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Original Article

BEHAVIORAL STUDIES OF WISTAR RATS IN ROTENONE INDUCED MODEL OF PARKINSON'S DISEASE

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ABSTRACT

Objective: The objective of the study was to determine the behavioral activities of Wistar rats induced with rotenone.

Methods: Thirty-six male Wistar rats were taken for the study and divided into six groups of six rats each. Group-I is the vehicle-treated, Group–II animals were induced with rotenone (3 mg/kg/bwt) by i. p. Group-III were co-treated with rotenone and L-DOPA (10 mg/kg/bwt) orally, Group-IV were co-treated with rotenone and quercetin (25 mg/kg/bwt) orally, Group-V were co-treated with rotenone and hesperidin (50 mg/kg/bwt) orally, Group-VI were treated with rotenone, quercetin and hesperidin in the same dosage regime for 60 d. The behavioural tests, such as open field test, ladder climbing test and hanging wire test were performed. The biochemical parameters such as urea, creatinine and activities of ALT and AST were also analysed.

Results: All data are expressed as the mean±SD. Disability was noted in the behaviour of rats induced with Parkinson's disease (PD). The deficits in behavioral activity were significantly changed when compared with an induced group ($p<0.001$) and biochemical parameters due to rotenone were significantly ($p<0.001$) restored by co-treatment with quercetin and hesperidin.

Conclusion: In our *in vivo* study, we have demonstrated the combination of quercetin and hesperidin to serve as neuroprotective compounds by improving the behavioral abnormalities and restoring the biochemical parameters. Hence, these powerful antioxidants may protect brain cells.

Keywords: Rotenone, Parkinson's disease, Quercetin, Hesperidin, L-DOPA, Behavioural study, Biochemical parameters

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INTRODUCTION

Parkinson's disease (PD) is one of the most common chronic neurodegenerative disorder. It is characterized by a variety of motor impairment like bradykinesia, rigidity, tremor and postural instability. Pathologically, Parkinson's disease(PD) is characterized by the severe loss of dopaminergic (DAergic) neurons in the substatina nigra pars compacta (SNpc) and the presence of Lewy bodies (LBs),which are present in neurons of the central nervous system [1, 3].

Mitochondria is involved in electron transport chain and rotenone is interfering in this pathway and it leads to mitochondrial damage. It inhibits the electron transfer from the center of iron-sulfur in complex I to ubiquinone. Rotenone interferes with the ATP synthesis [4].

Rotenone is toxic to mammals and humans and dangerous to insects and fishes. Rotenone is toxic to erythrocytes *in vitro* [5]. Rotenone-induced oxidative damage causes dopaminergic neuronal death [6] neuronal death occur in Parkinson's diseases [7].

Once L-DOPA has entered the central nervous system, it is converted into dopamine by the enzyme aromatic L-amino acid decarboxylase also known as L-DOPA. Besides the central nervous system, L-DOPA is also converted into dopamine within the peripheral nervous system [8].

Further, chronic administration of rotenone, a well-characterized high affinity potent and specific inhibitor of mitochondrial complex-1[9] has been demonstrated to produce nigrostriatal dopaminergic neurodegeneration [10].

In Parkinson's disease which is a degenerative disease of the central nervous system manifested by a depletion of dopamine-producing cells the oxidative stress, inflammation and mitochondrial dysfunction have been implied as major contributors to the brain cell loss. The findings from *in vitro* and *in vivo* studies have demonstrated that hesperidin is able to attenuate reduction in levels of cellular antioxidant enzymes, dopamine and prosurvival proteins all of which would indicate its usefulness in Parkinson's disease treatment [11, 13].

Quercetin it's have a strong antioxidant. It's is present in apples and in vegetables appear to protect brain cells against oxidative stress, a tissue-damaging process linked with Alzheimer's disease and other neurodegenerative diseases [14]. Quercetin via its COMT and MAO enzymes inhibiting properties might potentiate the anti-catabolic effect of L-DOPA plus carbidopa treatment. That fore quercetin could serve as an effective adjunct to L-DOPA therapy in Parkinson's disease [15].

