

Study of the Combined Effect of Silymarin, Quercetin, and Hesperidin on 3-nitro Propionic Acid-induced Rat Model of Huntington's Disease

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ABSTRACT

This investigation proposes to encapsulate how the combined effects of silymarin, quercetin, and hesperidin impact the abilities of rats with Huntington's disease (HD). Male Wistar rats were administered 3-NP through intraperitoneal injections. Various behavioral measures, including muscle grip strength, locomotor activity, and a string test, were assessed. After 22 days, assessments of lipid peroxidation, glutathione levels, superoxide dismutase, catalase, succinate dehydrogenase (Complex II) activity, lactate dehydrogenase (Complex IV) activity, and the determination of interleukin-6 levels was performed. Administering 3-NP dosage of 10 mg/kg body weight for 21 days outcomes in a substantial rise in learning and memory impairments that closely resembled those seen in HD. Within specific treatment groups, it was observed that quercetin significantly enhanced muscle coordination and demonstrated a noteworthy upsurge in various behavioral measures. After silymarin, quercetin, and hesperidin were combined, there were significant improvements in key biochemical markers such as glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and SDH activity induced by 3-NP. These improvements led to a decrease in neuronal damage and apoptosis in brain. Each bioflavonoid successfully reinstated the stages of biochemical markers, for example, GSH, SOD, CAT, and SDH, while reducing the activity of malondialdehyde (MDA) and lactate dehydrogenase (LDH). However, after these bioflavonoids were combined, their collective impact was more potent Compared to what each achieved individually.

Keywords: Bioflavonoids, Cognitive function, Huntington's disease, Monotherapy, Neurodegenerative disorder.

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INTRODUCTION

Huntington's disease (HD) is rare neurological state regarded as in chorea, psychological and behavioral symptoms, and cognitive decline typically emerging between the ages of 30 and 50 years.^{1,2} The global prevalence is estimated between 5.96 to 13.7/100,000.³ While the precise pathogenic mechanisms behind HD are not fully understood, mitochondrial dysfunction, neuroinflammation, excitotoxicity, neurochemical imbalances, oxidative stress, and apoptosis are recognized as contributing factors.^{4,5} For many years, there was no FDA-approved medication specifically for treating chorea in HD. Tetrabenazine is an approved drug for HD-associated chorea, following its successful use in the TETRA-HD clinical trial.^{6,7} While tetrabenazine and deutetrabenazine are approved by FDA for HD-associated chorea, they come with limitations, including potential drug interactions and side effects.^{8,9} This mycotoxin, after being administered chronically (at a dosage of 10 mg/kg/day for 3-6 weeks), induces HD-like characteristics.¹⁰ Depending on the dose and duration, this animal model simulates the hyperkinetic and hypokinetic indications of HD.¹¹ It leads to cell death through a combination

of necrosis and apoptosis, mirroring what is observed in the HD brain. There is an initial wave of necrotic cell death shortly after 3-NP administration, followed by slow apoptosis, allowing the evaluation of both early and late HD phases.¹²

Bioflavonoids are a class of natural compounds with diverse phenolic structures having anti-inflammatory, antioxidant, antiviral, anti-carcinogenic, and anti-allergic potentials.¹³⁻¹⁵ Silymarin is a derivative obtained from milk thistle, is a flavonolignan with anti-inflammatory, cytoprotective, and anticarcinogenic properties. It primarily consists of three flavonolignans: silidianin, silychristine, and silybin.¹⁶ Silymarin inhibits LPS-induced microglial activation, reducing the TNF- α and nitric oxide (NO).¹⁷ It also protects dopaminergic neurons and SNC neurons.¹⁸

Quercetin, a type of flavonol found in various fruits and vegetables, has demonstrated the ability to reverse the inhibition of the respiratory chain complex caused by 3-NP. Longer management through quercetin has shown promise in improving motor performance and increasing muscle mass in the early stages of aging.^{19,20}

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Hesperidin, another bioflavonoid derived from citrus byproducts, possesses a wide range of pharmacological properties and medicinal applications.²¹ Hesperidin has been found to inhibit the elevation of TNF- α , reduce apoptosis, and mitigate excitotoxicity.²² Furthermore, hesperidin's ability to decrease malondialdehyde (MDA) levels and enhance catalase (CAT) activity through oral administration suggests its potential in treating HD, possibly by intervening in the microglial path over and done with the inactivation of microglial cells.²³

This investigation aims to explore the combined effects of silymarin, quercetin, and hesperidin in a male wistar rat model of HD's induction through 3-NP, based on the potential positive impact of the mentioned bioflavonoids on the condition.

