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ESTABLISHING NEUROPROTECTIVE INSIGHTS: IN-VIVO EXPLORATION OF QUERCETIN'S THERAPEUTIC POTENTIAL IN AUTISM INDUCED RATS MODEL

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Abstract
To relate the neuroprotective potential of Quercetin within the Autism-Induced neurodegenerative rats model and introduce novel insights of serum Brain-derived neurotrophic factor BDNF levels. Following the approval of the study, in-vivo experiments were conducted. Twenty-five healthy male rats, seven weeks old, of the Sprague-Dawley strain were selected and to categorize them five groups were formed. Group I was the physiological / non-induced rats where n=5 while other groups of various sizes of n=20 were administrated with intraperitoneal injections of propionic acid (250 mg/kg/day for 5 days). Group II (induced with PPA), Groups III, IV, and V used Quercetin at concentrations of 100 mg/kg, 200 mg/kg, and 400mg/kg respectively a regular diet for a total of 24 days. The effect of quercetin was determined in terms of BDNF level by enzyme-linked immunosorbent assay ELISA. Statistical analysis was conducted where One-way ANOVA (Post hocTurkey test) was used. The levels of BDNF were observed to be back in the quercetin-treated groups (III, IV and V) as compared with group (II)inferring that Quercetin therapy can heal the autism-like symptoms. The levels measured BDNF (ng/ml) were as follows, Group I (13.1 ± 0.3), II (5.1 ±0.2), III (9.8 ±0.3), IV (8.0 ±0.3) and V (10.1 ±0.3). The group induced by the treatment with PPA was PPA+200 mg/kg Quercetin. It also showed the difference between the groups using a one-way ANOVA. To be specific, BDNF levels in Groups III, IV and V were higher than in Group II (PPA-induced) as shown by the Posthoc Turkeys test at a significance level of p<0. 05. Quercetin was found to interact with PPA-induced Autism Mouse models proportional to the recovery in serum BNDF levels.

Keywords: *Autism, BDNF, Neurodegeneration, Propionic acid (PPA), Quercetin*

INTRODUCTION

A group of neurodevelopmental disorders known as autism spectrum disorder are distinguished by severe deficits in social interactions, trouble communicating, and repetitive activities (1). ASD is now estimated to impact 1 in 54 children in the United States, with a greater frequency in boys than in girls (2). ASD is a complicated and multidimensional etiology that is caused by a confluence of genetic, environmental, and neurological variables. It is beneficial for exploring potential treatments to reduce the neurodevelopment issues in Autism (3). One kind of bioflavonoid quercetin that can be detected in oranges, lemons, and grapefruits has caused a great amount of interest because of its various pharmacological properties (4). Quercetin has a strong neuroprotective profile through capabilities to modify most of the signaling pathways, reduce the level of oxidative stress, and inhibit neuroinflammation (5). In the body of the publications of the past two years, there was evidence that quercetin affects the level of Brain-derived

Neurotrophic Factor (BDNF) in the blood, which is the protein that has a critical role in the brain structural plasticity, neurodegeneration, and cognition (6).

Thus, BDNF plays a critical role as far as the development and survival of neurons in the neurological system to be supported and maintained (7). All these pathways promote synaptic plasticity as well as neuronal survival and differentiation and all these facets enforce neuroprotection. BDNF assists in strengthening the features of synaptic connections hence rectifying the synaptic abnormalities and the cognitive dysfunctions associated with the ASD (8). Moreover, through regulation of the respective BDNF levels for synapse, BDNF impacts on synaptic plasticity. BDNF reduces oxidative stress and neuroinflammation, which are the components of the neurodegenerative disease including the ASD. Harmful effects of Reactive Oxygen Species ROS are reduced by antioxidant as well as anti-inflammatory activity (9, 10). Sprague-Dawley (S-D) rats were chosen for this research due to their well-documented neurodevelopmental responses in experimental models and their genetic consistency which provides for the reproducibility of results. In addition, S-D rats have predictable behavior patterns and uniformly robust physiological response, making them an excellent model to study changes induced by ASD and therapeutic interventions. Thus, this work contributes to the growing literature on the role of phytochemicals as potential therapeutic treatments for neurodevelopmental disorders through an analysis of quercetins' impact on BDNF levels in a PPA-induced autism model. The results highlighted the role that quercetin in supporting neurodevelopmental health and may open the door to the creation of novel, all-natural treatment approaches for ASD.

