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Combination effect of silymarin, quercetin, and hesperidin on anxiety and depression in 3 nitropropionic acid-induced rat model of huntington's disease

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Abstract

Huntington's disease is an autosomal-dominant, progressive neurodegenerative disorder with chorea, incoordination, cognitive decline, and behavioral difficulties, whereas bioflavonoids have the potential to promote anxiety and depression. The aim of this study was to investigate and summarize the synergistic effect of silymarin, quercetin, and hesperidin on anxiety and depression in 3-nitropropionic acid (3-NP)-induced rat model of Huntington's disease. We divided animals into eight groups ($n = 6$) for different combination treatment with said bioflavonoids along with 3-NP. Anxiety and depression such as symptoms were assessed on 21st day post-treatment with elevated plus maze, light, and dark model and force swim test. Dopamine and serotonin level in the brain striatum were also measured after completion of behavioral analysis as these neurotransmitters are involved in anxiety and depression like behavior. We found that intraperitoneal administration of 3-NP (10 mg/kg B/W) for 21 days leads to anxiety and depression such as symptoms in male Wistar rats, whereas combinations of bioflavonoids ameliorate the symptoms. At the end of the study, we concluded that combination of silymarin, quercetin, and hesperidin is more effective in ameliorating anxiety and depression as compared to mono therapy.

Keywords: 3 nitropropionic acid, Anxiety, Bioflavonoid, Depression, Huntington's disease

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Introduction

The purpose of this study is to explore the combination effect of silymarin, quercetin, and hesperidin on 3-NP-induced anxiety and depression in rat model of Huntington's disease (HD).

HD is an autosomal, dominantly inherited neurodegenerative disorder resulting from the expansion of a CAG trinucleotide repeat more than 35 times which are inversely related to age.[1,2] Various psychiatric and behavioral symptoms can occur in HD, including irritability, aggression, obsessions, compulsive behaviors, anxiety, or psychosis with depression which has a significant effect on quality of life in HD patients.[3-5] Anxiety appears to be associated with depression, suicide, irritability, quality of life, pain, illness beliefs, and coping styles.[6] Diagnosis is based on clinical manifestations in conjunction with positive family history, and confirmatory genetic testing.[7] There are no effective therapies available to treat the cognitive symptoms; therefore, cognitive dysfunction determines the quality of life of the HD patient.[8] Tetrabenazine is the only FDA-approved pharmacological agent for treatment of chorea associated with HD, but long-term use leads to depression and anxiety.[9] Although there are many compounds which showed effect in animal models of HD, no agent has been shown to modify the disease. Therapeutic interventions in HD focus on symptomatic treatment of motor, behavioral, and psychiatric disturbances.

Various toxins and genetic animal models are being used for testing of novel and safe therapies for HD. 3 nitropropionic acid (3-NP)-induced rat model of HD is found to be most suitable preclinical model.[10] Systemic administration 3-NP, an inhibitor of the mitochondrial citric acid cycle, results symptoms resembling that of HD including irritability, aggression, obsessions, compulsive behaviors, anxiety, or psychosis with depression which has a significant effect on quality of life in HD patients.[11,12]

Flavonoids are a family of polyphenolic compounds found ubiquitously in fruits and vegetables as well as in food products and beverages derived from plants.[13] These flavonoids have many therapeutic properties, such as anti-microbial, anti-oxidant, anti-inflammatory, and immune-modulatory.[14] The potential roles of flavonoids in neurodegenerative disorders were confirmed by many studies which may be due to ability of suppression of the microglial activation and reduction of neurotoxicity induced by neurotoxic species released by microglia.[15] Flavonoids are key compounds for the development of a new generation of therapeutic agents that are clinically effective in treating neurodegenerative diseases.[16]

Among natural products, silymarin, classified within the group of flavonolignans and isolated from milk thistle plant *Silybum marianum*.[17] Neuroprotective effect of silymarinin rat brain may be due to antioxidant property to attenuate oxidative neuronal damage associated with various neurodegenerative diseases, lower damage to dopaminergic neurons through inhibiting lipopolysaccharide-induced activation of microglia, reduce production of inflammatory mediators, prevent lipid peroxidation,

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and promote regenerative processes.[18-21] Silymarin treatment increased anxiety-like behavior in open field, elevated plus maze (EPM), and light–dark box in adult mice which shows development of brain and behavior.[22]