Free radicals produced during oxidative stress due to inhibition of mitochondrial complex-1 could be responsible for the oxidative damage generated in dopamine (DA) metabolism, which further yields reactive oxygen species (ROS) [16,17,18] resulting in various diseases. In this study, we investigated the neuroprotective effect of quecetin and hesperidin against rotenone-induced animal model for Parkinson's disease (PD) by analysing biochemical changes along with a behavioral alteration in a rat model.

MATERIALS AND METHODS

Animal design

Adults Wistar albino rats (Male), weighing between 150-180g were maintained at centre for laboratory animal technology and research, Sathyabama University-India. The animals were maintained in standard environmental conditions and provided with commercial food and water *ad libitum.* All protocols were approved by the institutional animal ethics committee of the Sathyabama University India. [IAEC NO: SU/CLATR/IAEC/VI/032/2016].

Drugs and chemicals

Rotenone, quercetin and hesperidin were purchased from sigma Aldrich. All other chemicals used were of analytical grade.

Experimental methodology

The animals were equally divided into six experimental groups. Each group contains six animals.

Group-I Animal served as a vehical control (DMSO+corn oil).

Group-II Rats that received rotenone (ROT) intra peritoneally (i. p) administered a single daily dose of (3 mg/kg/bwt) for 60 d.

Group-III Animals was treated with a dose of rotenone at 3 mg/kg b. wt+L-DOPA (10 mg/kg b. wt) for 60 d daily.

Group-IV Animals were treated daily with rotenone (3 mg/kg b. wt i. p) and oral administation of quercetin (20 mg/kg b. wt) for 60 d daily.

Group-V Animals were daily treated with single dose of rotenone at 3 mg/kg b. wt and administration of hesperidin (50 mg/kg b. wt) for 60 d.

Group-VI Rat served as a co-treated daily with a single dose of rotenone+quercetin+hesperidin as mentioned above.

Behavioral studies

Open field test

The open field test was carried out to [19] provide measures of locomotion, exploration and anxiety. Each rat was placed into the centre of an open field apparatus (a circular wooden box with a diameter 72x72 cm with 36 cm height with floor divided into 16 regions). Rats were assessed individually for 5 min, and three parameters were analyzed (i) Ambulation: Number of gride lines the rat crossed with four paws. (ii) Rearing: Number of times the rat stood on their hind paws and (iii) Grooming: Duration of time the rat spent licking or scratching itself while stationary.

The apparatus was cleared with a 5% ethanol solution before behavioral testing to eliminate possible bias due to odors left by the previous rat.

Ladder climbing test

The grip walk apparatus with the 2 cm apart was inclined to a 45 ° angle. The rat was placed in the ladder and made to walk on the apparatus and movement was noticed. The score represents the muscles activity [20].

Hanging wire test

This test was used to assess forelimb strength. The apparatus consisted of a stainless steel wire (90 cm) length, 3 mm in diameter, fixed horizontally between two vertical supports and 60 cm above a soft padded surface. The wire hang test was carried out on the rat and was forced to grasp the central position of the wire with its forepaws. The latency(s) to fall from the wire to the flat soft pad was measured. When the latency time was over 120s, the rat was released from the wire and the time was recorded as 120s. The trial was conducted three times for each rat and the longest duration was the value used for evaluation. The resting pause between consecutive attempts was 3 min [21].

Biochemical parameters

Experimental rats were anesthetized. A blood sample was withdrawn by retino orbital and allowed to clot for 30 min by keeping undisturbed at room temperature serum was separated by centrifugation. Aspartate and alanine transaminases (AST and ALT) were assayed according to the method of King J [22]. Serum urea was determined by the method of Natelson. S [23]. Serum creatinine was determined in the method of Jaffe [24] respectively.

Statistical analysis

Results were expressed as mean±Standard. Statistical significance was determined by one-way (ANOVA). The data obtained from the studies were analyzed using student's t-test. P values less than P<0.05 were considered significant.

