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Impact of genistein on oxidative stress biomarkers in erythrocytes: A study in IPD patients

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The pathophysiology of Idiopathic Parkinson's disease (IPD), a progressive neurodegenerative condition, is extensively affected by oxidative stress. IPD is a multifactorial disease in which oxidative stress damage the systemic cellular system, along with the central nervous system. However, the peripheral biomarkers imbalance and therapeutic responses in IPD remain restricted. In this study, erythrocytes from healthy control and IPD patients were used to evaluate the impact of Genistein on oxidative stress biomarkers. The assessment was conducted on medically appropriate blood samples collected from 95 subjects out of which (n=45) are healthy control and (n=50) are IPD patients. The impact of genistein were assessed against oxidative stress induced by 10mM H_2O_2, evaluated by quantifying the levels of MDA, GSH, SOD and Catalase after co-incubation of erythrocytes with genistein (10^{-7}M to 10^{-5}M) and H_2O_2. The outcomes showed elevated MDA levels and SOD activity (P<0.001) and decreased catalase activity and GSH levels (P<0.001) after incubation with H_2O_2. Genistein, when administered *in vitro*, effectively mitigated oxidative stress-induced damage in red blood cells from all individuals. These findings, validate the present study by providing genistein's systemic antioxidant effectiveness and reinforcing the significance of erythrocyte-based oxidative indicators in IPD. This study helps to fulfil the gap of growing demand for reliable, non-invasive biomarkers to evaluate the oxidative stress in IPD.

Keywords: Catalase, Glutathione, Hemocompatibility, Malondialdehyde, Reactive oxygen species

Parkinson's disease (PD) is a gradual neuro-degenerative state primarily described by the loss and deterioration of dopamine-producing neurons in the area of the substantia nigra and the subsequent depletion of striatal dopamine. PD is known as the most common neurodegenerative disorder after Alzheimer's disease, with an expected global dominance exceeding six million individuals and approximations suggesting a doubling of this figure within the coming decades[1]. PD is perceived frequently in older adults, and its occurrence increases abruptly after the age of 60, although cases with an earlier onset are being reported more frequently. Among the different clinical types, Idiopathic Parkinson's disease (IPD) remains the most common, accounting for roughly 80-85% of all diagnosed cases[2]. Unlike genetic forms of the disease, IPD does not stem from a single known cause; instead, it is thought to occur from a combination of age-related changes, environmental exposures, mitochondrial dysfunction and inherited susceptibilities that together drive the gradual loss of dopamine-producing neurons. The word "idiopathic" highlights this complicated multifaceted origin, where the disease cannot be entirely explained by a single genetic mutation or environmental trigger[3].

From a pathogenic perspective, IPD is linked with a significant reduction in dopamine levels in the striatum due to a consistent and selective degeneration of dopaminergic neurons inside the substantia nigra pars compacta. The characteristic motor symptoms of the disease, including resting tremors, bradykinesia (slow movement), muscular rigidity and postural instability are caused by this dopamine deficiency. Patients frequently have a wide range of non-motor sequelae in addition to these well-known motor related symptoms, such as autonomic function disruptions, cognitive decline and mood-related problems[4]. On a microscopic feature of the disease, Lewy bodies those are found as the intracytoplasmic aggregates made mostly of abnormal folding of synuclein within the afflicted neurons. Despite

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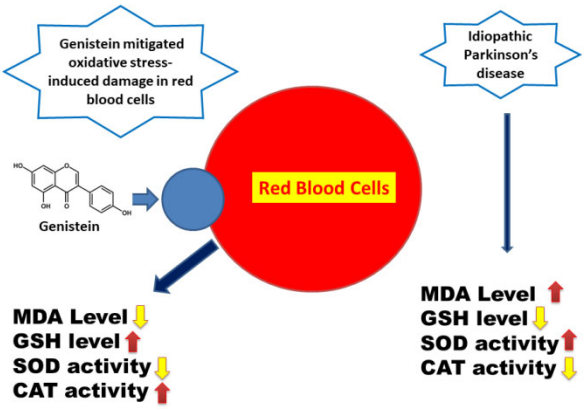

Graphical abstract

significant advancements in the development of promising treatments like levodopa, and dopamine receptor agonists, none of the new treatments have been indicated to inhibit or delay the underlying neurodegenerative process, highlighting the ongoing need for interventions that alter the progression of the disease[5].

A growing number of research highlights oxidative stress as a significant component behind IPD pathogenesis. Because dopamine metabolism naturally produces free radical species, including as hydrogen peroxide and superoxide anions, dopaminergic neurons are particularly susceptible to oxidative injury[3]. Over time, this persistent oxidative environment stimulates lipid peroxidation, protein modification and DNA damage, events that exacerbate mitochondrial impairment and eventually lead to neuronal apoptosis.

In recent years, peripheral oxidative stress markers have gained attention because they provide a marginally invasive way to track redox changes that may parallel events in the central nervous system. Among these peripheral cells, erythrocytes have been demonstrated to be a useful model. Although they are anucleate and not part of the nervous system, erythrocytes are constantly subjected to elevated oxygen levels, and the high concentration of polyunsaturated fatty acids in their membranes renders them especially prone to oxidative damage[8]. To preserve their structure and function, the antioxidant defense mechanism in erythrocytes is maintained by compromising enzymes like superoxide dismutase, glutathione and catalase. Earlier studies have verified that oxidative stress markers in erythrocytes mirror the body's overall redox balance and correlate with the progression of neurodegenerative disease[9]. Because erythrocytes can be easily obtained through simple blood draws, they represent a cost-effective and practical means of monitoring disease progression, assessing treatment processes and evaluating the potential of antioxidant-based therapies. This approach is particularly advantageous in IPD, where direct examination of brain tissue is not possible in living patients[10].

