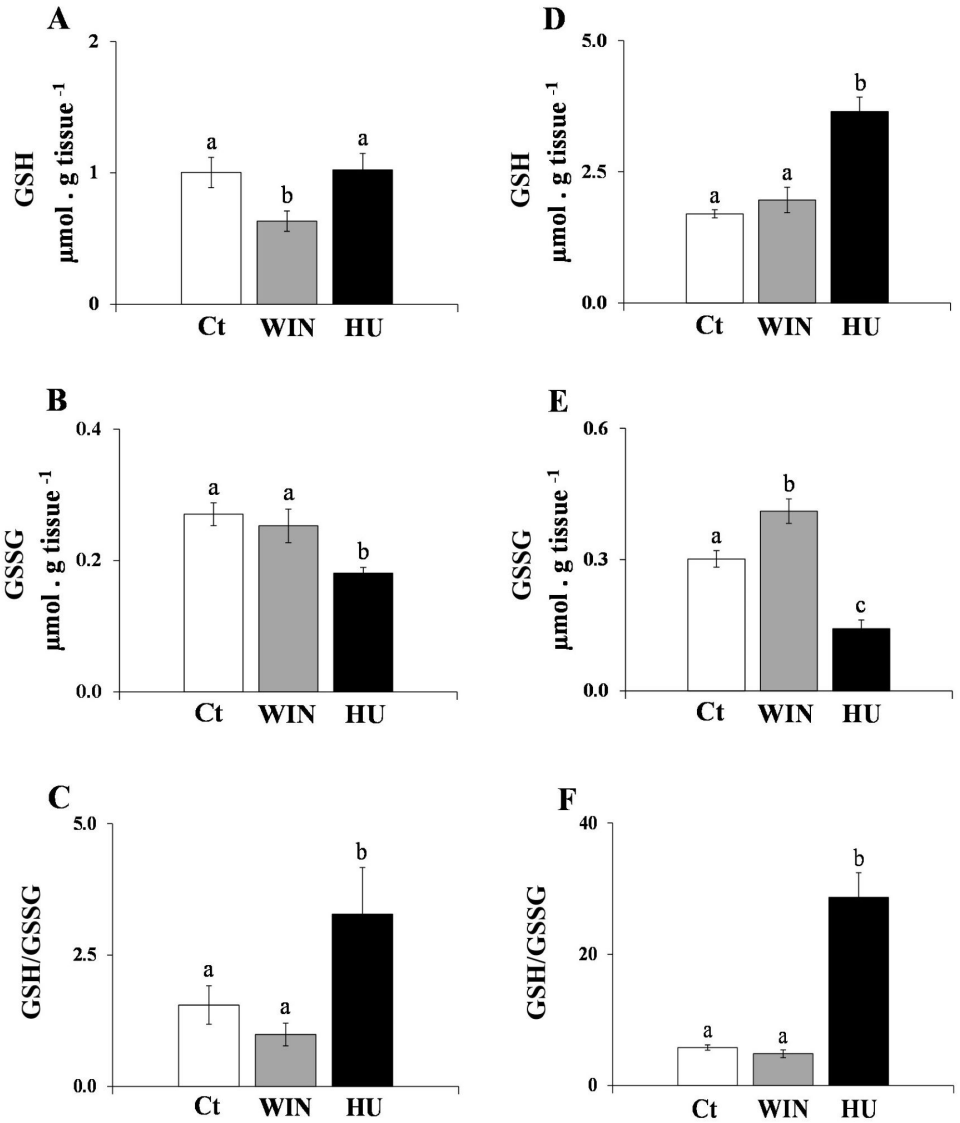

FIGURE 3 | Hepatic and cardiac levels of reduced (GSH) (**A** and **D**, respectively) and oxidized (GSSG) (**B** and **E**, respectively) glutathione, and GSH/GSSG ratio (**C** and **F**, respectively) of *Brycon amazonicus* from control (Ct, n = 15), CB1+CB2-treated group (WIN, n = 15), and CB2-treated group (HU, n = 15). Mean values ± SE. Different letters indicate significant differences between groups ($p < 0.05$).

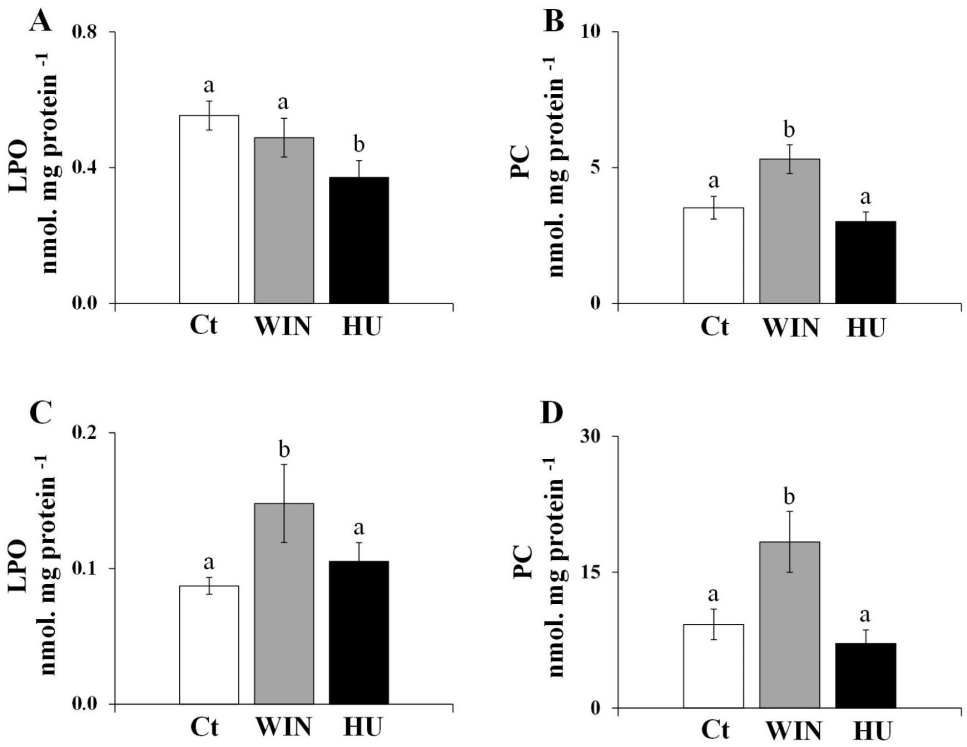

FIGURE 4 | Lipid peroxidation (LPO) and protein carbonyl (PC) levels in the liver (**A** and **B**, respectively) and the heart (**C** and **D**, respectively) of *Brycon amazonicus* from control (Ct, n = 15), CB1+CB2-treated group (WIN, n = 15), and CB2-treated group (HU, n = 15). Mean values ± SE. Different letters indicate significant differences between groups ($p < 0.05$).