Quercetin, a plant pigment, is a potent antioxidant flavonoid and more specifically a flavonol, found mostly in onions, grapes, berries, cherries, broccoli, and citrus fruits.[23] It protects neurons from oxidative damage by reducing lipid peroxidation, dopaminergic neuronal loss, and apoptosis.[24-27] It also regulates the serotonergic and cholinergic neurotransmission which shows antianxiety and antidepressant effect and enhances memory.[28,29]

Many studies proved that hesperidin ameliorates anxiety in neurological disorders.[30,31] Hesperidin treatment reduces hemorrhage, inflammatory cell infiltration, tissue loss, and pro-inflammatory cytokines including tumor necrotic factor-α and interleukin-1β.[32,33] These finding suggest that the bioflavonoids mention above may have beneficial effect on HD and efficacy of these bioflavonoids against HD can be evaluated using 3-NP-induced rat model of HD.

The current medical therapies use pharmaceutical interventions with lifestyle modification to prevent or control HD, but no treatment is available to stop the progression of the disease. Therefore, the present study designed to investigates synergistic effect of combinatorial treatment of silymarin, quercetin, and hesperidin on anxiety and depression in 3-NP-induced HD in Wistar rats. A multi-fold increase in the number of *in vivo* and clinical studies for bioflavonoids against HD is the need of hour.

Materials and Methods

Drugs and Chemicals

Silymarin, quercetin, hesperidin, and 3-NP were purchased from Sigma–Aldrich, St. Louis, MO, USA. ELISA kit for testing of serotonin and dopamine was purchased from Krishgen biosystems, Mumbai, (MS) India. All other chemicals and biochemical reagents of analytical grade used in the study were purchased from local vendor, Pune (MS) India.

Experimental Animals

Male Wistar rats (200–250 g) were procured from Crystal biological solutions, Pune. Rats were randomly placed in rat cages with paddy husk bedding under standard laboratory conditions at temperature 23 ± 2°C with relative humidity 55 ± 10% under 12 h light and 12 h dark cycle throughout the experiment. Animals were provided with free access to water and standard laboratory feed. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee of Crystal biological solutions, Pune, constituted under Committee for Purpose of Control and Supervision of Experiments on Animals by Ministry of Environment and Forests, Government of India, New Delhi, India. Ethical guidelines were strictly followed during all the experimental procedures.

Preparation of Drugs and Chemicals

Silymarin (200 mg/kg B/W), quercetin (50 mg/kg B/W), and hesperidin (50 mg/kg B/W) were administered by oral gavage for 21 days as a suspension prepared in 0.5% Carboxymethylcellulose (CMC) (w/v) using mortar and pestle. 3-NP (10 mg/kg B/W) was administered Intraperitoneal, prepared freshly using normal saline, and administered for 21 days, 3-NP was injected 90 min after the administration of test drugs. All dosing were done in between 9 am and 11 am.

Experimental Design

After 1 week of acclimatization, animals were divided into eight groups and received treatment for 21 days. Group I (NC) – normal control received normal saline (1 ml/kg i.p.) and 0.5 % CMC (1 ml/100 g p.o.), Group II (HC) – Huntington control received 3-NP (10 mg/kg, i.p.) + 1 ml/g 0.5 % CMC p.o., and Group III (ST), IV (QT), and V (HT) received silymarin (200 mg/kg; p.o.), quercetin (50 mg/kg; p.o.), and hesperidin (50 mg/kg; p.o.), respectively, with concomitant administration of 3-NP. Group VI (S+Q+H T) received combination of silymarin (200 mg/kg; p.o.), quercetin (50 mg/kg; p.o.) and hesperidin (50 mg/kg; p.o.), Group VII (S+QT) received combination of silymarin (200 mg/kg; p.o.) and quercetin (50 mg/kg; p.o.), and Group VIII (S+HT) received combination of silymarin (200 mg/kg; p.o.) and hesperidin (50 mg/kg; p.o.) with concomitant administration of 3-NP (10 mg/kg i.p.). Doses of the test drugs and induction chemical were selected from previous literature.

Statistical Analysis

One-way analysis of variance followed by Dunnett's multiple comparison test.

Estimation of Behavioral Parameters

EPM

Apparatus was consisted of four arms in plus sign shape. Two opposite arms were open (50 × 10 cm) and the other two opposite arms were enclosed with walls (50 × 10 × 40 cm) elevated 50 cm from the floor. All animals were individually placed on the center of the plus-maze and allowed to freely explore the maze for 5 min and the time spent and number of entries in each of the two open arms and all four arms were recorded. % Time spent in open arms and % number of entries in open arms were calculated using following formulae.[34]

% Time spent in open arms = (open arms time spent/total time spent × 100)

% no. of entries in open arms = (open arms entries/total no. of entries × 100).