In recent years, nutraceuticals and naturally occurring plant-derived polyphenols have extended considerable attention as complementary approaches for treating neurodegenerative disorders. These bioactive substances, which are found in fruits, vegetables and legumes, have inherent anti-inflammatory, antioxidant and neuroprotective qualities that may support in thwarting a number of IPD-related degenerative processes[11]. Genistein, an isoflavone mostly present in foods made from soy, has become one of the most promising of the different chemicals[12]. Research has shown that genistein reduces pro-apoptotic signaling cascades, elevates endogenous antioxidant defense systems and affects mitochondrial activity. Mechanistically, genistein stimulates the production of antioxidant enzymes by activating transcription factors like Nrf2 and associated regulatory pathways, and it directly scavenges ROS through its hydroxyl groups. These findings are maintained by preclinical research[13]. Administration with genistein has been shown in cellular and animal models to help preserve mitochondrial integrity, reduces oxidative DNA damage and constraint the production of inflammatory cytokines. Genistein therapy has been connected to improved motor function, decreased lipid peroxidation, and elevated cell survival in dopaminergic cell cultures and rodent models of IPD[14]. The influence of genistein on peripheral oxidative stress markers, such as those measurable in erythrocytes, has not yet been thoroughly examined in patients with IPD, and despite this encouraging preclinical outcome, its benefits in human clinical settings is still restricted.

The present research is designed to systematically assess erythrocyte oxidative stress parameters, including MDA levels, GSH concentration, and enzymatic activities of SOD and Catalase in individuals diagnosed with IPD. These markers will be evaluated before and after genistein administration to determine whether changes in peripheral oxidative balance reflect potential therapeutic benefits[15]. By integrating molecular redox biology with clinical investigation, this study aims to expand our

understanding of dietary isoflavones as adjuncts in PD management and to promote the use of erythrocyte biomarkers as accessible, sensitive indicators of oxidative stress in neurodegenerative conditions[16].

The outcomes of this investigation may pave a way to novel, non-invasive ways to track IPD progression. Additionally, they focus the establishment of more determined antioxidant-based treatments, which eventually provide to the enhancement of IPD patient's general health. As oxidative stress is increasingly recognized as a major underwriting factor to neurodegenerative illnesses, methods like the one examined in this study are a significant step toward more comprehensive and enduring therapeutic approaches.

Materials and Methods

Study design and participants

This case-control investigation includes participants from the Movement Disorder Clinic at Pt. B.D.Sharma Post Graduate Institute of Medical Sciences, Rohtak, between January 2023 to October 2024. The study was approved by the Institutional Human Ethics Committee (Ref. No. CUH/IHEC/ 2023/07), with all procedures adhering to the declaration of Helsinki principles. 50 patients with clinically diagnosed IPD and 45 age-matched healthy controls were enrolled for the study following written informed consent. IPD analysis was recognized according to UK Parkinson's Disease Society Brain Bank criteria by qualified neurologists. Inclusion criteria comprised confirmed IPD diagnosis, age 50-75 years, stable antiparkinsonian therapy for a minimum of three months, and absence of any other neurological conditions. Exclusion criteria included secondary Parkinsonism, cerebrovascular disease history, concurrent malignancy, active infections, substance abuse, pregnancy or lactation, and antioxidant supplementation within three months. Control subjects were from healthy community volunteers, matched for age and gender, with no neurological disorders.

Blood collection and processing

3 mL of venous blood from healthy individuals was collected in EDTA vials. Samples underwent immediate processing to minimize *ex vivo* oxidation. The plasma was separated from the cellular component by centrifugation at 1800 × g for 10 min at 4°C. Packed red blood cells (PRBCs) were rinsed three times using ice-cold phosphate buffer saline (PBS; pH 7.4), isotonic with blood to eradicate residual plasma proteins and platelets. Erythrocytes were further suspended in Krebs-Ringer phosphate buffer supplemented with 5mM glucose. This suspension was divided into aliquots for baseline measurements and genistein treatment protocols.

Hemocompatibility assay

Hemocompatibility assay was conducted as established protocol by Sadhasivam and Durairaj, 2014[17]. For performing this assay erythrocytes were incubated with varying concentrations (10^{-3}M to 10^{-7}M) of genistein inside isotonic solutions of 0.9% NaCl. The erythrocytes were centrifuged at 1000 x g for 10 min at room temperature. Buffy coat and plasma were discarded and the pellet was washed with PBS three times. Then 100 µL of Milli Q water (positive control) was mixed with 100µl of erythrocytes, hemolysis of erythrocytes was induced mechanically. The test tubes were then incubated for 1 hour at 37°C. After incubation, the samples were centrifuged at 1000 × g for 5 min. The supernatant was collected and UV-spectrometer was used to quantify hemoglobin absorbance at 541 nm.

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

DPPH assay was performed as described by Kim, H.H. *et al* 2021[18]. Different concentrations of genistein were serially diluted at 2 µg/mL using methanol as solvent. Then 1 mL of each concentration was added to different test tubes and marked accordingly. 1 mL of 0.002% methanolic DPPH solution was mixed to each test tube, and 1 mL of methanol was added to make up the final volume upto 3 mL. The test tubes were covered properly and kept in dark place for 30 min. Then the optical densities were measured using the spectrophotometric measurement at a wavelength of 517 nm.

Induction of oxidative stress and *in vitro* treatment with genistein

The *in vitro* impact of genistein on erythrocyte intracellular GSH content, MDA levels and activity of endogenous enzymes such as Catalase and SOD was measured using a standard procedure. The collected blood from two different groups (healthy and diseased) was rinsed 2 to 3 times with PBS. Oxidative stress was induced in the experiment by treating washed erythrocytes with hydrogen peroxide (H_2O_2) during incubation at a concentration of 10 mM at 37°C for 15 min. The selected dose and exposure time for H_2O_2 were adopted from a previously published

study that employed the same conditions to generate oxidative stress in erythrocytes. The influence of genistein at different concentrations, 10^{-7}M to 10^{-5}M (final concentration), was evaluated by co-incubating erythrocytes with genistein and H_2O_2 for 60 min at 37°C. Control samples received same treatment. All experiments were performed in triplicate with appropriate blanks.