DISCUSSION

This is one of the first studies to demonstrate the effects of both cannabinoid receptor activation, CB1 and CB2, as well as selective activation of CB2, on the redox balance of two crucial organs: the liver and the heart, under physiological conditions. The comprehension of the antioxidative properties resulting from the activation of CB1 and CB2 receptors is crucial for employing agonists as a therapeutic approach in the prevention and treatment of disorders related to oxidative distress. This study demonstrated that acute treatment with the CB1/CB2 agonist WIN 55,212-2, resulted in a shift of the redox balance from eustress to distress, both in the liver and in the heart. On the other hand, the acute administration of the selective CB2 receptor agonist HU-308 exhibited antioxidant properties in both vital organs, contributing to the maintenance of oxidative eustress.

Cannabinoid receptors activate various intracellular signaling pathways, adding complexity and bias to our understanding of their role in regulating oxidative signaling and metabolic abnormalities (Ibsen *et al*., 2017; Soethoudt *et al*., 2017; Saroz *et al*., 2019). Although it is still unclear which specific CB1 and CB2 signaling pathways are activated, there is a growing acknowledgment that, under various pathological processes, the activation of CB1 receptors might trigger c-jun n-terminal kinase (JNK) and p38 mitogen activated protein kinase (p38 MAPK) signaling cascades, which

are known to be related in cell death, ROS generation, and inflammatory response, contributing to the development of cardiac and hepatic dysfunctions (Mukhopadhyay *et al*., 2010; Rajesh *et al*., 2012; Tian *et al*., 2017; Kim *et al*., 2020; Jorgačević *et al*., 2021). Conversely, CB2 signaling demonstrates the ability to selectively activate the protein kinase 1 and 2 (ERK1/2) nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. These pathways are intricately associated with antifibrogenic and anti-inflammatory functions, ultimately resulting in cardio- and hepatoprotective effects (Lépicier *et al*., 2003; Montecucco *et al*., 2009; Teixeira-Clerc *et al*., 2010; Montecucco, Di Marzo, 2012; Steffens, Pacher, 2012; Wang *et al*., 2014; Hashiesh *et al*., 2021). Therefore, CB2 stimulation appears to dynamically modulate numerous pathophysiological processes and is implicated in the regulation of various pathological conditions (Tabrizi *et al*., 2016; Shang, Tang, 2017; Hashiesh *et al*., 2021).

Our results demonstrated that CB2 activation modulates the balance between oxidative eustress and distress even under physiological conditions, which would be highly relevant for hepatic and cardiac survival when homeostasis is lost. Considering that ROS plays a pivotal role in cell function (Sinenko *et al*., 2021; Sharma *et al*., 2023), the positive modulation of CB2-induced oxidative eustress contributes to tissue integrity and the normal functioning of physiological processes. Redox signaling refers to oxidation/reduction modifications of cellular signaling components, capable of regulate gene expression, excitation-contraction coupling, regeneration, cell growth, migration, differentiation, and apoptosis (Sack *et al*., 2017; Dubois-Deruy *et al*., 2020). Consequently, the production and maintenance of controlled levels of intracellular ROS mediated by CB2 receptor activation, preserving redox homeostasis, holds the potential to ameliorate or prevent disease progression.

The treatment of HU-308 reduced ROS production in the liver, thereby mitigating LPO injuries and preserving PC levels in both cardiac and hepatic tissues. In zebrafish *Danio rerio* (Hamilton, 1822), inhibition of CB2 receptor activity using the antagonist AM630 disrupts liver function, suggesting that the endocannabinoid system is involved in the regulation of lipid homeostasis (Liu *et al*., 2016). The antioxidant effect observed upon CB2 activation in matrinxã was associated with an increase in the GSH/GSSG ratio, achieved by elevating GSH levels and/or inhibiting GSSG production, all while preserving the activities of antioxidant enzymes. Previous studies have reported that stimulation of CB2 receptors by synthetic agonists promotes cardioprotection and hepatoprotection through downstream activation via Nrf2 pathways (Li *et al*., 2016; Hashiesh *et al*., 2021; More *et al*., 2024). This activation leads to the inhibition of oxidative stress, prevention of apoptotic cell death and fibrosis, reduced lesion area, and attenuation of the release of inflammatory cytokines in ischemia/reperfusion or toxicant-induced liver injury (Rajesh *et al*., 2007; Montecucco *et al*., 2009; Louvet *et al*., 2011; Li *et al*., 2014; Wu *et al*., 2019; Yu *et al*., 2019; González-Candia *et al*., 2022). Nrf2 is a redox-sensitive transcription factor responsible for maintaining redox homeostasis by modulating antioxidant-response element (ARE)-dependent transcription and subsequently expressing enzymes involved in antioxidant defense (Kang *et al*., 2020). This process enhances the antioxidant capacity, both enzymatic and non-enzymatic, in a compensatory manner in response to an increase in ROS (González-Candia *et al*., 2022).

By reducing ROS levels, HU-308 also safeguards GSH levels, preventing their oxidation, as evidenced by the increased GSH/GSSG ratio in cardiac and hepatic tissues.