Light and dark model

The instrument was consisted of 2 parts, 1/3 with opaque walls and a covered (dark compartment), whereas the remaining 2/3 was open and illuminated (light compartment). The door between the two compartments permits rats to move from one side to another. Each rat was released in the light compartment and observed for 5 min. Time spent and number of entries in light compartment were recorded.[35,36]

Forced swim test

Rats were forced to swim in water filled cylinder (24–30°C) from which they cannot escape. A depth of 30 cm is commonly recommended so that animal should not touch the bottom with its tail or feet.

Animals were observed continuously for 5 min and duration as well as time of immobility was measured. Any animal that sinks below the surface was removed from the water immediately.[37,38]

Biochemical Estimation

After completion of behavioral testing on 21st day, all rats were sacrificed by cervical dislocation and brain was removed. Brain striatum was identified, separated, weighed, and used for all biochemical estimation.

Preparation of brain homogenate

Using brains striatum 10% (w/v), tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 g for 15 min. Aliquots of the supernatant were separated and used for biochemical estimation.[39]

Estimation of serotonin level in brain striatum

Estimation of serotonin level was done by ELISA method (Krishgen biosystems kit). Procedure and calculation were carried out as per the instruction given by manufacturer and expressed as ng/mg tissue sample.

Estimation of dopamine level in brain striatum

Estimation of dopamine level was done by ELISA method (Krishgen biosystems kit). Procedure and calculation were carried out as per the instruction given by manufacturer and dopamine level was expressed as ng/mg tissue sample.

Results

EPM

% time spent in open arm

Intraperitoneal administration of 3-NP for 21 days significantly reduced ($P < 0.0001$) % time spent in open arms in HC rats when compared with NC rats. Silymarin-treated rats (200 mg/kg), quercetin-treated rats (50 mg/kg), and hesperidin-treated rats (50 mg/kg) showed significant increase $P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively, in % time spent in open arms as compared to HC rats. Administration of silymarin (200 mg/kg p.o.) in combination with either quercetin (50 mg/kg p.o.) or hesperidin (50 mg/kg p.o.) produced significant increase $P < 0.01$ and $P < 0.001$ in % time spent in open arms when compared with HC rats. Further, combination treatment of silymarin (200 mg/kg p.o.) with quercetin (50 mg/kg p.o.) and hesperidin (50 mg/kg p.o.) more significantly increased ($P < 0.0001$) % time spent open arms when compared to HC rats [Figure 1].

% no. of entries in open arms

Intraperitoneal administration of 3-NP for 21 days significantly reduced ($P < 0.0001$) % no. of entries in open arms in HC rats when compared with NC rats. Silymarin-treated rats (200 mg/kg) and hesperidin-treated rats (50 mg/kg) showed significant increase $P < 0.01$ and $P < 0.05$, respectively, in % no. of entries in open arms, but quercetin-treated rats (50 mg/kg) did not produced any significance increase in % no. of entries in open arms as compared to HC rats. Administration of silymarin (200 mg/kg p.o.) in combination with either quercetin (50 mg/kg p.o.) or hesperidin (50 mg/kg p.o.) produced same significant increase ($P < 0.01$) in % no. of entries in open arms when compared with HC rats. Further, combination treatment of silymarin (200 mg/kg p.o.) with quercetin (50 mg/kg p.o.) and hesperidin (50 mg/kg p.o.) more significantly increased ($P < 0.001$) % no. of entries in open arms when compared to HC rats [Figure 2].