MATERIAL AND METHODS

Materials

Hesperidin, Silymarin, quercetin, and 3-NP were bought from Sigma Aldrich, India. ELISA kits used on behalf of assessing serotonin and dopamine levels were acquired from Krishgen Biosystems in Mumbai, India. A diagnostic kit for measuring lactate dehydrogenase (LDH) was obtained from Tulip Diagnostics, also based in Mumbai, India. With the exception of cases where specific sources are mentioned.

Animals Used

Male Wistar rats (200–250 gms) were purchased from Crystal Biological Solutions, India. These rats were maintained in standard laboratory conditions, with the temperature kept at $25 \pm 2^\circ\text{C}$, RH at $60 \pm 5\%$, and a light-dark cycle of 12 hours each. Entirely investigational techniques and protocols underwent rigorous scrutiny and received approval from the Institutional Animal Ethics Committee (IAEC) at Crystal Biological Solutions in Pune. This ethical board operates in compliance with the procedures recognized by the CPCSEA, New Delhi.

Preparation of Drugs and Chemicals

Quercetin 50 mg/kg, silymarin 200 mg/kg and hesperidin 50mg/kg were given oral route towards rats for a duration of 21 days. These substances were given as a suspension prepared in a 0.5% (w/v) carboxymethylcellulose (CMC) utilizing a mortar and pestle. Meanwhile, 3-NP (10 mg/kg BW) was freshly prepared utilizing normal saline and administered via intraperitoneal injection for the same 21-day period. The injection of 3-NP took place 90 minutes after test drugs were administered and all dosing occurred between 9 am and 11 am.

Experiment Utilizing Animals

Afterward a one-week adaptation period, rats were separated into eight different groups, as well as each group underwent a 21-day treatment regimen as follows:

Group 1: The normal control group was given a 1mL/kg intraperitoneal injection of normal saline (NS) and 1-mL/100g of 1% carboxymethyl cellulose (CMC) via oral (orally) administration. Group 2: The Huntington control group was given a 10 mg/kg dosage of 3-NP via I.P. injection and 1-mL/100g of 0.5% (CMC) via oral administration.

Group 3 (ST), Group 4 (QT), and Group 5 (HT): These groups were treated with silymarin (200 mg/kg, orally), quercetin (50 mg/kg, orally), and hesperidin (50 mg/kg, orally), correspondingly, while simultaneously receiving 3-NP (10 mg/kg) through I.P. injection.

Group 6: This group was administered a combination of silymarin (200 mg/kg, orally), quercetin (50 mg/kg, orally), and hesperidin (50 mg/kg, orally).

Group 7: This group was administered a combination of silymarin (200 mg/kg, orally) and quercetin (50 mg/kg, orally). Group-8: This group was administered a combination of silymarin (200 mg/kg, orally) and hesperidin (50 mg/kg, orally) while simultaneously being administered 3-NP (10 mg/kg) through I.P. injection.

Evaluation of Behavioral Parameters Muscle Grip Strength (Rota Rod Apparatus)

Motor coordination and balances of all the animals were evaluated, they underwent testing utilizing the Rota rod apparatus. Each rat was positioned on a rotating rod with 7 cm diameter, set at a speed of 25 rpm. The average performance results were documented as the duration in seconds that each rat remained on the rotating rod, with a supreme allowable time of 180 seconds.²⁴

Locomotor Activity

Animals were positioned within an actophotometer, wherever beams of light interacted with photoelectric cells, and their baseline action score was calculated over 5-minute duration. This measurement was registered as the count of light beams interrupted during their locomotion.²⁵

String Test

This test is employed to check the rodents' grip strength.²⁶ The test was conducted following the procedure outlined by Shear *et al.*, and the results were conveyed as the duration (in seconds) that the rat could maintain its grip on a steel wire.²⁷

Assessment of Biochemical Parameters in Brain Striatum

Following the conclusion of tasks such as assessing muscle grip strength, locomotor activity, and the string test on the 22nd day, all the rats were humanely euthanized via cervical dislocation for subsequent biochemical analysis. The rat's brain was then carefully dissected, and the striatum was isolated, identified, and weighed.