This is of great practical importance because GSH plays vital roles in shielding cells from oxidative harm, neutralizing the toxicity of foreign chemical electrophiles, and preserving redox balance (Forman *et al*., 2009; Atalay *et al*., 2020). Fouad *et al*. (2013) observed a significant reduction in lipid peroxidation (LPO), maintenance of GSH levels, and decreased production of tumor necrosis factor-alpha (TNF-α) and nitric oxide (NO) in the cardiac tissue of rats exposed to doxorubicin cardiotoxicity following treatment with cannabidiol, which has been associated with CB2 activation (Martínez-Pinilla *et al*., 2017). Cannabidiol supplementation at 100 and 250 mg kg^{-1} in high soybean oil diets significantly increased liver antioxidant defenses, including peroxidase and superoxide dismutase activities and total antioxidant capacity, while concurrently reducing lipid peroxidation and inhibiting inflammatory gene expression in large yellow croaker *Larimichthys crocea* (Richardson, 1846) (Wang *et al*., 2023). Cannabidiol treatment (5 mg/kg, i.p.) also provided protection against oxidative stress induced by the organophosphate insecticide chlorpyrifos in the brain of goldfish *Carassius auratus* (Linnaeus, 1758) (Gómez-Vega, González-Mantilla, 2023).

In our previous investigation, utilizing the fish species *B. amazonicus* as an experimental model, HU-308 treatment significantly enhanced ventricular contractility, inducing positive inotropic and lusitropic responses attributed to increased cAMP levels and Ca^{2+}-handling protein expression (Gonçalves *et al*., 2024). The preservation of redox homeostasis and prevention of oxidative distress in cardiomyocytes following HU-308 treatment could also contribute to an enhancement in cardiac performance. Given the pivotal role of oxidative distress in driving contractile dysfunction, the activation of antioxidant systems, especially the GSH system, is crucial for protecting cardiomyocytes from oxidative damage (Tan *et al*., 2023). Excessive ROS can damage all major cellular components (DNA, proteins, and lipids), resulting in altered Ca^{2+} regulation and activation of pathways associated with apoptosis, promotion of fibrosis, and inflammatory responses (Aimo *et al*., 2020). For instance, ryanodine receptors, which release Ca^{2+} from the sarcoplasmic reticulum, demonstrate sensitivity to changes in cellular redox status, showing inhibition under conditions characterized by a high GSH/GSSG ratio and activation in the presence of a low GSH/GSSG ratio (Poluektov *et al*., 2019).

Conversely, the non-selective agonist WIN 55,212-2 induced oxidative distress, as represented by elevated levels of LPO and/or PC in the liver and heart. Previous research has also shown that specific CB1 stimulation directly induces intracellular ROS generation and initiates inflammatory responses through the p38-MAPK pathway in various tissues (Han *et al*., 2009; Mukhopadhyay *et al*., 2010; Rajesh *et al*., 2010; Tiyerili *et al*., 2010; Zoppi *et al*., 2011; Guillamat-Prats *et al*., 2019), resulting in an exacerbation of redox imbalance. Additionally, the CB1+CB2 treatment increased the activity of antioxidant enzymes CAT and GPx while inhibiting GR activity in both tissues. In the heart, it also triggered increased activities of SOD and GST, while causing a decrease in their activities in the liver. The increased activity of enzymes such as CAT, GPx, and SOD indicates the generation of ROS, primarily the superoxide anion and hydrogen peroxide. Given their inhibitory effect on oxyradical formation, the SOD-CAT and GPx systems constitute the primary defenses against oxygen toxicity (Ighodaro, Akinloye, 2018). The reduced activity of hepatic SOD could be related to the excessive production of hydrogen peroxide (Perry *et al*., 2010; Gottfredsen *et al*., 2013). The inhibition of hepatic and cardiac GR activity following CB1+CB2 agonist treatment is intricately

linked to the increased cardiac GSSG levels or the reduction of hepatic GSH content and GST activity. GST catalyzes the conjugation of GSH with electrophiles, thereby protecting organisms against genotoxic and carcinogenic compounds (Lushchak, 2012). Under these conditions, GSH depletion and GST inhibition made the hepatocyte more vulnerable to oxidative damage, consequently leading to higher levels of PC.

Corroborating our findings, CB1 blockade attenuated hepatic oxidative/nitrosative stress parameters and improved liver histology in mice with nonalcoholic fatty liver disease (Jorgačević *et al*., 2015). On the other hand, CB1 overexpression induces hepatic lipid accumulation in larval and adult zebrafish and promotes lipotoxicity (Pai *et al*., 2013). Several studies also provide evidence indicating that the stimulation of CB1 receptors can either promote or facilitate oxidative distress, inflammation, and cell death in models of cardiomyopathies (Mukhopadhyay *et al*., 2007, 2010; Rajesh *et al*., 2012), as well as in human coronary artery endothelial cells (Rajesh *et al*., 2010).

Because WIN 55,212-2 functions as a non-selective agonist for CB1 and CB2 receptors, it appears that CB1 receptor activation may have triggered a compensatory mechanism to counterbalance the positive effects of CB2 receptor activation. This phenomenon has been documented in cases of both acute and chronic kidney diseases, where the activation of CB2 induces anti-inflammatory responses and reduces fibrosis, whereas signaling through the CB1 receptor induces oxidative distress, inflammation, and cell apoptosis (Barutta *et al*., 2018; Hokmabadi *et al*., 2023).

This study is the first to investigate how cannabinoid receptor activation affects the balance between oxidative distress and eustress in healthy animals, revealing an interaction between the endocannabinoid system and the antioxidant defense system under physiological conditions (Fig. 5). The modulation through CB2 can effectively counteract disruptions in redox homeostasis, mitigating ROS formation and preventing oxidative damage. On the other hand, CB1 activation led to an imbalance resulting in adverse outcomes and oxidative distress, which can potentially contribute to the progress and/or aggravation of various diseases. Therefore, CB2 receptor emerges as a promising therapeutic target for developing strategies focused on enhancing antioxidant defense, redirecting inflammatory signaling, facilitating hypoxia adaptation, and enhancing the overall health status of fish.