Estimation of malondialdehyde levels

The levels of malondialdehyde in erythrocytes were evaluated using the method by Esterbauer and Cheeseman[19]. 200 µL of packed red blood cells (PRBCs) was mixed in 3 mL of PBS buffer. 1 mL of the suspension was further dissolved in 1 mL of 10% trichloroacetic acid (TCA). The mixture was then centrifuged at 5000 g for 5 min. 1 mL of 0.067% solution of thiobarbituric acid (TBA) was added to the supernatant and incubated at 90°C for approximately 20 min in the water bath. This solution was then allowed to rest and left undisturbed for cooling. Absorbance was measured at 532 nm. A standard calibration curve was utilized to determine the total MDA levels in the erythrocytes. The units used for MDA level expression were in nmol/ mL of PRBCs, as described by Rizvi *et al*[20].

Estimation of GSH levels

Reduced glutathione (GSH) levels in erythrocytes were quantified using the conventional method described by Rahman *et. al* 2006[21]. This assay is dependent on the capacity of the sulphydryl (-SH) group in GSH to reduce 5,5-dithiobis-2-nitrobenzoic acid (DTNB), commonly known as Ellman's reagent, resulting in the formation of a yellow-coloured anionic product, 2-nitro-5-thiobenzoic acid (TNB). Packed RBCs were deproteinized with a 5% *w/v* solution of metaphosphoric acid, followed by thorough homogenization. The mixture was then centrifuged at 3000 × g at 4°C for 10 min to preserve GSH stability. In a 96-well microplate, 20 µL of PBS was added to all wells. Subsequently, 20 µl of the deproteinized sample supernatants were added into each well. A freshly prepared 0.067% DTNB solution in 1% sodium citrate (120 µL) was put into the wells filled with samples and PBS. Absorbance was measured immediately at 412 nm using a microplate reader, for 3 min at an interval of 30 seconds. Then the rate of TNB formation was calculated, and GSH concentrations were assessed from a standard calibration curve. Results were expressed as nanomoles of GSH per millilitre of PRBCs (nmol/mL/PRBCs).

Estimation of catalase activity

Catalase activity was evaluated using the method described by Aebi 1984[22]. This assay is based on the assumption of the decomposition of hydrogen peroxide. For this, PRBCs were washed and haemolysed by diluting with four volumes of distilled water to obtain a 5% haematocrit. Then, hemolysate was prepared using 2 mL of phosphate buffer (50 mM and pH 7.0) with a 1:100 dilution and a volume 20 µL. The reaction was then initiated by mixing the hemolysate with hydrogen peroxide. The total reaction volume was 3 mL, containing 2 mL of the diluted hemolysate and 1mL of 30 mM H_2O_2 prepared in phosphate buffer. A blank was prepared using 2 mL of phosphate buffer without hemolysate and 1 mL of 30 mM H_2O_2 prepared in phosphate buffer. The decrease in absorbance at 240 nm, corresponding to H_2O_2 decomposition, was recorded every 30 sec for 3 min at 20°C (room temperature) using a UV-visible spectrophotometer. Catalase activity was denoted as the rate of change in absorbance per min per mg of protein.

Estimation of superoxide dismutase activity

The SOD Activity was estimated by the enzyme's capacity to prevent the photoreduction of nitroblue tetrazolium (NBT) by superoxide radicals, which results in the formation of formazan, as described by Rukmini, D'Souza, *et al* 2004[23]. In this assay, washed RBCs were lysed and treated with chloroform at 4°C for 15 min. The resulting supernatant was treated for enzymatic analysis. The final 3ml mixture contained the following components: phosphate buffer (pH 7.8), 75 mM EDTA, 13 mM methionine, 50 µM NBT, and 1.3 µM riboflavin. The reaction mixture was thoroughly mixed and immediately exposed to fluorescent light for 10 min which led to superoxide generation. The absorbance was then calculated at 560 nm using a UV-visible spectrophotometer. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT photoreduction under the assay conditions.

Statistical analysis

Data were evaluated using GraphPad Prism software (version 8.0). Unpaired student's t-tests were used for healthy control and patient groups, while paired t-tests were applied to assess pre- and post-

treatment differences. Data are presented as mean ± standard deviation, with p-values less than 0.05 considered statistically significant.

Results

The genistein hemocompatibility was assessed across a range of concentration from 10^{-3} M to 10^{-7} M using human erythrocytes. PBS is used as the negative control demonstrated negligible hemolysis (0%) and milli Q water is considered as positive control exhibited complete hemolysis (100%). Genistein at 10^{-3}M displayed mild hemolysis whereas doses from 10^{-4} M to 10^{-7} M are hemocompatible (Fig. 1). Therefore, according to the outcome of this assay, erythrocytes can safely be exposed to genistein at lower concentrations.

The antioxidant capacity of genistein was evaluated utilizing the DPPH scavenging assay at various concentrations ranging from 10^{-3} M to 10^{-7} M (Fig. 2).

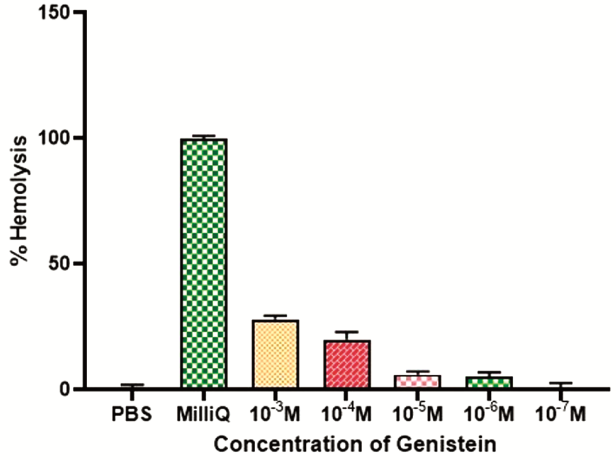

Fig. 1 — % Hemolysis activity of genistein at different concentrations. Values are expressed as mean ± SD