Preparation of Brain Homogenate

A 10% tissue homogenate was organized by utilizing 0.1 M phosphate buffer with a pH of 7.4 and 0.1 mM EDTA. The solution was centrifuged at 14,000 rpm for 30 minutes at a temperature of 4°C. Supernatant fractions obtained were separated and used for biochemical analysis.²⁸

Antioxidant parameters

Estimation of lipid peroxidation

A mixture of 2 mL of thiobarbituric acid reaction solution was combined with 0.1 mL of a tissue sample, which consisted of 1-mL of a 10% trichloroacetic acid solution and 1-mL of a

0.67% TBAR. The resultant blend was then subjected to heat in a boiling water bath for 30 min as well as subsequently cooled in ice bath for 10 minutes. Subsequently chilling, the blend was centrifuged for 10 minutes at 4830 times the force of gravity ($\times g$) towards separating the supernatant, which was then calculated for absorbance at 532 nm. The outcomes were communicated in nanomoles of MDA/gram wet tissue weight.²⁹

Reduced glutathione estimation

Tissue homogenate of equal volumes and 10% trichloroacetic acid were combined and subjected to centrifugation for 20 minutes, leading to the separation of 1-mL of supernatant. The remaining liquid, known as the supernatant, was combined with 3 mL of 0.2 M phosphate buffer with a pH of 8, along with 0.5 mL of a DTNB reagent (0.6 mM in 0.2 M phosphate buffer). After thorough mixing, the absorbance was measured at 412 nm. The outcomes were expressed in nanomoles of GSH per gram of wet tissue weight.³⁰

Estimation of superoxide dismutase action

A blend was created by combining 0.1 mL of the sample, 1.2 mL of sodium pyrophosphate buffer with a pH of 8.3 (0.052 M), 0.1 mL of phenazine methosulphate (186 μ M), 0.3 mL of nitro blue tetrazolium (300 μ M), and 0.2 mL of NADH (750 μ M). The reaction was initiated by adding NADH, and after an incubation period of 90 seconds at 30°C, the reaction was halted by adding 1-mL of glacial acetic acid. After letting the blend sit for duration of 10 minutes, the chromogen's color intensity was assessed at a wavelength of 560 nm, and this measurement was compared to a reference or blank sample.³¹

Catalase activity

To start the reaction, 1-mL of a 30 mM H₂O₂ solution was blended with 1.9 mL of a 0.05 M phosphate buffer at pH 7, and the reaction was started utilizing adding 0.1 mL of the homogenate. The decline in absorbance resulting from the breakdown of H₂O₂ in the mixture was calculated utilizing a spectrophotometer at 240 nm at 1-minute intervals. The action of catalase was quantified in units of catalase per gram of wet tissue weight, and the calculations were done.³²

Mitochondrial Enzyme Action

Succinate dehydrogenase (Complex II) action estimation

To evaluate succinate dehydrogenase activity, a mitochondrial suspension (0.05 mL) was introduced into a reaction mixture comprising 1.5 mL of phosphate buffer (0.2 M, pH 7.8), 0.2 mL of succinic acid (0.6 M, pH 7.8), 0.3 mL of 1% w/v BSA, and 0.1 mL of potassium ferricyanide (0.3 M). The reduction in absorbance at 420 nm was continuously tracked for 3 minutes, using water as a reference. The outcomes were quantified as nanomoles of succinate oxidized per minute per gram of wet tissue weight.

Estimation of lactate dehydrogenase (Complex IV) action

Lactate dehydrogenase (LDH) action in rat brain homogenate was assessed utilizing a diagnostic kit from Tulip Diagnostics. The reagents were added, thoroughly mixed, and the initial absorbance was recorded after 1 minute. Subsequent

absorbance readings were taken at 1-minute intervals for 2 and 3 minutes. The average change in absorbance per minute was computed and reported as IU/L.

Estimation of Proinflammatory Cytokines

Interleukin 6 level

Interleukin 6 levels were quantified utilizing the ELISA method. The procedure and calculations were conducted following the manufacturer's provided instructions and reported as picomoles per milligram of tissue sample.