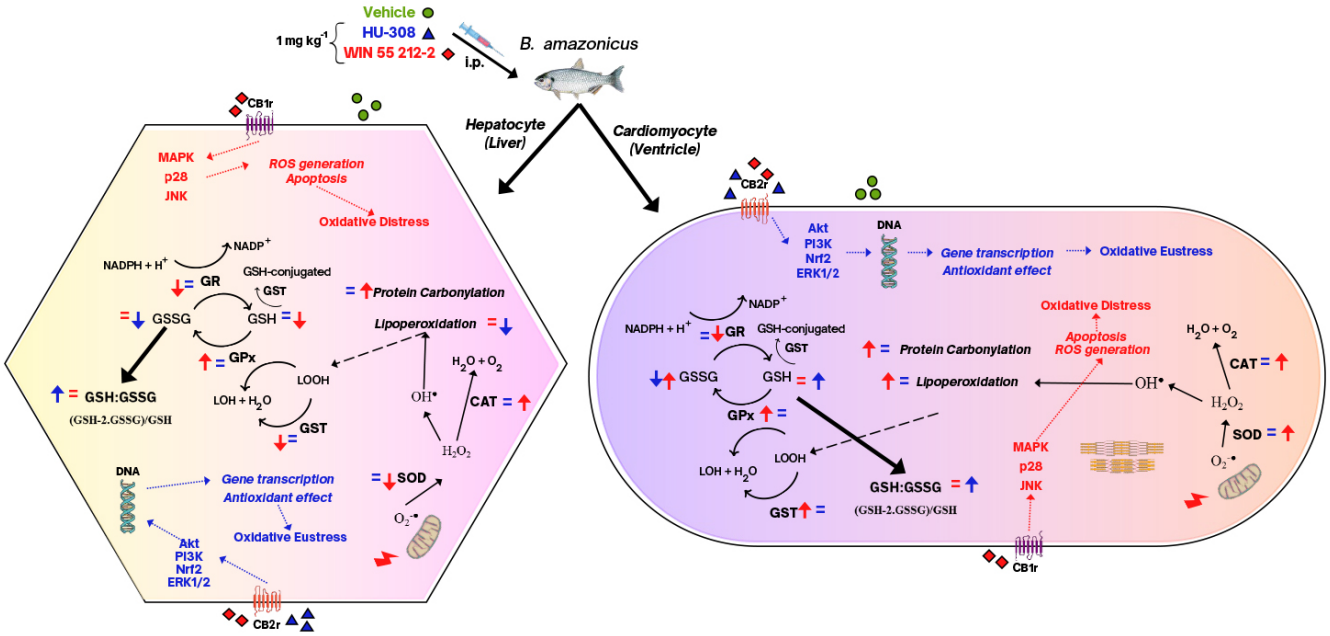

FIGURE 5 | The diagram illustrates the impact of both CB1+CB2 and CB2 activation on oxidative stress in liver (right side) and heart (left side) cells of *Brycon amazonicus*. Arrows indicate increases or decreases in non-enzymatic antioxidant levels, antioxidant enzymatic activities, and oxidative stress biomarkers. Equal symbols (=) denote the constancy of these parameters. Blue symbols represent the CB2-treated group (HU) exhibiting oxidative eustress effects, whereas red symbols denote the CB1+ CB2-treated group (WIN) demonstrating oxidative distress effects. Red and blue dotted arrows indicate the potential signaling pathways of CB1 and CB2 receptors, respectively. CB1: cannabinoid receptor type 1; CB2: cannabinoid receptor type 2; GSH: reduced glutathione; GSSG: oxidized glutathione; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; GR: glutathione reductase; GST: glutathione-S-transferase; LPO: lipoperoxidation level; PC: Protein carbonyl content.

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REFERENCES

- **Aebi H.** Catalase. In: Bergmeyer HU, editor. Methods of enzymatic analysis. NewYork: Academic Press Inc.; 1974. p.673–80. https://doi.org/10.1016/b978-0-12-091302-2.50032-3
- **Aimo A, Castiglione V, Borrelli C, Saccaro LF, Franzini M, Masi S *et al.*** Oxidative stress and inflammation in the evolution of heart failure: from pathophysiology to therapeutic strategies. Eur J Prev Cardiol. 2020; 27(5):494–510. https://doi.org/10.1177/2047487319870344
- **Anderson ME.** Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol. 1985; 113:548–55. https://doi.org/10.1016/S0076-6879(85)13073-9
- **Atalay S, Jarocka-Karpowicz I, Skrzydlewska E.** Antioxidative and anti-inflammatory properties of cannabidiol. Antioxidants. 2020; 9(1):21. https://doi.org/10.3390/antiox9010021