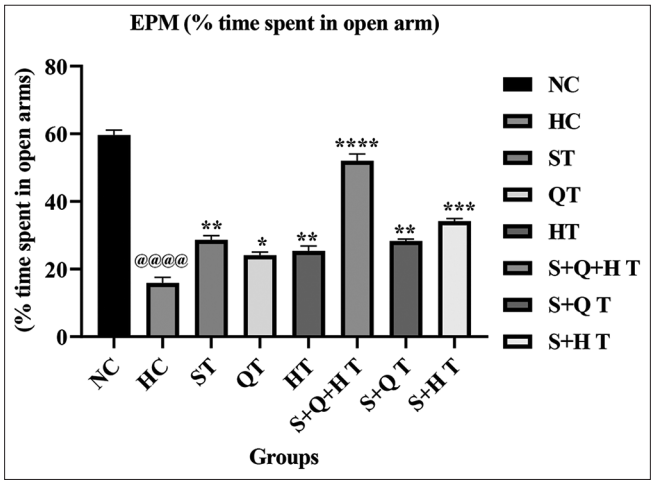

Figure 1: Effect of combination of silymarin, quercetin, and hesperidin on % time pends in elevated plus maze. Results are expressed as mean ± SEM ($n = 6$). Data were analyzed by one-way analysis of variance followed by Dunnett's multiple comparison test. @@@@$P < 0.0001$ when Huntington control compared with NC rats, *$P < 0.05$, **$P < 0.01$, *** $P < 0.001$, and ****$P < 0.0001$ when treatment groups compared with Huntington control, ST: Silymarin treated, QT: Quercetin Treated, HT: Hesperidin treated, S+Q+HT: Silymarin + Quercetin + Hesperidin treated, S+QT: Silymarin + Quercetin treated, and S+HT: Silymarin + Hesperidin treated

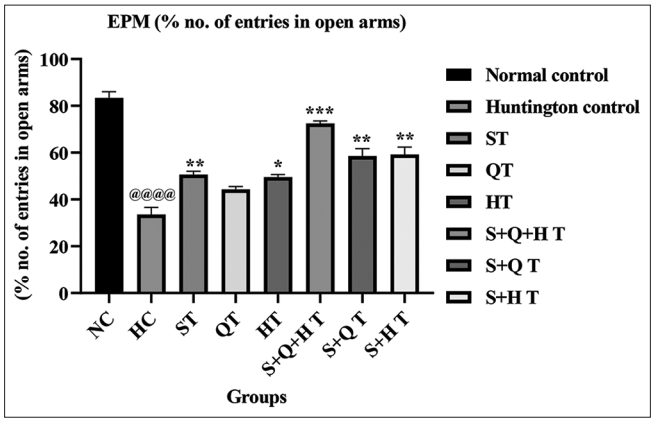

Figure 2: Effect of combination of silymarin, quercetin, and hesperidin on % number of entries in open arm in elevated plus maze. Results are expressed as mean ± SEM ($n = 6$). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. @@@@$P < 0.0001$ when Huntington control compared with NC rats, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ when treatment groups compared with Huntington control, ST: Silymarin treated, QT: Quercetin Treated, HT: Hesperidin treated, S+Q+HT: Silymarin + Quercetin + Hesperidin treated, S+QT: Silymarin + Quercetin treated, and S+HT: Silymarin + Hesperidin treated

Light and Dark Model

Time spent in light compartment

Intraperitoneal administration of 3-NP for 21 days exhibited significant depletion ($P < 0.001$) in time spent in light compartment in HC rats when compared with NC rats. Administration of silymarin (200 mg/kg) significantly increases ($P < 0.05$) time spent in light compartment as compare to HC rats, but administration of quercetin (50 mg/kg) or hesperidin (50 mg/kg) as a single drug therapy did not cause any significant changes in time spent in light compartment as compare to HC rats. Administration of silymarin (200 mg/kg p.o.) in combination with either quercetin (50 mg/kg p.o.) or hesperidin (50 mg/kg p.o.) produces same significant increased (0.01) time spent in light compartment when compared with HC rats. Further, combination treatment of silymarin (200 mg/kg p.o.) with quercetin (50 mg/kg p.o.) and hesperidin (50 mg/kg p.o.) more significantly increased ($P < 0.001$) time spent in light compartment when compared to HC rats [Figure 3].

Number of entries in light compartment

Intraperitoneal administration of 3-NP for 21 days exhibited significant depletion ($P < 0.001$) in no. of entries in light compartment in HC rats when compared with NC rats. Administration of silymarin (200 mg/kg) or hesperidin (50 mg/kg) significantly increased ($P < 0.05$) no. of entries in light compartment as compare to HC rats, but administration of quercetin (50 mg/kg) did not cause any significant changes in no. of entries in light compartment as compare to HC rats. Administration of silymarin (200 mg/kg p.o.) in combination with either quercetin (50 mg/kg p.o.) or Hesperidin (50 mg/kg p.o.) produce significant increased (0.01) no. of entries in light compartment when compared with HC rats Further, combination treatment of silymarin (200 mg/kg p.o.) with quercetin (50 mg/kg p.o.) and hesperidin (50 mg/kg p.o.) more significantly increased ($P < 0.001$) time spent in light compartment when compared to HC rats [Figure 4].