RESULT AND DISCUSSION

Quercetin has been shown to improve learning as well as memory through escalating GSH levels and reducing OH amount.³³ It not only inhibits acetylcholinesterase but also decreases MDA levels.³⁴ Hesperidin (with its antioxidant and mitochondrial maintenance properties) has shown neuroprotective effects in a neuroblastoma cell line, benefiting memory retrieval and recognition memory consolidation by increasing the activity of antioxidant enzymes and GSH levels while decreasing MDA in the hippocampal region. The Rota rod test is employed to evaluate motor coordination and fatigue resistance in rodents, both of which are adversely affected as HD progresses, leading to altered muscle movements and diminished muscle grip strength.^{35,36} In this study, silymarin, quercetin, and Hesperidin individually upsurged fall-off time, with quercetin showing a notably pronounced effect. Combining silymarin with quercetin or Hesperidin, or utilizing all 3 together, resulted in significant improvements in fall-off time compared to the combined of silymarin and Hesperidin.³⁷⁻⁴²

Muscle Grip Strength (Rota Rod Apparatus)

Administering 3-NP intraperitoneally to rats with HD for 21 days led to a substantial decrease (*p*-value <0.001) in the time it took for them to fall off compared to the normal control (NC) group. However, the groups treated with silymarin (200 mg/kg), quercetin, and hesperidin (50 mg/kg) showed noteworthy upsurges (*p*-value <0.05), (*p*-value <0.01), and (*p*-value <0.05), correspondingly, in fall-off time compared towards the HC group. Combining silymarin (200 mg/kg, orally) with either quercetin or hesperidin (50 mg/kg, orally) led to a substantial upsurge (*p*-value <0.001), and *p*-value <0.01, separately, in fall-off time after Comparing to the HC group. Furthermore, the combination of silymarin (200 mg/kg, orally) with quercetin and hesperidin (50 mg/kg, orally) showed an even further significant upsurge (*p*-value <0.001) in fall-off time compared to the HD rats as shown in Figure 1. The outcomes are presented as mean \pm SEM (n = 6), and statistical investigation was accomplished utilizing one-way ANOVA monitored through Dunnett's multiple comparison test.

The Huntington control rats exhibited significant differences after comparing to the NC rats at a *p*-value <0.001. Regarding the treatment groups, the stages of significance comparing towards the Huntington control were as follows: **p* <0.05, ***p* <0.01, and ****p* <0.001.

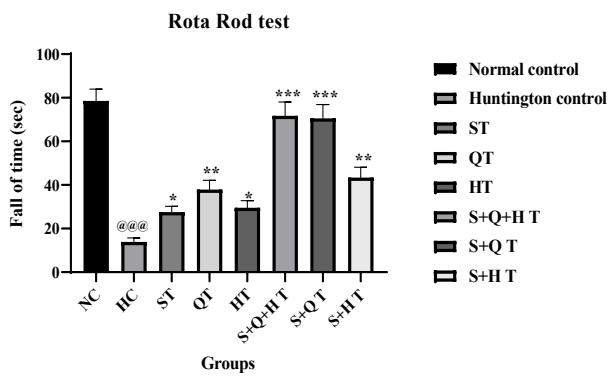


Figure 1: Result of combination of silymarin, quercetin, and hesperidin on fall off time (sec) by Rota rod test

Locomotor Activity

The intraperitoneal administrated of 3-NP over 21 days resulted in a substantial decrease ($p\text{-value} < 0.001$) in the number of beams cut in the HC group compared towards the NC group. However, the ST group (200 mg/kg) and the QT group (50 mg/kg) both exhibited a noteworthy upsurge in the number of beams cut ($p\text{-value} < 0.05$ and 0.01, separately). In contrast, the HT group did not show a noteworthy upsurge in the number of beams cut after equated to the HC group. Furthermore, after silymarin (200 mg/kg, orally) was administered in combined with either quercetin or hesperidin (50 mg/kg, orally), it led to a substantial upsurge in the number of beams cut ($p\text{-value} < 0.001$ and 0.05, separately). Comparing towards the HC group. Notably, the combination of Silymarin (200 mg/kg, orally) with Quercetin and Hesperidin (50 mg/kg, orally) resulted in a further pronounced upsurge ($p\text{-value} < 0.001$) in the number of beams cut while comparing to the HC group as shown in Figure 2.

Locomotor activity is an indicator of central nervous system excitability, and reduced activity is associated with CNS depression. As HD advances, motor function impairment can lead to altered muscle movements and reduced locomotor activity.⁴³ In this study, silymarin, quercetin, and hesperidin individually upsurged the number of locomotions. Combining silymarin with quercetin or hesperidin, or utilizing all three together, led to a significant upsurge in the total of locomotions compared to the combined of silymarin and hesperidin.