- **Barutta F, Bruno G, Mastrocola R, Bellini S, Gruden G.** The role of cannabinoid signaling in acute and chronic kidney diseases. Kidney Int. 2018; 94(2):252–58. https://doi.org/10.1016/j.kint.2018.01.024
- **Carlberg I, Mannervik B.** Glutathione reductase. Methods Enzymol. 1985; 113:484–90. https://doi.org/10.1016/S0076-6879(85)13062-4
- **Castillo PE, Younts TJ, Chávez AE, Hashimotodani Y.** Endocannabinoid signaling and synaptic function. Neuron. 2012; 76(1):70–81. https://doi.org/10.1016/j.neuron.2012.09.020
- **Cunha Bastos VLF, Salles JB, Valente RH, León IR, Perales J, Dantas RF *et al*.** Cytosolic glutathione peroxidase from liver of pacu (*Piaractus mesopotamicus*), a hypoxia-tolerant fish of the Pantanal. Biochimie. 2007; 89(11):1332–42. https://doi.org/10.1016/j.biochi.2007.04.003
- **Dubois-Deruy E, Peugnet V, Turkieh A, Pinet F.** Oxidative stress in cardiovascular diseases. Antioxidants. 2020; 9(9):864. https://doi.org/10.3390/antiox9090864
- **Flohé L, Ötting F.** Superoxide dismutase assays. Methods Enzymol. 1984; 105:93–104. https://doi.org/10.1016/S0076-6879(84)05013-8
- **Forman HJ, Zhang H, Rinna A.** Glutathione: overview of its protective roles, measurement, and biosynthesis. Mol Aspects Med. 2009; 30(1–2):1–12. https://doi.org/10.1016/j.mam.2008.08.006
- **Fouad AA, Albuali WH, Al-Mulhim AS, Jresat I.** Cardioprotective effect of cannabidiol in rats exposed to doxorubicin toxicity. Environ Toxicol Pharmacol. 2013; 36(2):347–57. https://doi.org/10.1016/j.etap.2013.04.018
- **Gómez-Vega AP, González-Mantilla JF.** Antioxidant effects of the phytocannabinoid cannabidiol (CBD) in the brain of chlorpyrifos-exposed goldfish (*Carassius auratus*). OARJLS. 2023; 6:8–16. https://doi.org/10.53022/oarjls.2023.6.1.0044
- **Gonçalves YC, Campos KCF, Vasconcelos ES, D'Almeida Eça BM, Rantin FT, Kalinin AL *et al*.** Activation of the cannabinoid type 2 (CB2) receptor improves cardiac contractile performance in fish, *Brycon amazonicus*. Comp Biochem Physiol C Toxicol Pharmacol. 2024; 277:109822. https://doi.org/10.1016/j.cbpc.2023.109822
- **González C, Herradón E, Abalo R, Vera G, Pérez-Nievas BG, Leza JC *et al*.** Cannabinoid/agonist WIN 55,212-2 reduces cardiac ischaemia–reperfusion injury in Zucker diabetic fatty rats: role of CB2 receptors and iNOS/eNOS. Diabetes Metab Res Rev. 2011; 27(4):331–40. https://doi.org/10.1002/dmrr.1176
- **González-Candia A, Candia AA, Paz A, Mobarec F, Urbina-Varela R, del Campo A *et al*.** Cardioprotective antioxidant and anti-inflammatory mechanisms induced by intermittent hypobaric hypoxia. Antioxidants. 2022; 11(6):1043. https://doi.org/10.3390/antiox11061043
- **Gottfredsen RH, Larsen UG, Enghild JJ, Petersen SV.** Hydrogen peroxide induces modifications of human extracellular superoxide dismutase that results in enzyme inhibition. Redox Biol. 2013; 1(1):24–31. https://doi.org/10.1016/j.redox.2012.12.004
- **Guillamat-Prats R, Rami M, Herzig S, Steffens S.** Endocannabinoid signalling in atherosclerosis and related metabolic complications. J Thromb Haemost. 2019; 119(4):567–75. https://doi.org/10.1055/s-0039-1678738
- **Habig WH, Pabst MJ, Jakoby WB.** Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974; 249(22):7130–39. https://doi.org/10.1016/S0021-9258(19)42083-8
- **Halliwell B, Gutteridge JM.** Free radicals in biology and medicine, fifth ed. New York: Oxford University Press; 2015.
- **Han KH, Lim S, Ryu J, Lee C-W, Kim Y, Kang J-H *et al*.** CB1 and CB2 cannabinoid receptors differentially regulate the production of reactive oxygen species by macrophages. Cardiovasc Res. 2009; 84(3):378–86. https://doi.org/10.1093/cvr/cvp240

- **Hashiesh HM, Sharma C, Goyal SN, Jha NK, Ojha S.** Pharmacological properties, therapeutic potential and molecular mechanisms of JWH133, a CB2 receptor-selective agonist. Front Pharmacol. 2021; 12:702675. https://doi.org/10.3389/fphar.2021.702675
- **Hokmabadi V, Khalili A, Hashemi SA, Hedayatyanfard K, Parvari S, Changizi-Ashtiyani S *et al*.** Cannabidiol interacts with the FXR/Nrf2 pathway and changes the CB1/CB2 receptors ratio in gentamicin-induced kidney injury in rats. Iran J Basic Med Sci. 2023; 26(3):343–50. https://doi.org/10.22038/IJBMS.2023.67998.14867
- **Hoseinifar SH, Yousefi S, Van Doan H, Ashouri G, Gioacchini G, Maradonna F *et al*.** Oxidative stress and antioxidant defense in fish: the implications of probiotic, prebiotic, and synbiotics. Rev Fish Sci Aquac. 2020; 29(2):198–217. https://doi.org/10.1080/23308249.2020.1795616
- **Ibsen MS, Connor M, Glass M.** Cannabinoid CB1 and CB2 receptor signaling and bias. Cannabis Cannabinoid Res. 2017; 2(1):48–60. https://doi.org/10.1089/can.2016.0037
- **Ighodaro OM, Akinloye OA.** First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. Alex J Med. 2018; 54(4):287–93. https://doi.org/10.1016/j.ajme.2017.09.001
- **Jiang Z-Y, Hunt JV, Wolff SP.** Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem. 1992; 202(1):384–89. https://doi.org/10.1016/0003-2697(92)90122-N
- **Jorgačević B, Mladenović D, Ninković M, Vesković M, Dragutinović V, Vatazević A *et al*.** Rimonabant improves oxidative/nitrosative stress in mice with nonalcoholic fatty liver disease. Oxid Med Cell Longev. 2015; 842108. https://doi.org/10.1155/2015/842108
- **Jorgačević B, Vučević D, Samardžić J, Mladenović D, Vesković M, Vukićević D *et al*.** The effect of CB1 antagonism on hepatic oxidative/nitrosative stress and inflammation in nonalcoholic fatty liver disease. Curr Med Chem. 2021; 28(1):169–80. https://doi.org/10.2174/0929867327666200303122734
- **Kang T-C.** Nuclear factor-erythroid 2-related factor 2 (Nrf2) and mitochondrial dynamics/mitophagy in neurological diseases. Antioxidants. 2020; 9(7):617. https://doi.org/10.3390/antiox9070617
- **Kim Y, Gautam S, Aseer KR, Kim J, Chandrasekaran P, Mazucanti CH *et al*.** Hepatocyte cannabinoid 1 receptor nullification alleviates toxin-induced liver damage via NF-κB signaling. Cell Death Dis. 2020; 11:1044. https://doi.org/10.1038/s41419-020-03261-8
- **Kruger NJ.** The Bradford method for protein quantitation. Methods Mol Biol. 1994; 32:9–15. https://doi.org/10.1385/0-89603-268-X:9
- **Lépicier P, Bouchard J-F, Lagneux C, Lamontagne D.** Endocannabinoids protect the rat isolated heart against ischaemia. Br J Pharmacol. 2003; 139(4):805–15. https://doi.org/10.1038/sj.bjp.0705313
- **Li Q, Guo H-C, Maslov LN, Qiao X-W, Zhou J-J, Zhang Y.** Mitochondrial permeability transition pore plays a role in the cardioprotection of cb2 receptor against ischemia-reperfusion injury. Can J Physiol Pharmacol. 2014; 92(3):205–14. https://doi.org/10.1139/cjpp-2013-0293
- **Li X, Han D, Tian Z, Gao B, Fan M, Li C *et al*.** Activation of cannabinoid receptor type II by AM1241 ameliorates myocardial fibrosis via Nrf2-mediated inhibition of TGF-β1/smad3 pathway in myocardial infarction mice. Cell Physiol Biochem. 2016; 39(4):1521–36. https://doi.org/10.1159/000447855
- **Liu LY, Alexa K, Cortes M, Schatzman-Bone S, Kim AJ, Mukhopadhyay B *et al*.** Cannabinoid receptor signaling regulates liver development and metabolism. Development. 2016; 143:609–22. https://doi.org/10.1242/dev.121731