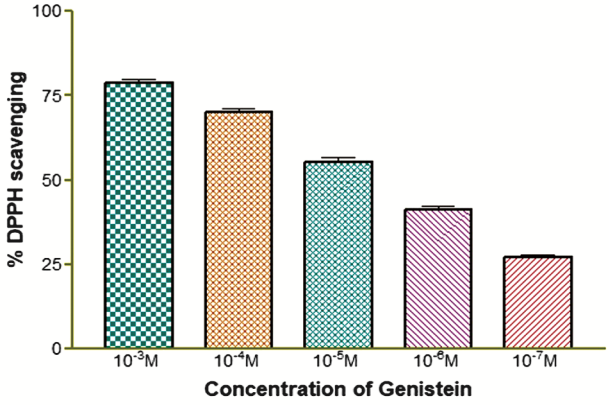

Fig. 2 — % DPPH activity of genistein at different concentrations. Values are expressed as mean ± SD

A dose-dependent response was recognized, with the highest activity 10^{-3}M, showing approximately 78% scavenging. The activity significantly decreased with lower concentrations. The lowest activity was observed at nearly 28% at 10^{-7}M. The findings support the free radical scavenging capabilities of genistein, which diminish with concentration.

Under conditions of oxidative stress, the erythrocyte membrane becomes highly vulnerable to lipid peroxidation, during which the double bonds of polyunsaturated fatty acids are broken, resulting in the production of MDA[24]. The outcomes of our study indicated the effect of genistein on H_2O_2-induced lipid peroxidation in erythrocytes from healthy controls and IPD patients. MDA levels were substantially elevated in the erythrocytes treated with H_2O_2 alone, in comparison to untreated controls in both healthy as well as IPD groups (Fig. 3). H_2O_2 exposure further elevated MDA levels while genistein treatment reduced MDA in a concentration-dependent manner, though levels remained slightly elevated compared to healthy controls. These effects suggest that genistein mitigates oxidative stress-induced lipid peroxidation in both healthy and diseased erythrocytes.

The glutathione (GSH) levels showed distinct patterns between healthy control and individuals with IPD following exposure to oxidative stress and subsequent treatment with genistein (Fig. 4). In healthy control, H_2O_2 treatment alone markedly reduced GSH

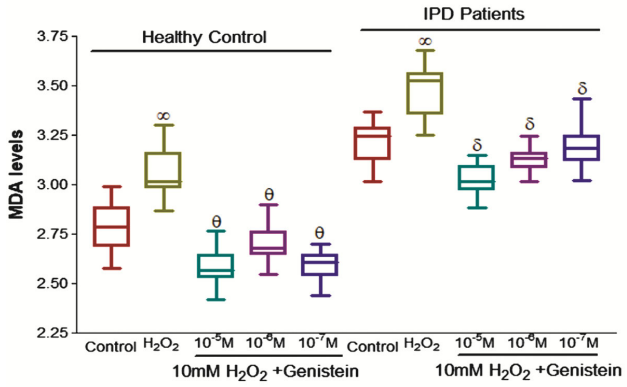

Fig. 3 — Effect of genistein administration (10^{-7}M to 10^{-5}M final concentration) on red blood cells. MDA levels in healthy control (n=45) and IPD patients (n=50). The MDA content was calculated as nmol mL^{-1} Packed Red Blood Cells (PRBC). Data are expressed as mean ± SD. $P<0.001$ compared to 10mM H_2O_2 treated with respective control in healthy control and IPD patients. ∞ $P<0.001$ 10 mM H_2O_2 compared to control without any treatment. δ $P<0.001$ genistein treated compared to 10mM H_2O_2 of healthy control. θ $p < 0.001$ genistein treated compared to 10mM of IPD patients. IPD; Idiopathic parkinson disease, MDA: Malondialdehyde

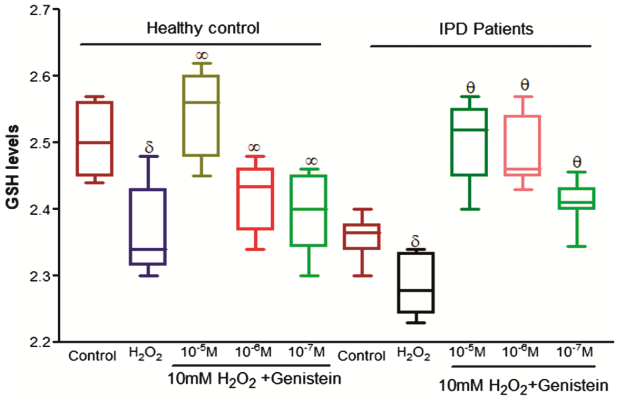

Fig. 4 — Effect of genistein administration (10^{-7}M to 10^{-5}M final concentration) on red blood cells GSH levels in healthy control (n=45) and IPD patients (n=50). The levels of the GSH content was calculated as mg mL^{-1} Packed Red Blood Cells (PRBC). Data are expressed as mean ± SD. $P< 0.001$ compared to 10mM H_2O_2 treated with respective control in healthy control and IPD patients. ∞ $P< 0.001$ 10 mM H_2O_2 compared to control without any treatment. δ $P< 0.001$ genistein treated compared to 10mM H_2O_2 of healthy control. θ $p < 0.001$ genistein treated compared to 10mM of IPD patients. IPD; Idiopathic parkinson disease, GSH; glutathione

