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Effect of Naringenin on 3-NP Induced Huntington's disease like Symptoms by Estimations of Motor Co-ordination and Behavioral Parameters

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Abstract: ***The main aim of this study was to investigate the effect of Naringenin, a flavonoid on 3-Nitropropionic acid (3-NP)-induced Huntington's disease like symptoms by estimations of motor co-ordination and behavioral parameters. 3-NP is an irreversible inhibitor of complex II in the mitochondria. 3-NP-induced neurodegeneration has been widely used as an animal model of Huntington's disease (HD). It replicates the pathology of HD by causing oxidative stress. Naringenin is a polyphenolic compound, a bioflavonoid, known to have a neuroprotective effect in a rat model of Alzheimer's disease. In the present study, the neuroprotective effect of Naringenin on 3-NP induced oxidative stress in the rat was determined by behavioral parameters. Rats were induced with 3-NP (15 mg/kg) intraperitoneally for 21 days and rats induced with 3-NP were treated with Naringenin (25mg/kg and 75mg/kg) for 21 days. 3-NP caused a decline in motor function in the neurological score, locomotor activity, and impaired rotarod activity. Naringenin treatment significantly improved grip strength indicating an improvement in motor performance, alterations in % spontaneous alternations. These findings suggest the antioxidant potential of Naringenin flavonoid against 3-Nitropionic acid induced neurotoxicity. However, more investigations are required to elucidate the cellular mechanisms of Naringenin against 3-Nitropropionic acid induced Huntington's disease like symptoms.***

Keywords: Naringenin, 3-Nitropropionic acid, Huntington's disease, Oxidative Stress.

INTRODUCTION

Neurodegenerative diseases are the heterogeneous group of disorders characterized by the progressive and selective loss of neuronal systems. Mitochondrial dysfunction causes vulnerability to oxidative stress and the activation of downstream cell death pathways that lead to neuronal apoptosis (Lin and Beal, 2006). Huntington's disease (HD) is an autosomal dominant, inherited the neurodegenerative disorder, clinically characterized by involuntary choreic movements, cognitive impairment, and dementia (Hannan, 2005). It is caused by an expanded CAG trinucleotide repeat (.38) leading to the synthesis of an aberrant protein (mutant huntingtin) with an expanded N-terminal polyglutamine (polyQ) tract (Zuhike.C et al., 1993).

3-Nitropropionic acid (3-NP), a natural environmental toxin causes selective neuronal degeneration in the striatum and reproduces the brain lesions in laboratory animals as observed in HD patients (Brouillet et al., 2005). 3-NP inhibits the succinate dehydrogenase enzyme of mitochondrial respiratory chain complex II. Oxidative stress is one of the major deleterious events in 3-NP-induced neurodegeneration. The mechanism by which 3-NP induces neurodegeneration involves mitochondrial membrane depolarization, energy depletion, oxidative stress and enhanced mitochondrial-dependent apoptosis (Rosenstock et al., 2004).

Although various parts of the brain are affected, 3-NP-induces specific striatal damage, a characteristic exploited to produce an experimental model of Huntington's disease (Tsang et al., 2009). 3-NP depleted the levels of antioxidant enzyme profiles, the activity of ATPases and enhances ROS levels in the striatum (Nam et al., 2005; Sandhir et al., 2010). Oxidative stress is hypothesized to play a vital role in 3- NP-induced neuronal apoptosis (Liot et al., 2009). Therapeutic strategies aimed at preventing or delaying ROS-induced apoptosis might be a reasonable choice for the treatment of neurodegenerative disease (Uttara et al., 2009). Among various therapeutic strategies, one of the possible ways is to augment or fortify endogenous defence against oxidative stress through the dietary or pharmacological intake of antioxidants. Emerging evidence suggests that the dietary phytochemicals, in particular, flavonoids, exert beneficial effects in the CNS by protecting neurons against oxidative stress mediated cell death (Huang and Zhang, 2010).