RESULTS

Open field test

(a) Ambulation frequency

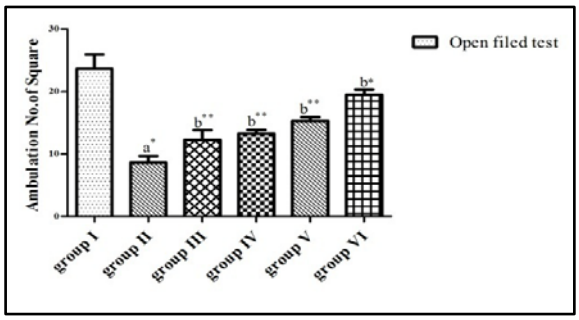
The present results exposed a significant (P<0.001) decrease in ambulation frequency in rotenone-induced group as compared with vehicle group (fig. 1a). L-DOPA+rotenone, quercetin+rotenone and hesperidin+rotenone group showed significantly increased ambulation frequency as compared to rotenone group. The combination group showed higher ambulation frequency when compared to both quercetin and hesperidin (group VI).

(b) Rearing frequency

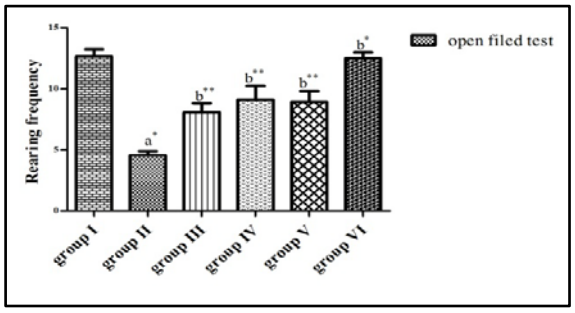
The results showed that the rearing frequency was significantly (P<0.001) decreased in rotenone-induced group when compared with vehicle control group (fig. 1b). The current results indicated that L-DOPA+rotenone, quercetin+rotenone and hesperidin+rotenone group was significantly increased when compared with rotenone group. The combination group also co-treated with rotenone, indicated increased rearing frequency when compared with rotenone group.

(c) Grooming

The results showed that the grooming frequency was significantly (P<0.001) decreased in rotenone-induced group when compared with vehicle control. The current results indicated that L-DOPA+rotenone, quercetin+rotenone and hesperidin+rotenone co-treated group was significantly increased when compared with rotenone group. The combination group also indicated increased grooming frequency when compared with rotenone group (fig. 1c).



(a)



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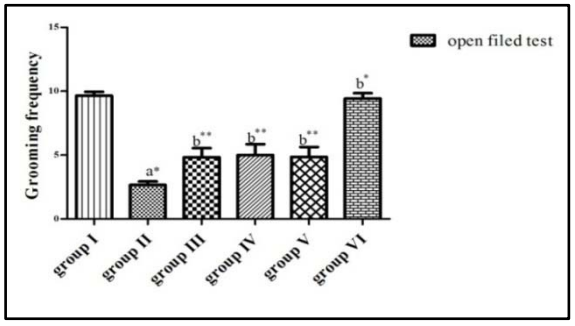


(c)

Fig. 1: Shows the behavioural activity (open field test) of experimental rats, (a) Ambulation (b) Rearing and (c) Grooming. Each value is expressed as mean±SD, n=6. Group I: Vehicle-treated rats, Group II: Rotenone induced rats (3 mg/kg/b. wt), Group III: Rotenone (3 mg/kg/b. wt)+L-DOPA (10 mg/kg/b. wt), Group IV: Quercetin (20 mg/kg/b. wt)+rotenone (3 mg/kg/b. wt), Group V: Hesperidin (50 mg/kg/b. wt)+rotenone (3 mg/kg/b. wt),Group VI: Quercetin (20 mg/kg/b. wt)+hesperidin (50 mg/kg/b. wt)+rotenone (3 mg/kg/b. wt). Statistical significance: *p<0.001, **p<0.01. Comparison: a-as compared with group I, b-as compared with group II

Ladder climbing test

The results showed significantly (p<0.001) increased value in the induced group in compared with vehicle control. In another group, the results score show that L-DOPA+rotenone, quercetin+rotenone, hesperidin+rotenone co-treated group was decreased significantly when compared to the induced group. In combination group, results indicate decreased the time for climbing time compared with the induced group.