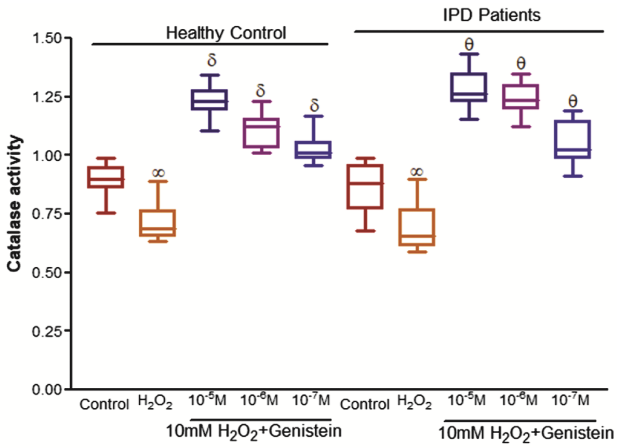

Fig. 5 — Effect of genistein administration (10^{-7} M to 10^{-5} M final concentration) on red blood cells catalase activity in healthy control (n=45) and IPD patients (n=50). The activity of the catalase enzyme was calculated as change in absorbance per min per mg of protein. Data are expressed as mean ± SD. $P< 0.001$ compared to 10mM H_2O_2 treated with respective control in healthy control and IPD patients. ∞ $P< 0.001$ 10mM H_2O_2 compared to control without any treatment. δ $P< 0.001$ genistein treated compared to 10mM H_2O_2 of healthy control. θ $p < 0.001$ genistein treated compared to 10 mM of IPD patients. IPD; Idiopathic parkinson disease

levels in comparison to control group. However, co-treatment with genistein at concentrations of 10^{-5} M and 10^{-6} M significantly restored GSH levels, reaching values comparable to or even exceeding the baseline control. A concentration of 10^{-7} M genistein indicated only a partial protective effect, with GSH levels remaining lower than those seen in higher genistein doses (10^{-5} M and 10^{-6} M). A comparable trend was observed in erythrocytes derived from IPD patients. H_2O_2 alone caused a distinct depletion of GSH, while genistein co-treatment notably ameliorated this decline in a concentration-dependent manner. Particularly, 10^{-5}M genistein yielded the most ample recovery of GSH levels in patient samples, suggesting robust antioxidant action even in the disease context.

Catalase activity displayed a distinct modulation by oxidative stress and subsequent genistein treatment in both healthy control and IPD erythrocytes (Fig. 5). In healthy control samples, exposure to H_2O_2 alone, caused a marked decline in catalase activity compared with untreated cells. Co-incubation with genistein, however resulted in a significant dose-dependent recovery. The most robust enhancement was observed at 10^{-5}M genistein, where catalase activity elevated beyond baseline control levels, followed by slightly lower improvements at 10^{-6} M and 10^{-7} M. A similar profile was evident in erythrocytes derived from IPD patients. H_2O_2 exposure reduced catalase activity relative to their baseline, yet co-treatment with genistein significantly reversed this decline. Among the tested doses, 10^{-5} M genistein constantly created the most extensive improvement while 10^{-6} M and 10^{-7} M offered moderate protection.

SOD activity demonstrated marked variations in response to oxidative stress and genistein intervention in both healthy and IPD groups. In healthy erythrocytes, exposure to H_2O_2 alone caused a significant reduction in SOD activity compared with the untreated control. Co-treatment with genistein resulted in a clear dose-dependent recovery, with the highest activity observed at 10^{-5} M. Genistein at 10^{-6} M and 10^{-7} M also improved SOD levels compared with H_2O_2 alone, though to a lesser extent. In erythrocytes from IPD patients, the same pattern emerged. These findings demonstrate that genistein effectively counteracts oxidative suppression of SOD in both healthy and diseased cells (Fig. 6).

Discussion

Idiopathic parkinson disease (IPD) is a progressive, persistent disorder of the peripheral and central nervous system, mainly affecting motor function due to degeneration of dopaminergic neurons in *substantia nigra*[25]. In IPD, the steady decline of dopamine-producing neurons is commonly linked with impaired mitochondrial function, increased

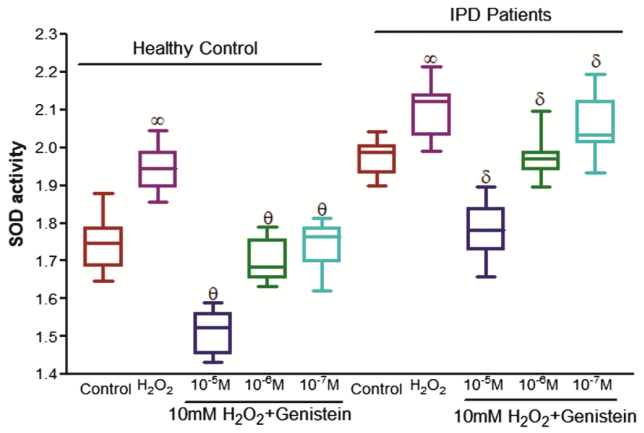

Fig. 6 — Effect of genistein administration (10^{-7}M to 10^{-5}M final concentration) on red blood cells catalase activity in healthy control (n=30) and IPD patients (n=36). The activity of the catalase enzyme was calculated as change in absorbance per min per mg of protein. Data are expressed as mean ± SD. $P< 0.001$ compared to 10mM H_2O_2 treated with respective control in healthy control and IPD patients. ∞ $P< 0.001$ 10mM H_2O_2 compared to control without any treatment. δ $P< 0.001$ genistein treated compared to 10mM H_2O_2 of healthy control. θ $p < 0.001$ genistein treated compared to 10mM of IPD patients. IPD; Idiopathic parkinson disease

oxidative damage, the accumulation of abnormal proteins such as alpha-synuclein and chronic inflammation in the brain. These are some of the key contributors to the disease's advancement[26]. The major hurdle in understanding the symptoms of disease is its complexity. The actual mechanism behind the disease is yet unknown. Although the disease is multifactorial but oxidative stress plays a fundamental role in the pathogenesis of IPD and is closely associated with the progressive degeneration of dopaminergic neurons. Oxidative stress is a central biochemical characteristic of neurodegenerative disorders, including IPD and is closely linked with elevated lipid peroxidation leading to membrane instability, enzyme inactivation and amplification of oxidative injury. There are several advantages of erythrocytes using as a cellular models for oxidative stress biomarkers. The fundamental advantage is the convenient accessibility of RBCs from peripheral blood, making them easily available and non-invasive. The RBCs can be easily separated from other components of the blood. Erythrocytes are indicator of many cellular processes such as maintaining pH, vascular tone and redox homeostasis. Because, RBC analysis is relatively cost-effective, it is a best model to study oxidative stress biomarkers in various studies. Erythrocytes serve as accessible peripheral indicators of oxidative status, and the present data reinforce their utility as a model for evaluating potential neuroprotective agents[27].