- **Louvet A, Teixeira-Clerc F, Chobert M-N, Deveaux V, Pavoine C, Zimmer A *et al.*** Cannabinoid CB2 receptors protect against alcoholic liver disease by regulating Kupffer cell polarization in mice. Hepatology. 2011; 54(4):1217–26. https://doi.org/10.1002/hep.24524
- **Lushchak VI.** Glutathione homeostasis and functions: potential targets for medical interventions. J Amino Acids. 2012; 736837. https://doi.org/10.1155/2012/736837
- **Marmelo I, Dias M, Grade A, Pousão-Ferreira P, Diniz MS, Marques A *et al*.** Immunomodulatory and antioxidant effects of functional aquafeeds biofortified with whole *Laminaria digitata* in juvenile gilthead seabream (*Sparus aurata*). Front Mar Sci. 2024; 11:1325244. https://doi.org/10.3389/fmars.2024.1325244
- **Martínez-Pinilla E, Varani K, Reyes-Resina I, Angelats E, Vincenzi F, Ferreiro-Vera C, *et al.*** Binding and signaling studies disclose a potential allosteric site for cannabidiol in cannabinoid CB_2 receptors. Front Pharmacol. 2017; 8:744. https://doi.org/10.3389/fphar.2017.00744
- **Montecucco F, Di Marzo V.** At the heart of the matter: the endocannabinoid system in cardiovascular function and dysfunction. Trends Pharmacol Sci. 2012; 33(6):331–40. https://doi.org/10.1016/j.tips.2012.03.002
- **Montecucco F, Lenglet S, Braunersreuther V, Burger F, Pelli G, Bertolotto M *et al*.** CB2 Cannabinoid receptor activation is cardioprotective in a mouse model of ischemia/reperfusion. J Mol Cell Cardiol. 2009; 46(5):612–20. https://doi.org/10.1016/j.yjmcc.2008.12.014
- **More SA, Deore RS, Pawar HD, Sharma C, Nakhate KT, Rathod SS *et al.*** CB2 cannabinoid receptor as a potential target in myocardial infarction: exploration of molecular pathogenesis and therapeutic strategies. Int J Mol Sci. 2024; 25(3):1683. https://doi.org/10.3390/ijms25031683
- **Mukhopadhyay P, Bátkai S, Rajesh M, Czifra N, Harvey-White J, Haskó G *et al.*** Pharmacological inhibition of CB1 cannabinoid receptor protects against doxorubicin-induced cardiotoxicity. J Am Coll Cardiol. 2007; 50(6):528–36. https://doi.org/10.1016/j.jacc.2007.03.057
- **Mukhopadhyay P, Rajesh M, Bátkai S, Patel V, Kashiwaya Y, Liaudet L *et al.*** CB1 cannabinoid receptors promote oxidative stress and cell death in murine models of doxorubicin-induced cardiomyopathy and in human cardiomyocytes. Cardiovasc Res. 2010; 85:773–84. https://doi.org/10.1093/cvr/cvp369
- **Nakamura W, Hosoda S, Hayashi K.** Purification and properties of rat liver glutathione peroxidase. Biochim Biophys Acta. 1974; 358(2):251–61. https://doi.org/10.1016/0005-2744(74)90455-0
- **Niki E.** Oxidative stress and antioxidants: distress or eustress? Arch Biochem Biophys. 2016; 595:19–24. https://doi.org/10.1016/j.abb.2015.11.017
- **Okegbe C, Sakhtah H, Sekedat MD, Price-Whelan A, Dietrich LEP.** Redox eustress: roles for redox-active metabolites in bacterial signaling and behavior. Antioxid Redox Signal. 2012; 16(7):658–67. https://doi.org/10.1089/ars.2011.4249
- **Pai W-Y, Hsu C-C, Lai C-Y, Chang T-Z, Tsai Y-L, Her GM.** Cannabinoid receptor 1 promotes hepatic lipid accumulation and lipotoxicity through the induction of SREBP-1c expression in zebrafish. Transgenic Res. 2013; 22:823–38. https://doi.org/10.1007/s11248-012-9685-0
- **Paloczi J, Varga ZV, Hasko G, Pacher P**. Neuroprotection in oxidative stress-related neurodegenerative diseases: role of endocannabinoid system modulation. Antioxid Redox Signal. 2018; 29(1):75–108. https://doi.org/10.1089/ars.2017.7144
- **Pereira R, Costa M, Velasco C, Cunha LM, Lima RC, Baião LF *et al.*** Comparative analysis between synthetic vitamin E and natural antioxidant sources from tomato, carrot and coriander in diets for market-sized *Dicentrarchus labrax*. Antioxidants. 2022; 11(4):636. https://doi.org/10.3390/antiox11040636
- **Perry JJP, Shin DS, Getzoff ED, Tainer JA.** The structural biochemistry of the superoxide dismutases. Biochim Biophys Acta. 2010; 1804(2):245–62. https://doi.org/10.1016/j.bbapap.2009.11.004