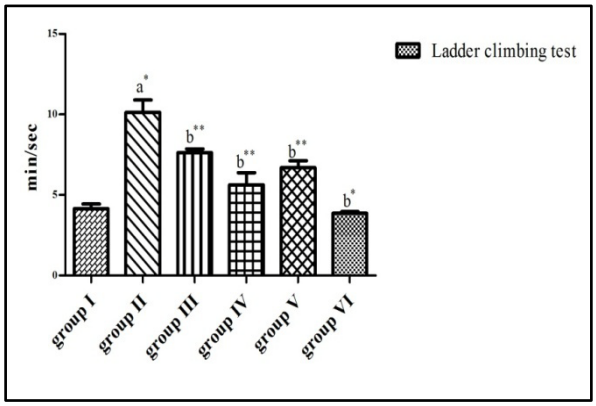


Fig. 2: Shows the behavioral activity (ladder climbing test) of experimental rats. Each value is expressed as mean±SD,n=6. Group I: Vehicle treated rats, Group II: Rotenone induced rats (3 mg/kg/b. wt), Group III: Rotenone (3 mg/kg/b. wt)+L-DOPA (10 mg/kg. b. wt), GroupIV: Quercetin (20 mg/kg/b. wt)+rotenone (3 mg/kg/b. wt), Group V: Hesperidin (50 mg/kg/b. wt)+rotenone (3 mg/kg/b. wt), Group VI: Quercetin (20 mg/kg/b. wt)+hesperidin (50 mg/kg/b. wt)+rotenone(3 mg/kg/b. wt). Statistical significance: *p<0.001, **p<0.01. Comparison a-as compared with group I, b-as compared with group II

Hanging wire test

Hanging time was significantly (p<0.001) decreased in the induced group when compared with vehicle control. The hanging time was improved in the treated group compared with the rotenone-induced group. Combination group retained the muscle strength in hanging wire test.

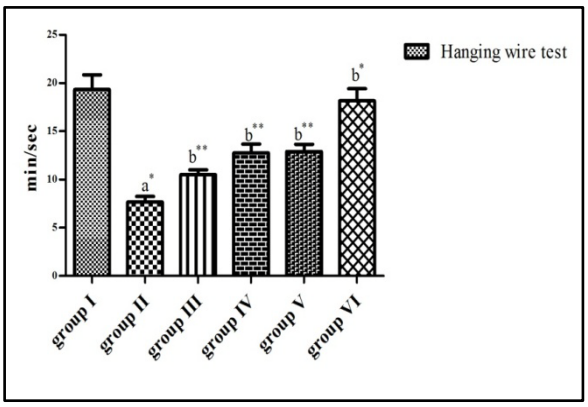
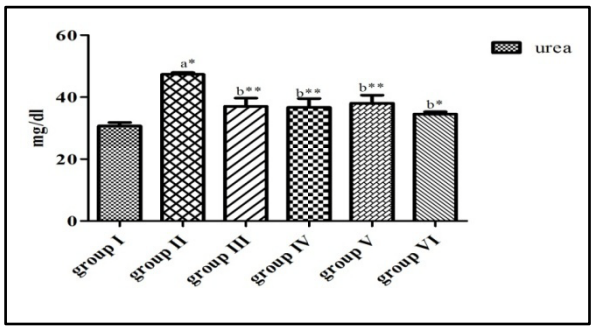