In this study conducted on RBCs, H_2O_2 exposure substantially increased MDA levels in erythrocytes, confirming the generation of oxidative stress. Particularly, genistein treatment attenuated this increase in a dose-dependent manner in both healthy controls and samples obtained from IPD patients. The reduction in MDA indicates that genistein effectively neutralizes reactive oxygen species and shields membrane lipids from oxidative damage[28]. These results align with previous studies describing the antioxidant capacity of flavonoids, which not only directly neutralize free radicals but also modulate intracellular signaling pathways to upregulate endogenous antioxidant defenses[29]. It is elevated oxidative state underscores its potential as a treatment option to diminishes oxidative damage linked to neurodegeneration. Analogously, our findings demonstrate that genistein exerts a potent protective influence against oxidative stress in erythrocytes, evident through the restoration of GSH levels after H_2O_2 challenge[30]. This influence was seen in both healthy and IPD patients derived erythrocytes, suggesting that genistein may have translational importance as a treatment adjuvant. The prominent depletion of GSH upon exposure to H_2O_2 emphasizes the vulnerability of red blood cells to oxidative stress , a process that mirrors systemic oxidative stress reported in neurodegenerative disorders[31]. These changes are peripheral markers of systemic oxidative stress in addition to damaging the integrity of the erythrocyte membrane. Various signalling pathways, such as Nrf 2/ Keap1 antioxidant defense system, NF-κB-mediated inflammatory signaling, MAPK stress-activated kinases and P13K/Akt survival signaling, are activated in cerebral tissues when redox dysregulation occurs. Core pathogenic events that underlie neurodegenerative diseases including as oxidative damage, neuroinflammation, mitochondrial impairment and apoptotic cell death are promoted by the convergence of these redox-sensitive pathways[32-34]. The ability of genistein to counteract this decline suggests that its antioxidant properties are sufficiently potent to bolster the cellular redox buffering capacity. Among the tested concentrations, 10^{-5}M genistein consistently offered the most evident rescue effect, indicating a dose-dependent response likely linked to its capacity to scavenge reactive oxygen species and modulate intracellular signaling

pathways that sustain glutathione homeostasis[35]. In IPD, systemic oxidative stress and impaired redox balance are widely recognized contributors to neuronal damage. By restoring GSH levels in these cells, genistein may indirectly reflect its capacity to enhance antioxidant defenses in neuronal tissues, offering a compelling avenue for future clinical exploration[36]. Similarly, the observed changes in catalase activity reinforce the notion that oxidative stress profoundly affects the redox balance in erythrocytes and that genistein has the capacity to counteract this damage. The modifications in the activity of catalase against the induced oxidative stress by H_2O_2 provide significant insights as it is crucial enzyme. Genistein showed antioxidant effect and also having capacity to modulate intracellular redox signalling pathways to restore catalase activity[37]. Attenuation of NF-κB and MAPK (JNK/p38) pathways restricts oxidative and inflammatory responses, whereas, Nrf2/Keap1/ARE signaling regulation increase transcription of antioxidant enzymes such as catalase[38]. Additionally, by preserving redox balance, PI3K/Akt pathway activation may support cellular survival. In line with past research on flavonoid-mediated redox control, these coordinated signaling events work together to shield erythrocytes from oxidative damage and aid in the recovery of catalase[39].

The data demonstrated the decreased SOD activity, the data unequivocally validate that oxidative stress induced by H_2O_2 deteriorates the antioxidant defense mechanism in erythrocytes. Since SOD is a crucial enzyme that neutralizes superoxide radicals, its decline indicates a compromised ability of the cell to control oxidative damage. Genistein co-treatment prominently restored and enhanced SOD activity in concentration-dependent manner[40]. The proclaimed effect at 10^{-5}M suggests that genistein not only protects SOD from oxidative inactivation but may also stimulate cellular mechanisms that maintain or upregulate enzymatic function. This aligns with reports that genistein can directly scavenge reactive oxygen species and modulate redox-sensitive signaling pathways. Importantly, similar protective trends were observed in erythrocytes from IPD patients, which are often characterized by systemic oxidative imbalance[41]. Genistein's potential as a supportive treatment drug targeted at redox equilibrium restoration is underlined by its competence to elevate SOD activity in IPD. These peripheral findings may reflect a more general neuroprotective action because oxidative stress is an identified cause of

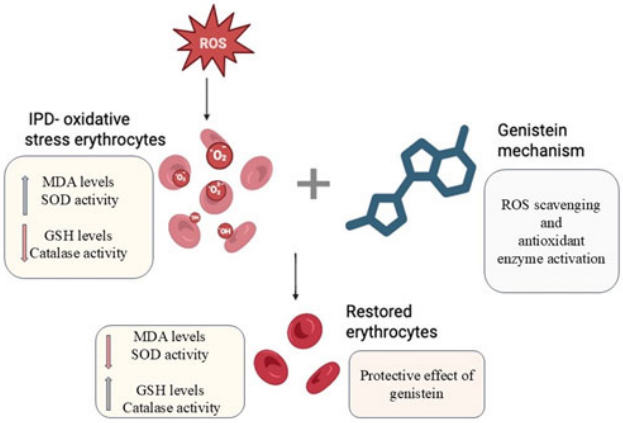

Fig. 7 — Protective effect of genistein on IPD erythrocytes under oxidative stress

neuronal damage in IPD. Overall, the conclusions demonstrate genistein's potential as a robust antioxidant intervention that can strengthen enzymatic defenses against oxidative damage in both healthy and pathological IPD conditions.