Force Swim Test (FST)

Time of immobility

Intraperitoneal administration of 3-NP for 21 days exhibited significant increase in $P < 0.0001$ in time of immobility in HC rats when compared with NC rats. Silymarin-treated rats (200 mg/kg), quercetin-treated rats (50 mg/kg), and hesperidin-treated group (50 mg/kg) show significant decreased in time of immobility $P < 0.05$, $P < 0.01$, and $P < 0.05$, respectively, as compared to HC rats. Administration of silymarin (200 mg/kg p.o.) in combination with either quercetin (50 mg/kg p.o.) or hesperidin (50 mg/kg p.o.) produced significant decrease in time of immobility ($P < 0.01$) when compared with HC rats. Further, combination treatment of Silymarin (200 mg/kg p.o.) with Quercetin (50 mg/kg p.o.) and hesperidin (50 mg/kg p.o.) more significantly decreased ($P < 0.001$) time of immobility when compared to HC rats [Figure 5].

Struggling Time

Intraperitoneal administration of 3-NP for 21 days exhibited significant decrease in ($P < 0.0001$) struggling time in HC rats when compared with NC rats. Silymarin-treated rats (200 mg/kg), quercetin-treated rats (50 mg/kg), and hesperidin-treated group (50 mg/kg) showed significant increase in struggling time $P < 0.05$, $P < 0.01$, and $P < 0.01$, respectively, as compared to HC rats. Administration of silymarin (200 mg/kg p.o.) in combination with either quercetin (50 mg/kg p.o.) or hesperidin (50 mg/kg p.o.) produced significant increase in struggling time $P < 0.001$ and 0.01, respectively, when compared with HC rats. Further, combination treatment of silymarin (200 mg/kg p.o.) with quercetin (50 mg/kg p.o.) and hesperidin (50 mg/kg p.o.) produced more

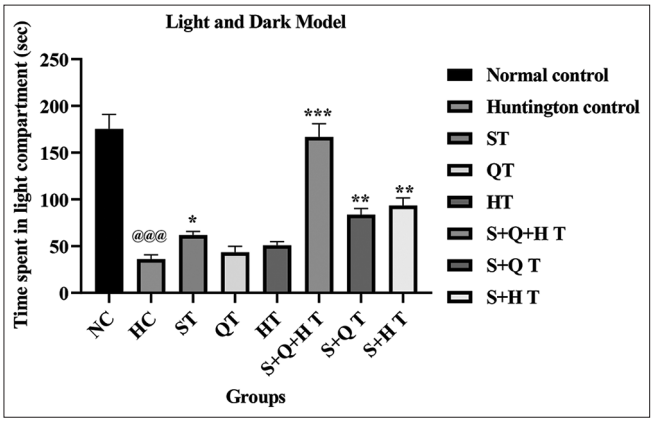

Figure 3: Effect of combination of silymarin, quercetin, and hesperidin on time spent in light compartment in light and dark model. Results are expressed as mean ± SEM ($n = 6$). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. @@@$P < 0.0001$ when Huntington control compared with NC rats, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ when Treatment groups compared with Huntington control, ST: Silymarin treated, QT: Quercetin Treated, HT: Hesperidin treated, S+Q+HT: Silymarin + Quercetin + Hesperidin treated, S+QT: Silymarin + Quercetin treated, and S+HT: Silymarin + Hesperidin treated

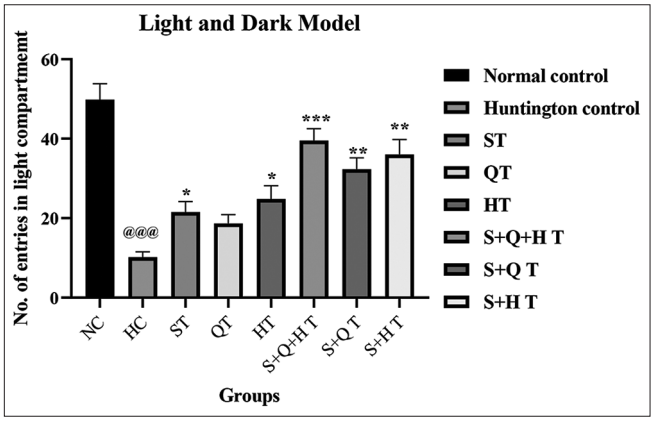

Figure 4: Effect of combination of silymarin, quercetin, and hesperidin on number of entries in light compartment in light and dark model. Results are expressed as mean ± SEM ($n = 6$). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. @@@$P < 0.001$ when Huntington control compared with NC rats, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ when treatment groups compared with Huntington control, ST: Silymarin treated, QT: Quercetin Treated, HT: Hesperidin treated, S+Q+H T: Silymarin + Quercetin + Hesperidin treated, S+Q T: Silymarin + Quercetin treated, and S+H T: Silymarin + Hesperidin treated

significant ($P < 0.0001$) increase in struggling time when compared to HC rats [Figure 6].