Fig. 3: Shows the behavioural activity (hanging wire test) of experimental rats. Each value is expressed as mean±SD, n=6. Group-I: Vehicle-treated rats, group-II: Rotenone induced rats (3 mg/kg/b. wt), group-III: Rotenone (3 mg/kg/b. wt)+L-DOPA (10 mg/kg b. wt), group IV: Quercetin (20 mg/kg b. wt)+rotenone (3 mg/kgb. wt), group V: Hesperidin (50 mg/kg b. wt)+rotenone (3 mg/kg b. wt), group VI: Quercetin (20 mg/kg b. wt)+hesperidin (50 mg/kg b. wt)+rotenone (3 mg/kg b. wt). Statistical significance: *p<0.001,p<0.01 Comparison: a-as compared with group I; b-as compared with group II**

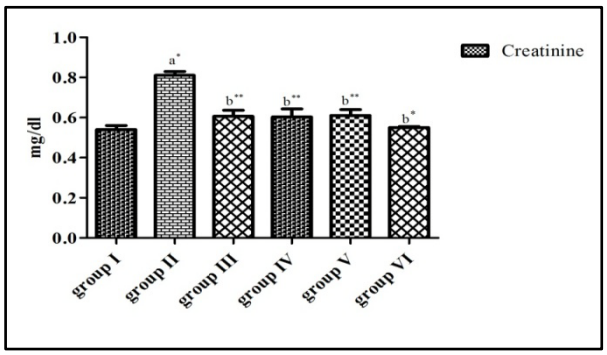
Biochemical parameters

Serum urea and creatinine

Serum urea level was found to be increased in rotenone-induced group when compared to the vehicle group (P<0.001 fig. 4a). L-DOPA+rotenone combination group was found to be significantly (P<0.01) decreased when compared to rotenone-induced group. Quercetin+rotenone and hesperidin+rotenone groups were found to be significantly (P<0.01) decreased when compared to rotenone-induced group. In the combination group it was found to be significantly (P<0.001) decreased when compared to rotenone-induced group. Serum creatinine level was found to be significantly (P<0.001) increased in rotenone-induced group compared to vehicle group. L-DOPA+rotenone combination group was found to be significantly (P<0.01 fig. 4b) decreased compared to rotenone group. Quercetin+rotenone and hesperidin+rotenone groups showed significantly (P<0.01) decreased in their level when compared to rotenone group. In the combination group was found to be significantly (P<0.001) decreased in level when compared to rotenone group.



(a)



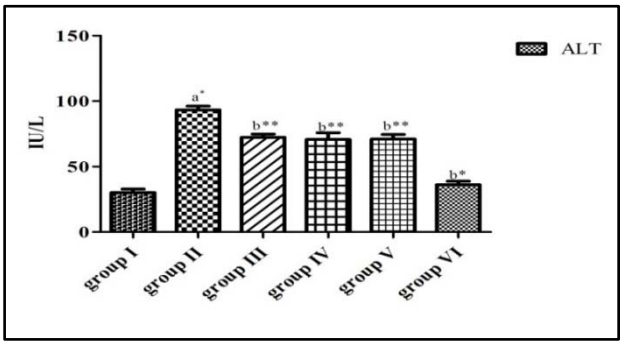
(b)

Fig. 4: Shows the biochemical parameters (a) urea and (b) Creatinine of experimental animals. Each value is expressed as mean±SD, n=6. Group-I: Vehicle treated rats, Group-II: Rotenone induced rats (3 mg/kg. b. wt), Group-III: L-DOPA (10 mg/kg/b. wt)+rotenone (3 mg/kg. b. wt),Group-IV: Quercetin (20 mg/kg. b. wt)+rotenone (3 mg/kg. b. wt),Group-V: Hesperidin (50 mg/kg. b. wt)+rotenone (3 mg/kg. b. wt),Group-VI: Quercetin (20 mg/kg. b. wt)+rotenone (3 mg/kg. b. wt)+Hesperidin (50 mg/kg. b. wt). Statistical significance: *p<0.001, **p<0.01, Comparison: a-as compared with group-I, b-as compared with group II