Limitations

While this study provides valuable insights into the protective effect of Genistein against the biomarkers of oxidative stress in erythrocytes of IPD patients, it also has certain limitations (Fig. 7). One of the study's main limitations is its small sample size. An increased sample size will improve the study's dependability. Lifestyle factors (*e.g.* eating habits, physical activity, sleep cycle) that are not taken into account for this study may cause oxidative levels to fluctuate. Individual variations in antioxidant consumption, genetic variables and environmental exposure are not quantified in this study.

Future perspectives

The present study reveals that Genistein is an efficient regulator of oxidative stress in human erythrocytes under experimentally produced redox imbalance, however, more findings are needed to increase its translational value. Deeper mechanistic understanding would come from illuminating the molecular mechanisms supporting genistein-mediated redox control, including its influence on mitochondrial function, antioxidant gene expression and redox-sensitive signaling pathways. Investigating combination treatments that combine genistein with other antioxidants or common anti-parkinsonian medications may improve redox homeostasis and treatment outcomes.

Conclusion

The existing study offers substantial experimental evidence for the antioxidant efficacy of genistein, an isoflavone produced from plants, in reducing damage caused by oxidative stress in human erythrocytes. We methodically assessed the impact of genistein on important oxidative stress indicators, such as catalase, superoxide dismutase, glutathione reductase and malondialdehyde, a consequence of lipid peroxidation, using both erythrocytes from healthy control and IPD patient's samples. Redox homeostasis was significantly compromised after hydrogen peroxide treatment. However, these disruptions were successfully and dose-dependently reversed by genistein co-treatment, with the strongest restoration of antioxidant status shown at a 10^{-5}M dosage. These effects were regularly noticed in erythrocytes from both healthy individuals and IPD patients. Genistein exhibits as a potential profiles serving as a therapeutic or preventive agent in oxidative stress-mediated illnesses by lowering oxidative damage indicators and restoring the activity of primary antioxidant defenses. Since oxidative stress is a crucial contributor to the pathogenesis of neurodegenerative disorders, these findings warrant further *in vivo* studies and clinical trials to explore genistein's neuroprotective potential and its utility in redox-targeted therapeutic strategies.

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Conflict of interest

All authors declare no conflict of interest.

References

1 Dionísio PA, Amaral JD & Rodrigues CMP, Oxidative stress and regulated cell death in Parkinson's disease. *Ageing Res Rev,* 67 (2021) 101263.

2 Singh A, Kukreti R, Saso L & Kukreti S, Oxidative Stress: a key modulator in neurodegenerative diseases. *Molecules* (Basel, Switzerland), 24 (2019) 1583.

3 Elfawy HA & Das B, Crosstalk between mitochondrial dysfunction, oxidative stress, and age-related neurodegenerative disease: Etiologies and therapeutic strategies. *Life Sci*, 218 (2019) 165.

4 Wadhwa R, Gupta R & Maurya PK, Oxidative Stress and Accelerated Aging in Neurodegenerative and Neuropsychiatric Disorder. *Curr Pharm Des*, 24 (2018) 4711.

5 Acharya P & Mathur MC, Oxidative stress in alopecia areata: a systematic review and meta-analysis. *Int J Dermatol*, 59 (2020) 434.

6 Trist BG, Hare DJ & Double KL, Oxidative stress in the aging substantia nigra and the etiology of Parkinson's disease. *Aging cell,* 18 (2019) e13031.

7 Riaz Z, Richardson GS, Jin H, Zenitsky G, Anantharam V, Kanthasamy A & Kanthasamy AG, Nuclear pore and nucleocytoplasmic transport impairment in oxidative stress-induced neurodegeneration: relevance to molecular mechanisms in Pathogenesis of Parkinson's and other related neurodegenerative diseases. *Mol Neurodegener*, 19 (2024) 87.

8 Raza C, Anjum R & Shakeel NUA, Parkinson's disease: Mechanisms, translational models and management strategies. *Life Sci,* 226 (2019) 77.

9 Imbriani P, Martella G, Bonsi P & Pisani A, Oxidative stress and synaptic dysfunction in rodent models of Parkinson's disease. *Neurobiol Dis,* 173 (2022) 105851.

10 Jomova K, Alomar SY, Valko R, Liska J, Nepovimova E, Kuca K & Valko M, Flavonoids and their role in oxidative stress, inflammation, and human diseases. *Chem Biol Interact*, 413 (2025) 111489.

11 Weng L, Zhang F, Wang R, Ma W & Song Y, A review on protective role of genistein against oxidative stress in diabetes and related complications. *Chem Biol Interact,* 310 (2019) 108665.

12 Shi B, Chen M, Xia Z, Tang W, Li Y, Qin C & Xu H, Genistein attenuates neuroinflammation and oxidative stress and improves cognitive impairment in a rat model of sepsis-associated encephalopathy: potential role of the Nrf2 signaling pathway. *Metab Brain Dis,* 38 (2023) 339.

13 Li Y, Zhang JJ, Chen RJ, Chen L, Chen S, Yang XF & Min JW, Genistein mitigates oxidative stress and inflammation by regulating Nrf2/HO-1 and NF-κB signaling pathways in hypoxic-ischemic brain damage in neonatal mice. *Ann Transl Med*, 10 (2022) 32.

14 Paramanik V, Kurrey K, Singh P, Tiwari S & Nisha, Roles of genistein in learning and memory during aging and neurological disorders. *Biogerontology*, 24 (2023) 329.

15 Açar Y, Ağagündüz D, De Cicco P & Capasso R, Corrigendum to "Flavonoids: Their putative neurological roles, epigenetic changes, and gut microbiota alterations in Parkinson's disease" [Biomed. Pharmacother. 168 (2023) 115788]. Biomedicine & pharmacotherapy. *Biomed Pharmacother*, 189 (2025) 118231.

16 Li R, Robinson M, Ding X, Geetha T, Al-Nakkash L, Broderick TL & Babu JR, Genistein: A focus on several neurodegenerative diseases. *J Food Biochem*, 46 (2022) e14155.

17 Fernández R, Berruecos C, Motta MCC & Velásquez D, Genotoxicity and Hemocompatibility of a novel calcium aluminate-based cement. *Eur Endod J*, 3 (2018) 87.