String Test

The injection of 3-NP for 21 days demonstrated a substantial decrease ($p\text{-value} < 0.0001$) in fall-off time in the HC group compared to the NC group. Conversely, the ST group (200 mg/kg), the QT group (50 mg/kg), and the HT group (50 mg/kg) exhibited upsurges in fall-off time ($p\text{-value} < 0.01$, 0.001, 0.05, separately) after comparing to the HC group. The administration of silymarin (200 mg/kg, orally) in combined with hesperidin (50 mg/kg, orally) resulted in a substantial upsurge in fall-off time ($p < 0.001$) compared to the HC group. Furthermore, the combined action of silymarin (200 mg/kg, orally) and quercetin (50 mg/kg, orally), either with or deprived

of hesperidin (50 mg/kg, orally), led to a significantly more pronounced upsurge ($p < 0.0001$) in fall-off time after compared towards the HC group, as illustrated in Figure 3.

The string test assesses rodent grip strength and is particularly relevant in HD due to the time off of the long-latency stretch reflex and delayed response to loading, resulting from reduced somatosensory input towards the cortex initiated through basal ganglia disruption.⁴⁴ In this study, the furthermore noteworthy surge in fall-off time was perceived in the combined group of silymarin, quercetin, and hesperidin, and the combined effect of silymarin with quercetin. Notably, quercetin demonstrated a remarkable upsurge in rat fall-off time after being administered individually.

Malondialdehyde

The intraperitoneal injection of 3-NP over 21 days resulted in an important upsurge ($p < 0.0001$) in MDA levels in the HC group compared to the NC group. In difference, the ST group (dosed at 200 mg/kg), QT group, and HT group (dosed at 50 mg/kg) exhibited significant decreases in MDA levels ($p\text{-value} < 0.01$, 0.01, 0.001, separately) after compared to the HC group. Furthermore, the combination of silymarin (200 mg/kg, orally) with quercetin and hesperidin (50 mg/kg, orally) exhibited a significant decrease ($p\text{-value} < 0.001$ and 0.0001) in MDA levels compared to the HC group. The combined action of silymarin (200 mg/kg, orally) with quercetin (50 mg/kg, orally) and hesperidin (50 mg/kg, orally) substantial upsurge MDA levels ($p < 0.0001$) compared to the HC group as depicted in Figure 4.

Malondialdehyde (MDA) is a marker of lipid peroxidation, and upsurged levels indicate elevated free radical generation,

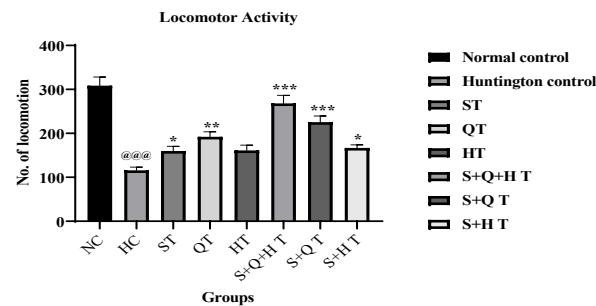


Figure 2: Study on the outcome of mixture of silymarin, quercetin, and hesperidin on no. of locomotion

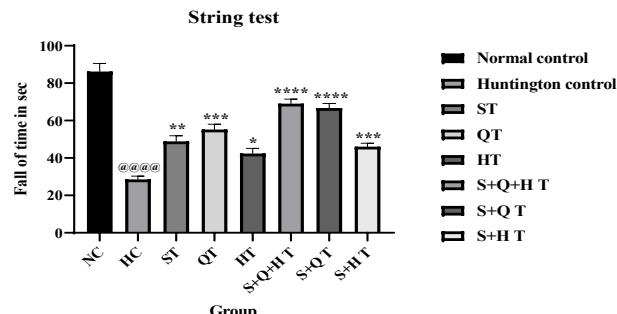


Figure 3: Study of the Effect of combined silymarin, quercetin, and hesperidin on fall time (sec) by String test

which can cause membrane damage and protein and DNA functional disturbances. In HD, an oxidative imbalance is apparent as elevated levels of lipid peroxidation and MDA due to mutant Htt inclusions in striatal neurons.⁴⁵ In this study, a momentous decrease in MDA levels, primarily in the combined group of silymarin, quercetin, and hesperidin, as the other groups exhibited no lipid peroxidation.⁴⁶⁻⁴⁸

Reduced Glutathione

The intraperitoneal injection of 3-NP over 21 days led to a significant decrease ($p < 0.001$) in the GSH level in the HC group compared to the direction of the NC group. In contrast, the ST group (dosed at 200 mg/kg), QT group, and HT group (dosed at 50 mg/kg) demonstrated significant upsurges in GSH levels ($p-value < 0.01, 0.01, 0.001$, separately) after compared to the HC group.