METHODOLOGY

After Research Ethical Committee approval (REC-785/12/07/2019), this inter-collaborative study and analysis (July, 2019 to June, 2020) was carried out in Animal House of affiliated Institutes interlinked with diagnostics centers in Punjab, Pakistan. Male rats (n=5x5=25) in good health (7 weeks old) with an average weight (28–35 grams) were involved. For all groups, Treatment periods (25 days) and experimental scheme (n=5x5) is as follows:

Group I: (Non-induced/Regular rats) Negative control group feed on a regular diet.
Group II: (Autism –induced) Positive control group (no treatment) on PPA only
Group III: (Autism-induced) Treatment group 1(100mg/kg Quercetin)
Group IV: (Autism-induced) Treatment group 2 (200mg/kg Quercetin)
Group V: (Autism-induced) Treatment group (400mg/kg Quercetin)

For the experiment, rats were acclimatized for one week then the experiment was started. They were thoughtfully handled to reduce stress to a minimum while doing regular things such as feeding, cage cleaning and administration of treatment. They were fed ad libitum a standard laboratory chow diet and water. The rats were housed under standard temperature and pressure (STP) conditions at a constant temperature of 22°C ± 2°C and 40–60% relative humidity on a 12-hour light/dark cycle.

Such environment was hygienic and cages were cleaned and sterilized regularly. An officer would check daily for discomfort or illness. Pain relief measures were administered unless the suffering could not be minimized with such measures; ethical considerations were taken care of. Intra-peritoneal injection of PPA 250 mg/kg/day in normal saline for five days to induce Autism with. Normal saline was used as the solvent for PPA due to its physiological compatibility with the body, ensuring safe and effective delivery without causing additional chemical reactions or irritation. After intra-peritoneal injection, rats were maintained under observation. BDNF levels were measured after giving rats various dosages of Quercetin for a whole day. BDNF levels: ELISA kits (Catalogue#KE00096-Proteintech-Germany) were used to record the BDNF levels for the quantitative measurement. Sterile blood was collected for quantitative determination of BDNF levels with assays performed according to the manufacturer's protocol.

Version 21 of SPSS will be used to analyze the data. Post hoc testing and analysis of ANOVA was used to analyze the variables. When $p<0.05$, statistical significance is reached.

RESULTS

Mean BDNF levels are presented in Fig. 1 and Table I as accumulative data were used in the evaluation. With regard to the neuro-degeneration recovery index in the treated rats, in group III, IV, and V, BDNF levels were elevated in all groups with the increase in Quercetin concentration, and therefore, Quercetin was confirmed as a neuro-protectant. The mean values of the BDNF levels that were statistically identified and compared with the non-significance of ($p<0.05$) are as follows: The BDNF levels were paralleled with the positive control group with maximum recovery in BDNF level of group V in ng/ml (10.1±0.3)

Table I. Quantitative measurements of Quercetin's therapeutic potential via increased BDNF (ng/ml) levels

Groups (Parameter)	**I (NC)**	**II (PC)**	**III (QT1)**	**IV (QT2)**	**V (QT3)**	**Statistics** (P-value)
BDNF Levels (ng/ml)	13.1 ±0.3	5.1 ± 0.2	9.8 ± 0.3	8.0 ± 0.3	10.1 ± 0.3	0.05
Doses and Treatments	On a regular diet only	On PPA only	100mg/kg Quercetin treatment	200mg/kg Quercetin treatment	400mg/kg Quercetin treatment	

Table I shows Accumulative mean values + standard error of BDNF levels (ng/ml). Results are accumulative mean NC= Negative control, PC= Positive control, QT1=100mg/kg of Quercetin, QT2= 200mg/kg Quercetin, and QT3=400mg/kg Quercetin. Mean values of BDNF levels were found significant with a p-value of ($p<0.05$).