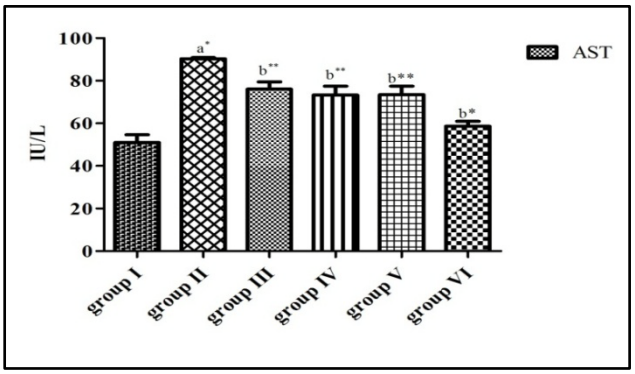
Serum ALT and AST

Serum ALT activity was found to be significantly (P<0.001 fig. 5a) increased in rotenone-induced group when compared to vehicle group. L-DOPA+rotenone combination group was found to be significantly (P<0.01) decreased when compared to be rotenone group. Quercetin+rotenone and hesperidin+rotenone groups were found to be significantly (P<0.01) decreased when compared to

rotenone group. The activity of ALT in quercetin+hesperidin+ rotenone group was found to be significantly (P<0.01) decreased when compared to rotenone group. Serum AST level was found to be significantly (P<0.001) increased in the rotenone-induced group when compared to be vehicle group. The activity of AST in L-DOPA+rotenone group was found to be significantly (P<0.01) decreased when compared to be rotenone group. Quercetin+rotenone and hesperidin+rotenone groups were found to be significantly (P<0.01 fig. 5b) decreased when compared to rotenone group. Finally, the quercetin+hesperidin+rotenone group was found to be significantly (P<0.001) decreased when compared to be rotenone group.



(a)



(b)

Fig. 5: Shows the biochemical parameters (a) ALT and (b) AST of experimental animals. Each value is expressed as mean±SD, n=6. Group-I: Vehicle treated rats, Group-II: Rotenone induced rats (3 mg/kg. b. wt), Group-III: L-DOPA (10 mg/kg/b. wt)+rotenone (3 mg/kg. b. wt),Group-IV: Quercetin (20 mg/kg. b. wt)+rotenone(3 mg/kg. b. wt), Group V: Hesperidin (50 mg/kg. b. wt)+rotenone (3 mg/kg. b. wt),Group-VI: Quercetin (20 mg/kg. b. wt)+rotenone (3 mg/kg. b. wt)+Hesperidin (50 mg/kg. b. wt). Statistical significance: *p<0.001,p<0.01. Comparison: a-as compared with group-I; b-as compared with group II**

DISCUSSION

Rotenone is a herbicide and insecticide which is the most potent member of the rotenoid family of neurotoxins that are found in tropical plants in nature. Increasing evidence has indicated that rotenone exposure is associated with an increased risk of developing Parkinson's disease (PD) [25].

The oxidation of dopamine, a transmitter of dopaminergic neurons, facilitates rotenone-development of neurotoxicity in rat substantia nigra [26] collectively rotenone model recapitulates most of the mechanisms thought to be importat in Parkinson's disease (PD) pathogenesis [27]. Rotenone-treated rats exhibited motor activity in the open field test and square bridge test along with a marked decrease in striatal dopamine level. In agreement rotenone-treated rats exhibit reduced mobility [28]. In this study, the protective effect of quercetin was investigated in a model of Parkinson's disease (PD). Previous studies have demonstrated that the unilateral damage of the nigrostriatal dopaminergic system through intrastriatal injection of 6-OHDA is followed by a reduction in the striatal dopaminergic postsynaptic receptors on the same side [29].

Neuroprotective effect of quercetin against neurotoxin-induced damage has already been reported in central nervous system. In addition, systemic administration of quercetin could protect hippocampal neurons against global ischemic consequences. There is also some evidence that following lesions and repetitive electrical stimulation of neuronal circuits, expression of matrix metalloproteinase (MMP) increases [30, 31].

Quercetin is a bioflavonoid with strong antioxidant properties. It inhibits PGE2 production, COX-2 expression, inducible nitric oxide synthase expression and nuclear factor-κB activation. So that the quercetin possess neuroprotective effect and may be useful in preventing oxidative damage in the brain as reported by Suryakantha *et al.* [32].