Serotonin Level

Intraperitoneal administration of 3-NP for 21 days exhibited significant decrease in ($P < 0.001$) serotonin level in HC rats when compared with NC rats. Silymarin-treated rats (200 mg/kg), quercetin-treated rats (50 mg/kg), and hesperidin-treated group (50 mg/kg) show significant increase in serotonin level $P < 0.01$, $P < 0.05$, and $P < 0.05$, respectively, as compared to HC rats. Administration of silymarin (200 mg/kg p.o.) in combination with either quercetin (50 mg/kg p.o.) or hesperidin (50 mg/kg p.o.) produced same significant increase in serotonin level ($P < 0.01$) when compared with HC rats. Further, combination treatment of silymarin (200 mg/kg p.o.) with quercetin (50 mg/kg p.o.) and hesperidin (50 mg/kg p.o.) produced more significant ($P < 0.001$) increase in serotonin level when compared to HC rats [Figure 7].

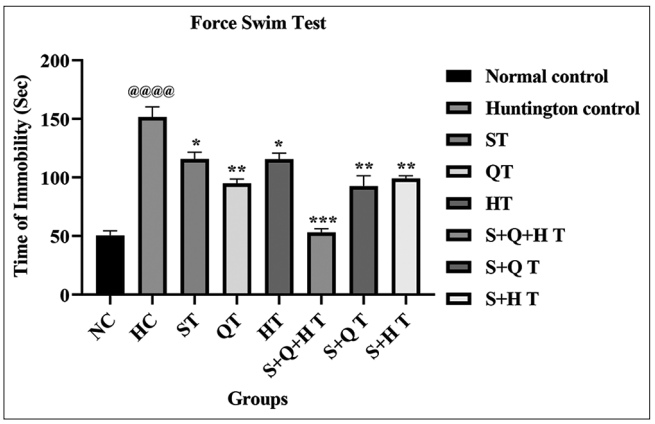

Figure 5: Effect of combination of silymarin, quercetin, and hesperidin on time of immobility in force swim test model. Results are expressed as mean ± SEM ($n = 6$). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. @@@@ $P < 0.0001$ when Huntington control compared with NC rats, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ when Treatment groups compared with Huntington control, ST: Silymarin treated, QT: Quercetin Treated, HT: Hesperidin treated, S+Q+H T: Silymarin + Quercetin + Hesperidin treated, S+Q T: Silymarin + Quercetin treated, and S+H T: Silymarin + Hesperidin treated

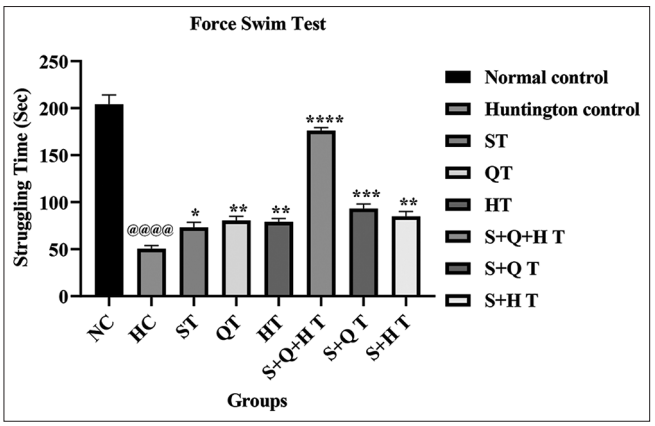

Figure 6: Effect of combination of silymarin, quercetin, and hesperidin on struggling time in force swim test model, results are expressed as mean ± SEM ($n = 6$). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. @@@@$P < 0.0001$ when Huntington control compared with NC rats, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ when treatment groups ompared with Huntington control, ST: Silymarin treated, QT: Quercetin Treated, HT: Hesperidin treated, S+Q+H T: Silymarin + Quercetin + Hesperidin treated, S+Q T: Silymarin + Quercetin treated, and S+H T: Silymarin + Hesperidin treated

Dopamine Level

Intraperitoneal administration of 3-NP for 21 days exhibited significant decrease in ($P < 0.0001$) dopamine level in HC rats when compared with NC rats. Silymarin-treated rats (200 mg/kg), quercetin-treated group (50 mg/kg), and hesperidin-treated group (50 mg/kg) showed significant increase in dopamine level $P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively, as compared to HC rats. Administration of silymarin (200 mg/kg p.o.) in combination with either quercetin (50 mg/kg p.o.) or hesperidin (50 mg/kg p.o.) produced significant increase in dopamine level $P < 0.001$ and $P < 0.001$, respectively, when compared with HC rats. Further, combination treatment of silymarin (200 mg/kg p.o.) with quercetin (50 mg/kg p.o.) and hesperidin (50 mg/kg p.o.) produced more significant ($P < 0.0001$) increase in dopamine level when compared to HC rats [Figure 8].