Furthermore, the combination of silymarin (200 mg/kg, orally) with quercetin and hesperidin (50 mg/kg, orally) resulted in a significant upsurge in GSH levels ($p-value < 0.001$ and 0.001, separately) compared to the HC group. Additionally, the combination of silymarin (200 mg/kg, orally) with quercetin and hesperidin (50 mg/kg, orally) led to a more pronounced upsurge ($p < 0.0001$) in GSH levels compared to the HC group as illustrated in Figure 5.

Superoxide Dismutase

The intraperitoneal injection of 3-NP over a 21-day period resulted in a significantly decrease ($p-value < 0.001$) in the

superoxide dismutase (SOD) level in the HC group after compared to the NC group. However, the silymarin group (administered at 200 mg/kg), the quercetin group (administered at 50 mg/kg), and the hesperidin group (administered at 50 mg/kg) exhibited significantly upsurges in SOD levels ($p-value < 0.01, 0.01, 0.001$), separately, after Comparing to the HC group. Furthermore, the administration of silymarin (200 mg/kg, orally) in combined with quercetin and hesperidin (50 mg/kg, orally) resulted in a significant upsurge in SOD levels ($p-value < 0.001$) and ($p-value < 0.0001$), separately, after compared to the HC group. Additionally, the combined treatment of silymarin (200 mg/kg, orally) with quercetin (50 mg/kg, orally) and hesperidin (50 mg/kg, orally) knowingly upsurge SOD levels ($p-value < 0.0001$) comparing towards the HC group as shown in Figure 6.

Catalase

The injection of 3-NP for 21 days resulted in a significant decrease ($p-value < 0.001$) in the catalase (CAT) level in the HC group after compared to the NC group. The ST group (administered at 200 mg/kg) and the HT group (administered at 50 mg/kg) exhibited a significant upsurge in CAT levels ($p-value < 0.01$), but the QT group (administered at 50 mg/kg) did not exhibit a significant upsurge in CAT levels compared to the HC group. Moreover, the administration of silymarin (200 mg/kg, orally) in combined with quercetin and hesperidin (50 mg/kg, orally) resulted in a significant upsurge ($p-value < 0.01$ and 0.001) in CAT levels, separately, after Comparing to the HC group. Furthermore, the combined of silymarin (200 mg/kg,

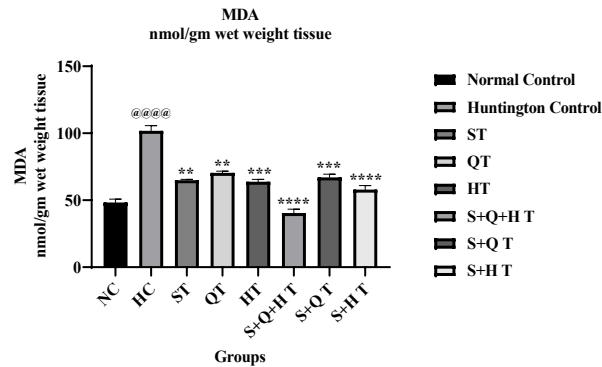


Figure 4: Result of MDA level

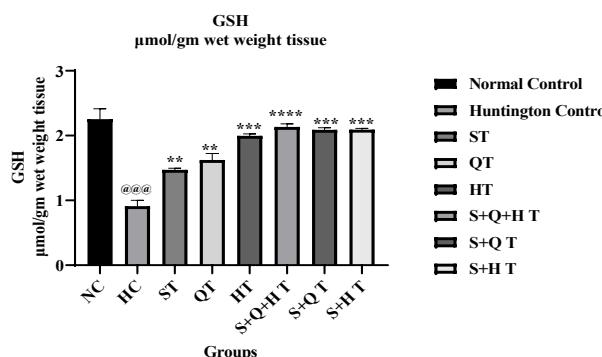


Figure 5: Study of the outcome of a blend of silymarin, quercetin, and hesperidin on reduced GSH