Muthukala *et al.*, reported that the quercetin have good replacement action for chemical therapeutic drugs as it has antioxidative and anti-inflammatory properties [33].

This pathway may be one candidate for the beneficial effect of quercetin in the present study and in this way the flavonoid could reduce the neuroplastic changes in neuronal circuits and augmented excitability in certain sites involved in epilepsy. On the other hand, quercetin and its derivatives in the body can selectively inhibit NMDA receptor functionality [in some ways acting as an antagonist] [34] and in this way exert their beneficial effect in some animal model of neural diseases like Parkinson's disease (PD).

The demonstration of the neuroprotective effect of quercetin in 6-OHDA model of Parkinson's disease in this study establishes a potential neural basis for the epidemiological association between quercetin consumption and a reduced risk of Parkinson's disease (PD) in future. To conclude these data establish a potential basis for the inverse association between quercetin administration and the development of Parkinson's disease and this may put forward flavonoids like quercetin as a noval treatment for this neurodegenerative disease [35].

A study suggested that depression is a most common psychiatric problem in Parkinson's disease [36]. Parkinson's disease symptoms show as increasing multidimensional disease, such as motor deficits [37]. The dopaminergic system it is one of the neurotransmitters and it plays on important role in behaviour model [38].

Hesperidin has been demonstrated to act as an antioxidant that stabilizes and thus prevent cell membrane from the damage in neurodegenerative diseases [39, 40]. The present study demonstrated that hesperidin acts as a protective agent through the behavioral and biochemical parameters just as in aging mice in the experimental model of Parkinson's disease induced by 6-OHDA [41]

The hesperidin has reduced the learing and memory deficits improved locomotor activity and increased glycogen synthase kinase-3B (GSK-3B) phosphorylation,anti-oxidative defence and mitochondrial complex I-IV enzymes activities in transgenic mice [42]. Chandran *et al.* [43] suggested that the Parkinson's disease there is behavioral dysfunction. Muscular coordination was affected by rotenone. Dinesh. T *et al.* [44] have demonstrated in the fish model the catalepsy activity was successfully induced by haloperidol. The hesperidin was found to possess therapeutic effect against Parkinson disease in 6-OHDA induced animal model. Hesperidin has reversed the 6-OHDA induced behavioral activity such as (Grip test, rotational test, swing test and catalepsy). Hesperidin exhibited changes in the biochemical parameters such as blood glucose, TG and protein as reported by Priya *et al.* [45].

The catalepsy or rigidity restricted movement or freezing of movement are exhibited by Parkinson's disease (PD) patients of drugs improves the locomotor activity in the PD patients [46]. During catalepsy, fish started showing aberrant swimming patterns like an upside down, arrow-like swimming, circular swimming and finally complete the catalepsy as reported by Seibt KJ *et al.* [47].

It increased the loss of nigrostriatal function by the administration of 6-OHDA in the increased a paw retraction test. This test shows the bilateral lesions produced changes in behavioural parameters [48].

The study reported by Michelle S *et al.*, [49] demonstrated a protective effect of hesperidin on the neurotoxicity induced by 6-OHDA in aged mice increasing the DA levels enzymatic and non-enzymatic activity, decreasing the ROS and improving the behavioral parameters.

Bhagyasree *et al.* [50] reported that the fruit *Anacardium Occidentale* was set to have the protective effect of against neurodegenerative disorder.

CONCLUSION

In our *in vivo* study, we have demonstrated the combination of quercetin and hesperidin to serve as neuroprotective compounds by improving the behavioral abnormalities and restoring the biochemical parameters. Hence, these powerful antioxidants may protect brain cells.

AUTHORS CONTRIBUTION

1. K. Srimathi Priyanga-Research scholar and first author

2. Dr. K. Vijayalakshmi-Corresponding author

3. R. Selvaraj-Sathyabama University, supporting for animal work

CONFLICT OF INTERESTS

Declared none

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