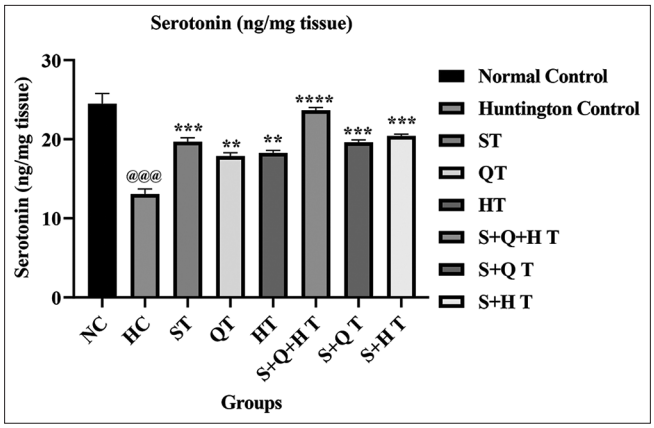

Figure 7: Effect of combination of silymarin, quercetin, and hesperidin on serotonin level, results are expressed as mean ± SEM ($n = 6$). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. @@@$P < 0.001$ when Huntington control compared with NC rats, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ when treatment groups compared with Huntington control group, ST: Silymarin treated, QT: Quercetin Treated, HT: Hesperidin treated, S+Q+H T: Silymarin + Quercetin + Hesperidin treated, S+Q T: Silymarin + Quercetin treated, and S+H T: Silymarin + Hesperidin treated

Discussion

Many studies proved that major depressive disorder is a major symptom of HD during the pre-motor symptomatic stages of the disease.[40] The diagnosis is difficult, but, usually, there is low self-esteem, feelings of guilt, and anxiety which may leads to suicidal nature.[41,42] The mechanism is not fully known, but abnormality in tryptophan mechanism leads to lower cerebrospinal fluid level of 5 hydroxy indole acetic acid which is the most accepted mechanism

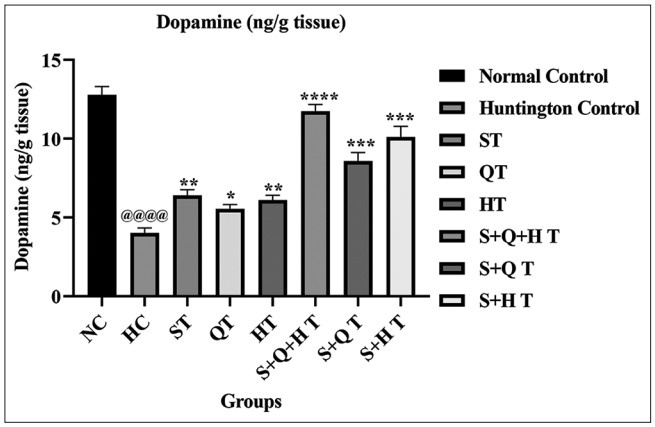

Figure 8: Effect of combination of silymarin, quercetin, and hesperidin on dopamine level, results are expressed as mean ± SEM ($n = 6$). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. $^{@@@@}P < 0.0001$ when Huntington control compared with NC rats, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ when treatment groups compared with Huntington control, ST: Silymarin treated, QT: Quercetin Treated, HT: Hesperidin treated, S+Q+H T: Silymarin + Quercetin + Hesperidin treated, S+Q T: Silymarin + Quercetin treated, and S+H T: Silymarin + Hesperidin treated

for suicidal tendency found in HD patient.[43] Moreover, treatment is complicated with the side effects of the drugs available such as tetrabenazine itself increase the risk of depression and suicide.[44] Despite of these, there are no estimated guidelines for treatment of depression and anxiety in patients with HD. Hence, our study provides a new insight into the protective effects of bioflavonoids in the treatment of anxiety and depression in HD.