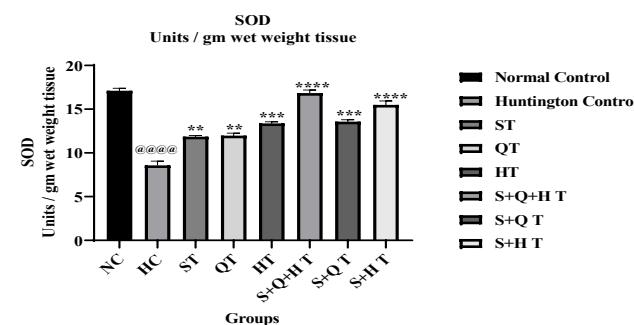


Figure 6: Result of a grouping of silymarin, quercetin, and hesperidin on SOD

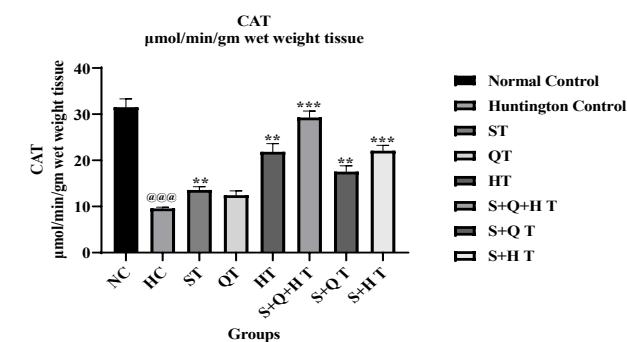


Figure 7: Result of CAT activity

orally) with quercetin and hesperidin (50 mg/kg, orally) further significantly restored ($p < 0.001$) CAT levels after compared to the HC group as shown in Figure 7.

Succinate dehydrogenase

The intraperitoneal injection of 3-NP over a period of 21 days resulted in a significantly decrease ($p < 0.0001$) in the succinate dehydrogenase (SDH) level in the HC group after Comparing to the NC group. However, the ST group (administered at 200 mg/kg), QT group (administered at 50 mg/kg), and HT group (administered at 50 mg/kg) exhibited significant upsurges in SDH levels (p -value < 0.001 , 0.01, 0.001 separately) after compared to the HC group. Furthermore, the administration of silymarin (200 mg/kg, orally) in combined with quercetin and hesperidin (50 mg/kg, orally) resulted in a significant upsurge in SDH levels (p -value < 0.001 and 0.001, separately) after Comparing to the HC group. Moreover, the combined treatment of silymarin (200 mg/kg, orally) with quercetin (50 mg/kg, orally) and hesperidin (50 mg/kg, orally) led to a more significant upsurge ($p < 0.0001$) in SDH levels after comparing to the HC group, as illustrated in Figure 8.

Lactate Dehydrogenase

The injection of 3-NP for 21 days resulted in a significant upsurge (p -value < 0.001) in the lactate dehydrogenase (LDH) level in the HC group after compared to the NC group. However, the ST group (administered at 200 mg/kg), QT group (administered at 50 mg/kg), and HT group (administered at 50 mg/kg) all indicated significantly decreases in LDH

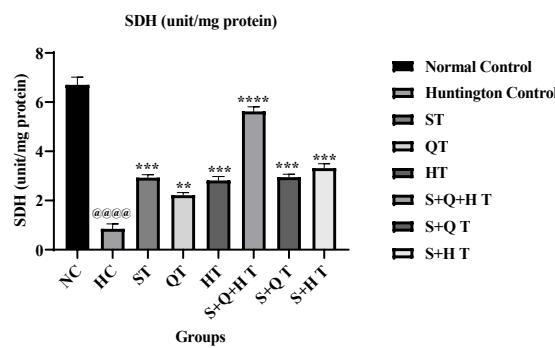


Figure 8: Result of a grouping of silymarin, quercetin, and hesperidin on SDH

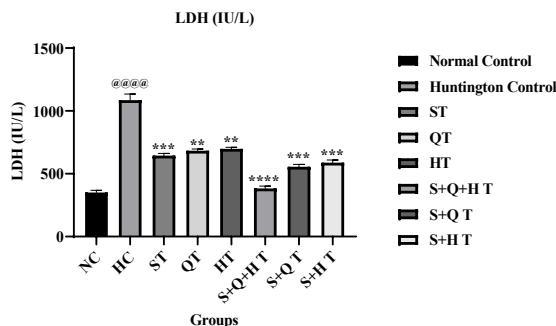


Figure 9: Outcome of a mixture of silymarin, quercetin, and hesperidin on lactate dehydrogenase