18 Rocha S, Gomes D, Lima M, Bronze-da-Rocha E & Santos-Silva A, Peroxiredoxin 2, glutathione peroxidase, and catalase in the cytosol and membrane of erythrocytes under H2O2-induced oxidative stress. *Free Radic Res*, 49 (2015) 990.

19 Esterbauer H & Cheeseman KH, Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods Enzymol*, 186 (1990) 407.

20 Hurşitoğlu O, Orhan F, Kurutaş EB, Doğaner A, Durmuş HT & Kopar H, Diagnostic Performance of Increased Malondialdehyde Level and Oxidative Stress in Patients with Schizophrenia. *Noro Psikiyatr Ars*, 58 (2021) 184.

21 Rahman I, Kode A & Biswas SK, Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nat Protocol,* 1 (2006) 3159.

22 Aebi H, Catalase *in vitro*. *Methods Enzymol,* 105 (1984) 121.

23 Rukmini MS, D'Souza B & D'Souza V, Superoxide dismutase and catalase activities and their correlation with malondialdehyde in schizophrenic patients. *Indian J Clin Biochem,* 19 (2004) 114.

24 Tolosa E, Garrido A, Scholz SW & Poewe W, Challenges in the diagnosis of Parkinson's disease. *Lancet Neurol,* 20 (2021) 385.

25 Correia Guedes L, Mestre T, Outeiro TF & Ferreira JJ, Are genetic and idiopathic forms of Parkinson's disease the same disease? *J Neurochem*, 152 (2020) 515.

26 Yadav S, Bhagat S, Singh S & Maurya PK, Exploring the anti-aging effect of dextran and polyethylene glycol-coated cerium oxide nanoparticles in erythrocytes. *Int J Biol Macromol*, 282 (2024):136700.

27 Reed TT, Lipid peroxidation and neurodegenerative disease. *Free Radic Biol Med,* 51 (2011) 1302.

28 Siddique YH, Naz F, Jyoti S, Ali F & Rahul, Effect of Genistein on the Transgenic Drosophila Model of Parkinson's Disease. *J Diet Suppl,* 16 (2019) 550.

29 Sharma D, Singh V, Kumar A & Singh TG, Genistein: A promising ally in combating neurodegenerative disorders. *Eur J Pharmacol,* 991 (2025) 177273.

30 Wu HC, Hu QL, Zhang SJ, Wang YM, Jin ZK, Lv LF, Zhang S, Zhen-Lin L, Hong-Lian W & Ou-Mei C, Neuroprotective effects of genistein on SH-SY5Y cells overexpressing A53T mutant α-synuclein. *Neural Regen Res*, 13 (2018) 1375.

31 Braxas H, Rafraf M, Karimi Hasanabad S & Jafarabadi MA, Effectiveness of Genistein Supplementation on Metabolic Factors and Antioxidant Status in Postmenopausal Women With Type 2 Diabetes Mellitus. *Can J Diabetes*, 43 (2019) 490.

32 Wen X, Tang S, Wan F, Zhong R, Chen L & Zhang H, The PI3K/Akt-Nrf2 Signaling Pathway and Mitophagy Synergistically Mediate Hydroxytyrosol to Alleviate Intestinal Oxidative Damage. *Int J Biol*, 20 (2024) 4258.

33 Guo J, Kong D, Luo J, Xiong T, Wang F, Deng M, Kong Z, Yang S, Da J, Chen C, Lan J, Chu L, Han G, Liu J, Tan Y & Zhang J, Orexin-A Attenuates the Inflammatory Response in Sepsis-Associated Encephalopathy by Modulating Oxidative Stress and Inhibiting the ERK/NF-κB Signaling Pathway in Microglia and Astrocytes. *CNS Neurosci Ther*, 30 (2024) e70096.

34 Verza FA, Das GC & Nishimura FG, The impact of oxidative stress and the NRF2-KEAP1-ARE signaling pathway on anticancer drug resistance. *Oncol Res*, 33 (2025) 1819.

35 Witayavanitkul N, Werawatganon D, Chayanupatkul M, Klaikeaw N, Sanguanrungsirikul S & Siriviriyakul P, Genistein and exercise modulated lipid peroxidation and improved steatohepatitis in ovariectomized rats. *BMC Complement Med Ther,* 20 (2020) 162.

36 Delgado CA, Poletto E, Vera LNP, Jacques CED, Vianna P, Reinhardt LS, Baldo G & Vargas CR, Effect of genistein and coenzyme Q10 in oxidative damage and mitochondrial membrane potential in an attenuated type II mucopolysaccharidosis cellular model. *Cell Biochem Funct,* 42 (2024) e3932.

37 Viña J, Borrás C & Mas-Bargues C, Genistein, A Phytoestrogen, Delays the Transition to Dementia in Prodromal Alzheimer's Disease Patients. *J Alzheimer's Dis*, 101 (2024) S275.

38 Li H, Weng Q, Gong S, Zhang W, Wang J, Huang Y, Li Y, Guo J & Lan T, Kaempferol prevents acetaminophen-induced liver injury by suppressing hepatocyte ferroptosis *via* Nrf2 pathway activation. *Food Funct*, 14(2023) 1884.

39 Ma L, Wu F, Shao Q, Chen G, Xu L & Lu F, Baicalin Alleviates Oxidative Stress and Inflammation in Diabetic Nephropathy *via* Nrf2 and MAPK Signaling Pathway. *Drug Des Devel Ther*, 15 (2021) 3207.

40 Wu C, Zhou S, Ma S & Suzuki K, Effect of Genistein Supplementation on Exercise-Induced Inflammation and Oxidative Stress in Mice Liver and Skeletal Muscle. *Medicina* (Kaunas, Lithuania), 57 (2021) 1028.

41 Tossetta G & Marzioni D, Natural and synthetic compounds in Ovarian Cancer: A focus on NRF2/KEAP1 pathway. *Pharmacol Res*, 183 (2022) 106365.