- **Petcu CD, Mihai OD, Tăpăloagă D, Gheorghe-Irimia R-A, Pogurschi EN *et al.*** Effects of plant-based antioxidants in animal diets and meat products: a review. Foods. 2023; 12(6):1334. https://doi.org/10.3390/foods12061334
- **Pisanti S, Picardi P, D'Alessandro A, Laezza C, Bifulco M.** The endocannabinoid signaling system in cancer. Trends Pharmacol Sci. 2013; 34(5):273–82. https://doi.org/10.1016/j.tips.2013.03.003
- **Poluektov YM, Petrushanko IY, Undrovinas NA, Lakunina VA, Khapchaev AY, Kapelko VI *et al.*** Glutathione-related substances maintain cardiomyocyte contractile function in hypoxic conditions. Sci Rep. 2019; 9:4872. https://doi.org/10.1038/s41598-019-41266-2
- **Rajesh M, Bátkai S, Kechrid M, Mukhopadhyay P, Lee W-S, Horváth B *et al.*** Cannabinoid 1 receptor promotes cardiac dysfunction, oxidative stress, inflammation, and fibrosis in diabetic cardiomyopathy. Diabetes. 2012; 61(3):716–27. https://doi.org/10.2337/db11-0477
- **Rajesh M, Mukhopadhyay P, Bátkai S, Haskó G, Liaudet L, Huffman JW *et al.*** CB2-receptor stimulation attenuates TNF-alpha-induced human endothelial cell activation, transendothelial migration of monocytes, and monocyte-endothelial adhesion. Am J Physiol Heart Circ Physiol. 2007; 293(4):2210–18. https://doi.org/10.1152/ajpheart.00688.2007
- **Rajesh M, Mukhopadhyay P, Haskó G, Liaudet L, Mackie K, Pacher P.** Cannabinoid-1 receptor activation induces reactive oxygen species-dependent and -independent mitogen-activated protein kinase activation and cell death in human coronary artery endothelial cells. Br J Pharmacol. 2010; 160(3):688–700. https://doi.org/10.1111/j.1476-5381.2010.00712.x
- **Reznick AZ, Packer L.** Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol. 1994; 233:357–63. https://doi.org/10.1016/S0076-6879(94)33041-7
- **Roach T, Stoggl W, Baur T, Kranner I.** Distress and eustress of reactive electrophiles and relevance to light stress acclimation via stimulation of thiol/disulphide-based redox defences. Free Radic Biol Med. 2018; 122:65–73. https://doi.org/10.1016/j.freeradbiomed.2018.03.030
- **Sack MN, Fyhrquist FY, Saijonmaa OJ, Fuster V, Kovacic JC.** Basic biology of oxidative stress and the cardiovascular system: Part 1 of a 3-part series. J Am Coll Cardiol. 2017; 70(2):196–211. https://doi.org/10.1016/j.jacc.2017.05.034
- **Saroz Y, Kho DT, Glass M, Graham ES, Grimsey NL.** Cannabinoid receptor 2 (CB_2) signals via G-alpha-s and induces IL-6 and IL-10 cytokine secretion in human primary leukocytes. ACS Pharmacol Transl Sci. 2019; 2(6):414–28. https://doi.org/10.1021/acsptsci.9b00049
- **Shang Y, Tang Y.** The central cannabinoid receptor type-2 (CB2) and chronic pain. Int J Neurosci. 2017; 127(9):812–23. https://doi.org/10.1080/00207454.2016.1257992
- **Sharma P, Nandave M, Nandave D, Yadav S, Vargas-De-La-Cruz C, Singh S *et al.*** Reactive oxygen species (ROS)-mediated oxidative stress in chronic liver diseases and its mitigation by medicinal plants. Am J Transl Res. 2023; 15(11):6321–41.
- **Sies H.** Oxidative stress: eustress and distress in redox homeostasis. In: Fink G, editor. Stress: physiology, biochemistry, and pathology. London: Academic Press; 2019. p.153–63. https://doi.org/10.1016/B978-0-12-813146-6.00013-8
- **Sies H.** Oxidative eustress and oxidative distress: introductory remarks. In: Sies H, editor. Oxidative stress. London: Academic Press; 2020. p.3–12. https://doi.org/10.1016/B978-0-12-818606-0.00001-8
- **Sies H.** Oxidative eustress: on constant alert for redox homeostasis. Redox Biol. 2021; 41:101867. https://doi.org/10.1016/j.redox.2021.101867
- **Sies H, Belousov VV, Chandel NS, Davies MJ, Jones DP, Mann GE *et al.*** Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. Nat Rev Mol Cell Biol. 2022; 23:499–515. https://doi.org/10.1038/s41580-022-00456-z