levels (p -value < 0.001 , 0.01, 0.001, separately) compared to the HC group. Furthermore, the administration of silymarin (200 mg/kg, orally) in combined with quercetin and hesperidin (50 mg/kg, orally) resulted in a significant decrease in LDH levels (p -value < 0.001 and 0.001, separately) after compared to the HC group. Moreover, the combined treatment of silymarin (200 mg/kg, orally) with quercetin (50 mg/kg, orally) and hesperidin (50 mg/kg, orally) led to a more significant restoration ($p < 0.0001$) of LDH levels after compared to the HC group, as depicted in Figure 9

Interleukin-6 (IL-6)

The administration of 3-NP for 21 days resulted in a significant upsurge (p -value < 0.0001) in the IL-6 level within the HC group after compared to the NC group. However, the ST group (administered at 200 mg/kg), QT group (administered at 50 mg/kg), and HT group (administered at 50 mg/kg) all exhibited significant decreases in IL-6 levels (p -value < 0.001 , 0.01, 0.001, separately) compared to the HC group.

Furthermore, the administration of silymarin (200 mg/kg, orally) in combined with quercetin and hesperidin (50 mg/kg, orally) resulted in a significant decrease in IL-6 levels (p -value < 0.001 and 0.0001, separately) after comparing to the HC group. Moreover, the combined treatment of silymarin (200 mg/kg, orally) with quercetin (50 mg/kg, orally) and hesperidin (50 mg/kg, orally) led to a more significant restoration ($p < 0.0001$) of IL-6 levels after compared to the HC group, as shown in Figure 10.

Huntington's disease is regarded as the degeneration of nerve cells in specific brain areas leading to a range of motor, psychiatric, and cognitive challenges.⁴⁹ Memory issues are commonly associated with HD, which can be further complicated by attention-related problems. While several therapeutic approaches have been explored for HD, for example, fetal neural transplantation, RNA interference, and transglutaminase inhibitors, a cure for neurodegenerative diseases remains elusive.⁵⁰

Recent research suggests that flavonoids, including silymarin, quercetin, and hesperidin, exhibit protective effects by defending neurons against neurotoxin-induced damage, reducing inflammation in neurons, and enhancing memory, learning, and cognitive function.⁵¹ These flavonoids operate

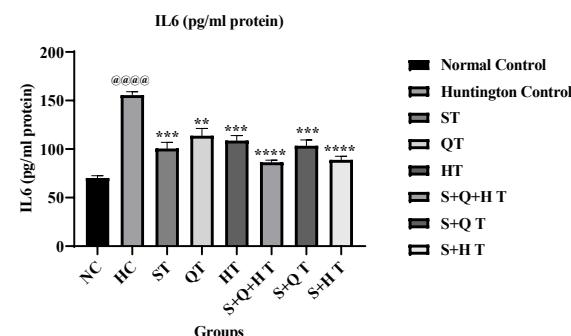


Figure 10: Result of combined silymarin, quercetin, and hesperidin on Interleukin 6 (IL6)

by inhibiting enzymes like acetylcholinesterase and beta-secretase, countering free radicals, and modulating signaling pathways crucial for cognitive and neuroprotective functions. Silymarin, specifically its active component silibinin, has shown promise as a neuroprotective agent, attributed to its capacity to mitigate oxidative stress, and influence processes such as beta-amyloid aggregation, inflammation, and cellular apoptosis in the brain.^{52,53} Silibinin has been found to enhance learning and memory.⁵⁴ Additionally, silymarin's antioxidant effects, possibly due to its antioxidant properties, have been demonstrated to enhance memory and address learning disorders.⁵¹⁻⁵⁴

CONCLUSION

Glutathione, an important endogenous antioxidant is found predominantly in its decreased form inside cells. It works by reacting with free radicals, preventing hydroxyl radical formation, and is transformed toward its oxidized form with the assistance of the enzyme glutathione peroxidase. Dysregulation of GSH metabolism in HD contributes to an imbalance in redox status. Reductions in GSH levels have been noted in the cortex of individuals with HD. This study highlighted that the most significant upsurge in GSH intensities occurred in the individual hesperidin set of animals, the combined group of all three, and the effect of silymarin with quercetin or hesperidin. SOD is a critical antioxidant enzyme involved in superoxide detoxification. In HD, cytosolic SOD activity decreases slightly.

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