As per the previous agreement, silymarin has been recently reported to exert neuroprotective activities against neurodegenerative diseases may be due to inhibition of neuroinflammation, apoptosis, and reduced TNF-α level.[45] The beneficial effects of silymarin in ameliorating memory impairments, anxiety, and depression were evaluated in rat model of post-traumatic stress disorder and neuroinflammation after mild traumatic brain injury in mice.[46,47] Quercetin has received the most attention as it provides supportive evidences for neuroprotective effects in neurodegenerative diseases.[48-50] Exact mechanism is not known but may be due to re-establishment of redox regulation of proteins and transcription factors; survival of signaling cascades; inhibition of kinases; and catechol oxidation products such as semiquinones, down-regulation of cytokines through nuclear factor, and quinones that could alter redox homeostasis, etc.[51,52] Moreover, hesperidin exerts anxiolytic-like and antidepressant-like effect through the modulation of cytokine production, neurotrophic factors levels, and dopaminergic innervation in the striatum.[31]

Based on these evidences, we used combinations of silymarin, quercetin, and hesperidin to study synergistic effect in alleviating anxiety and depression in 3-NP-induced rat model of HD.

In our study, we found that administration of 3-NP at a dose of 10 mg/kg for 21 days can significantly induce in rat model of HD. We used FST to evaluate the depression, whereas EPM and light and dark model help us to evaluate anxiety like behavior in rats. Along with this, we measured level of serotonin and dopamine in rat brain striatum responsible for anxiety and depression like symptoms in HD patients.

Forced swim test, one of the most widely used behavioral test to assess depressive-like behavior in animal models and for the screening of potential antidepressant treatments is based on the principle that animals develop an immobile posture in a non-escapable cylinder filled with water when they are in depressive phase. Increased immobility (floating) and decreased struggling time are interpreted as a behavioral correlate of negative mood, representing a kind of depression in the animal.[53] In our study, we evaluate duration of immobility as well as struggling time. Administration of 3-NP at a dose of 10 mg/kg significantly increased duration of immobility and decreased struggling time. silymarin, quercetin, and hesperidin as a single drug therapy was proven significantly effective, but quercetin was more effective as compared to other bioflavonoids. Combination of all three drugs was most effective in decreasing immobility and increasing struggling time as compared to any single drug therapy.

Light and dark model is one of the best animal models to measure the anxiety in animal. Reduced exposure of rats into light area considered as an anxiety like behaviour.[54] In our study, we found that only silymarin is significant as a single drug therapy to ameliorate anxiety, whereas combinations were found best. EPM is validated to assess the anti-anxiety effects of pharmacological agents. An increase in open arm activity (duration and/or entries) reflects anti-anxiety behavior.[55,56] In our study, all three bioflavonoids are significant in increasing open arm activity, but combinations were more significant.

Many hypotheses propose that diminished activity of serotonin pathways plays a role in the pathophysiology of depression and anxiety.[57-59] The previous study suggests that the level of serotonin decreases in HD which is one of the reasons for depression.[39,60] Many theories suggest that depletion of dopamine leads to major depressive disorders and *vice versa*.[61-63] In the early stages, dopamine neurotransmission is increases leading to hyperkinetic movements, whereas in the late-stage, dopamine deficits produce hypokinesia.[64] The exact mechanism is unknown but may include initial upregulation of dopamine neuron activity caused by the genetic mutation, reduced inhibition resulting from striatal medium spiny neuron loss, increased excitation from cortical inputs, and dopamine autoreceptor dysfunction.[65] In our study, we found that combination of these bioflavonoids is more significant to increase the serotonin and dopamine level as compare to single drug therapy.

There is no treatment that can halt or even slow down the progression of HD. The current effective treatments aim to ease the symptoms such as anxiety and depression of this disease. Results from these studies provide new viewpoints on the disease itself and, sometimes, novel approaches for its treatment, as well as for future research. Risk assessment and pharmacokinetics of flavonoids are essential parameters that need to be explored for their clinical use. Hence, a multi-fold increase in the number of *in vivo* and clinical studies is the need of the hour.

Conclusion

HD is a neurodegenerative genetic, in which striatum and hippocampus are mostly affected, because nerve cells of the striatum are the first to die as HD progresses. There is no treatment completely available to stop the progression of the disease. Experimental models have begun to uncover the pathways, thus help to understand the mechanisms implicated and allowing for the characterization of potential targets for new therapeutic strategies. Thus, our study,

we observed that administration of 3-NP for 14 days induced clinical symptoms like HD. Results indicated silymarin, quercetin, and hesperidin can increase exposure time in light area in EPM and increase struggling time in FST when used as monotherapy. The observations also depicted that elevated dopamine and serotonin level were responsible for depression. However, the combination treatment showed more significant improvement than the single drug therapy. Hence, we conclude that combination therapy of silymarin, quercetin, and hesperidin is more beneficial in anxiety and depression in HD as compared to mono therapy.

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