- **Sies H, Jones DP.** Oxidative stress. In: Fink G, editor. Encyclopedia of stress, 2nd edn. Amsterdam: Elsevier; 2007. p.45–48.
- **Sinenko SA, Starkova TY, Kuzmin AA, Tomilin AN.** Physiological signaling functions of reactive oxygen species in stem cells: from flies to man. Front Cell Dev Biol. 2021; 9:714370. https://doi.org/10.3389/fcell.2021.714370
- **Soethoudt M, Grether U, Fingerle J, Grim TW, Fezza F, Petrocellis L *et al.*** Cannabinoid CB2 receptor ligand profiling reveals biased signaling and off target activity. Nat Commun. 2017; 8:13958. https://doi.org/10.1038/ncomms13958
- **Solbrig MV, Fan Y, Hazelton P.** Prospects for cannabinoid therapies in viral encephalitis. Brain Res. 2013; 1537:273–82. https://doi.org/10.1016/j.brainres.2013.08.032
- **Song C, Sun C, Liu B, Xu P.** Oxidative stress in aquatic organisms. Antioxidants. 2023; 12(6):1223. https://doi.org/10.3390/antiox12061223
- **Steffens S, Pacher P.** Targeting cannabinoid receptor CB(2) in cardiovascular disorders: promises and controversies. Br J Pharmacol. 2012; 167(2):313–23. https://doi.org/10.1111/j.1476-5381.2012.02042.x
- **Tabrizi AM, Baraldi PG, Borea PA, Varani K.** Medicinal chemistry, pharmacology, and potential therapeutic benefits of cannabinoid CB2 receptor agonists. Chem Rev. 2016; 116(2):519–60. https://doi.org/10.1021/acs.chemrev.5b00411
- **Tan M, Yin Y, Ma X, Zhang J, Pan W, Tan M *et al.*** Glutathione system enhancement for cardiac protection: pharmacological options against oxidative stress and ferroptosis. Cell Death Dis. 2023; 14:131. https://doi.org/10.1038/s41419-023-05645-y
- **Teixeira-Clerc F, Belot M-P, Manin S, Deveaux V, Cadoudal T, Chobert M-N *et al.*** Beneficial paracrine effects of cannabinoid receptor 2 on liver injury and regeneration. Hepatology. 2010; 52(3):1046–59. https://doi.org/10.1002/hep.23779
- **Tian L, Li W, Yang L, Chang N, Fan X, Ji X *et al.*** Cannabinoid receptor 1 participates in liver inflammation by promoting M1 macrophage polarization via RhoA/NF-κB p65 and ERK1/2 pathways, respectively, in mouse liver fibrogenesis. Front Immunol. 2017; 8:1214. https://doi.org/10.3389/fimmu.2017.01214
- **Tiyerili V, Zimmer S, Jung S, Wassmann K, Naehle CP, Lütjohann D *et al.*** CB1 receptor inhibition leads to decreased vascular AT1 receptor expression, inhibition of oxidative stress and improved endothelial function. Basic Res Cardiol. 2010; 105:465–77. https://doi.org/10.1007/s00395-010-0090-7
- **Voicu V, Brehar F-M, Toader C, Covache-Busuioc R-A, Corlatescu AD, Bordeianu A *et al.*** Cannabinoids in medicine: a multifaceted exploration of types, therapeutic applications, and emerging opportunities in neurodegenerative diseases and cancer. Biomolecules. 2023; 13(9):1388. https://doi.org/10.3390/biom13091388
- **Wang P-F, Jiang L-S, Bu J, Huang X-J, Song W, Du Y-P *et al.*** Cannabinoid-2 receptor activation protects against infarct and ischemia-reperfusion heart injury. J Cardiovasc Pharmacol. 2012; 59(4):301–07. https://doi.org/10.1097/fjc.0b013e3182418997
- **Wang Y, Ma S, Wang Q, Hu W, Wang D, Li XJ *et al.*** Effects of cannabinoid receptor type 2 on endogenous myocardial regeneration by activating cardiac progenitor cells in mouse infarcted heart. Sci China Life Sci. 2014; 57:201–08. https://doi.org/10.1007/s11427-013-4604-z
- **Wang Z, Wang X, Li J, Gong T, Li Q, Bu X *et al.*** Effects of cannabidiol on growth performance, appetite, antioxidant capacity and liver inflammatory gene expression of juvenile large yellow croaker (*Larmichthys crocea*) fed diets with high soybean oil level. Aquaculture. 2023; 574:739658. https://doi.org/10.1016/j.aquaculture.2023.739658

- **Wu HM, Kim TH, Kim A, Koo JH, Joo MS, Kim SG.** Liver X receptor α-induced cannabinoid receptor 2 inhibits ubiquitin-specific peptidase 4 through miR-27b, protecting hepatocytes from TGF-β. Hepatol Commun. 2019; 3(10):1373–87. https://doi.org/10.1002/hep4.1415
- **Xin Q, Xu F, Taylor DH, Zhao JF, Wu J.** The impact of cannabinoid type 2 receptors (CB2Rs) in neuroprotection against neurological disorders. Acta Pharmacol Sin. 2020; 41:1507–18. https://doi.org/10.1038/s41401-020-00530-2
- **Yu W, Jin G, Zhang J, Wei W.** Selective activation of cannabinoid receptor 2 attenuates myocardial infarction via suppressing NLRP3 inflammasome. Inflammation. 2019; 42:904–14. https://doi.org/10.1007/s10753-018-0945-x
- **Zoppi S, Nievas BGP, Madrigal JLM, Manzanares J, Leza JC, García-Bueno B.** Regulatory role of cannabinoid receptor 1 in stress-induced excitotoxicity neuroinflammation. Neuropsychopharmacology. 2011; 36:805–18. https://doi.org/10.1038/npp.2010.214

AUTHORS' CONTRIBUTION

Suzana Luisa Alves Fernandes: Data curation, Formal analysis, Methodology, Visualization, Writing-original draft.

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Ana Lúcia Kalinin: Funding acquisition, Resources, Validation, Visualization, Writing-original draft, Writing-review and editing.

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ETHICAL STATEMENT

Experiments were approved by the Ethical Committee for Animal Use in Experiments of the Universidade Federal de São Carlos (CEUA number 4997170718).

COMPETING INTERESTS

The author declares no competing interests.

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