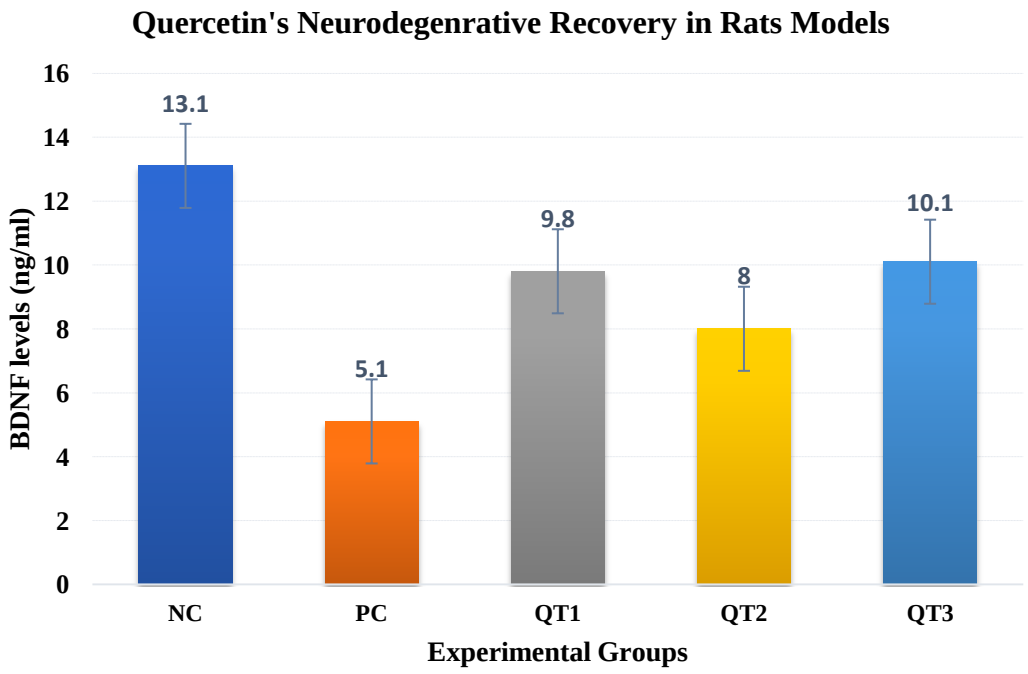

Fig. 1. Comparative analysis of *BDNF* levels in the experimental groups

NC = Negative control, **PC** = Positive control, **QT1** = Treatment group 1 (100mg/kg), **QT2** = Treatment group 2 (200mg/kg), and **QT3** = Treatment group 3 (400mg/kg).

BDNF levels (ng/ml) in the experimental groups:

- **Group I (NC)**: 13.1 ± 0.3
- **Group II (PC)**: 5.1 ± 0.2
- **Group III (QT1)**: 9.8 ± 0.3
- **Group IV (QT2)**: 8.0 ± 0.3
- **Group V (QT3)**: 10.1 ± 0.3

DISCUSSION

Autism spectrum disorder (ASD) is a complicated neurodevelopmental illness that is known to exhibit oxidative stress and inflammatory symptoms. Flavonoids, which are derived from plants and have

been extensively researched, are a class of compounds that have neuroprotective, anti-inflammatory, and antioxidant qualities (11). In an experiment, rodents were given valproic acid to cause symptoms similar to autism. The rats were then given flavonoids, which demonstrated antioxidant, anti-inflammatory, and neuroprotective qualities that helped to lessen the symptoms of autism (12). The current work demonstrates the significant neuroprotective effects of quercetin in a mice-based model of propionic acid-induced autism. With the intention of finding out the brain-derived neutrophilic factor (BDNF) levels. The results show that quercetin therapy, as opposed to PPA-induced groups, raises BDNF levels in treated groups, which in turn considerably lowers the symptoms of autism-like illnesses. According one study quercetin stimulates the BDNF pathway, which raises BDNF levels, and it also possesses neuroprotective and oxidative qualities (13). This outcome is in line with recent research that suggests natural compounds may be useful in treating neurodevelopmental problems.

Reduced BDNF levels have apparently been connected to a range of neuropsychiatric and neurodevelopmental problems, including autism disorders (14). In our investigation, the neurotoxic effects of PPA were clearly visible since the PPA-induced group's BDNF levels were considerably lower. The highest dose of quercetin (400 mg/kg) almost completely restored BDNF levels in a dose-dependent manner, indicating that quercetin's capacity to raise BDNF levels may govern its neuroprotective effects, at least in part (15).

These results exhibit that the distinctions of BDNF level in every group measured by one-way ANOVA ($p < 0.05$) and supported by the post hoc Turkeys test ($p < 0.05$) prove the efficacy of quercetin's effect. These statistical investigations indicate that 200 mg/kg and 400 mg/kg effectively enhance BDNF level, thus pointing to quercetin application for treating the ASD. A number of current investigations have highlighted the part BDNF plays in maintaining the health and function of the brain. Researchers have shown that drugs that increase BDNF levels may be able to lessen the symptoms associated with neurodevelopmental disorders (16, 17, 18). Likewise, our findings indicate that the naturally occurring flavonoid quercetin may significantly raise BDNF levels, mitigating the neurotoxic effects of PPA and improving behavioral outcomes in autistic models.

Quercetin's anti-inflammatory and antioxidant properties might be the cause of its neuroprotective effects (19). Quercetin has been shown to reduce oxidative stress and inflammation, two significant elements in the pathophysiology of ASD (20). Quercetin contributes to improvement of oxidative stress and drives the pro-inflammatory state that may create more favorable condition for brain activity. As it reduces the level of oxidative stress and also helps in regulating inflammation it may be good for overall health of the brain. This leads to the development of environment that could prevent the neurodegenerative diseases and deterioration of cognition. This would enhance neurobehavioral outcomes and boost BDNF production. These findings are consistent with other studies that suggested flavonoids, such as quercetin, may alter significant neurotrophic variables and mitigate the effects of neurotoxic shocks. These results from this in vivo study provide promising prospects for advancing quercetin as a therapeutic agent for neurodevelopmental disorders including ASD. Moving forward, clinical trials based on these findings are crucial to determine the safe and effective application of quercetin in humans. These results point to the possibility of neuropharmaceutical approaches to modulate BDNF and diminish neuroinflammation as well as oxidative stress to lead to novel treatments for ASD and other neurodevelopmental conditions.

CONCLUSION

Our study showed that the increased BNDF levels in a mouse model (PPA-induced autism) supported the neuroprotective effects of dose-dependent response of Quercetin. Our findings highlighted the Quercetin's therapeutic potential in managing the ASD.

Conflict of Interest:

Authors declared no conflict of interest.

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