Naringenin ((2S)-5,7-dihydroxy-2-(4-hydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one) a dietary flavonoid commonly found in citrus fruits exhibits diverse biological and pharmacological properties including hypocholesterolemic, antiestrogenic, hypolipidemic, antihypertensive, and anti-inflammatory activities (Bernini R *et al.*, 2003). Naringenin had recently received considerable attention as a neuroprotective agent (Kim et al., 2012). In the present study, an attempt has been made to evaluate the neuroprotective activity of Naringenin against 3- nitropropionic acid induced Huntington's disease like symptoms.

MATERIALS AND METHODS

Chemicals

3-Nitropropionic acid and Naringenin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade.

Animals

Male Wistar rats (250–300 g) were obtained from the Mahaveer Enterprises, Hyderabad, India. The animals were acclimatized to the laboratory conditions for a period of 2 weeks. The animals were housed under standard conditions of 12 h light/dark cycles and were given a standard rat feed (Hindustan Lever Ltd.,) and water *ad libitum.* The experiments were conducted according to ethical norms approved by the Institutional Animal Ethics Committee (IAEC) of ANU College of Pharmaceutical Sciences, constituted for the purpose of animal experimentation as per CPCSEA guidelines (Approval no: *ANUCPS/IAEC/AH/P/11/2017).* Care was taken to minimize animal suffering.

Experimental Procedure

The rats were divided into five groups (n = 6) as below:

Group I - Control- Rats administered with saline.

Group II - Rats administered with 3-Nitropropionic acid (15 mg/kg) intra-peritoneal (*i.p.*) for 21 days.

Group III - Rats treated with Naringenin (75 mg/kg) orally for 21 days.

Group IV- Rats simultaneously treated with 3-NP (15 mg/kg i.p.) & Naringenin (25 mg/kg) orally for 21 days.

Group V - Rats simultaneously treated with 3-NP (15 mg/kg i.p.) & Naringenin (75 mg/kg) orally for 21 days.

All the treatments were continued for 21 days and Group-II, IV, and V groups were treated with 3-NP (15 mg/kg/day, *i.p*) from 8th day to 21st day one hour after the regular treatment as above said. On the 21st day after 3 hr of treatment, the animals were assessed for various physical and behavioural parameters.

Motor co-ordination and behavioral assessments

- **Neurological scoring**

Neurological scoring was assessed to measure the motor disturbance induced by 3-NP, based on their normal ambulatory movements. The scoring was done as per previous literature: normal 0; general slowness of displacement due to mild hind limb impairment; 2 co-ordination loss and significant abnormality in gait; 3 hind limbs paralysis; 4, inability to move due to impairment in both forelimbs and hind limbs; 5 recumbency (Jung-Eun Park *et al.*, 2013).

- **Locomotor activity**

On 7th, 14th and 21st day of the experimental protocol all the animals were observed for locomotor activity. Animals were acclimatized for 5 min in the Photoactometre after that each animal was allowed to move freely in the Photoactometre for 5 min (300 sec) which was accessed with light sensitive photocells to count the motor activity digitally. The count/5 min was measured for each animal for their locomotor activity (Kumar *et al.*, 2011).

- **Rota rod**

The skeletal muscle relaxation together with taming or calming effect, these agents reduce anxiety and tension. The loss of muscle-grip is an indication of muscle relaxation. This effect can be easily studied in animals using an inclined plane or rotating rods. The difference in fall off time from the rotating rod between the normal and treated animal is taken as an index of muscle relaxation. The angle of the slope of the inclined plane or the rate of rotation of the rod should be adjusted such that a normal rat can stay on the plane or on the rod for an appreciable time (3-5 min) of time. Turn on the Rota-rod by selecting an appropriate speed (20-25 rpm). Place the animal one by one in to several compartments. Note down the 'fall off time' when the rat falls from the rotating rod. A normal group of rats generally falls off within 3-5 minutes. Later the treated groups are followed by noting the fall off time (Sandhir R *et al.*, 2010).

➢ **Elevated plus maze test**

Elevated plus-maze is the simplest apparatus to study anxiety response and the effect of almost all type of anti-anxiety agents. Exposure of the animals to novel maze alley evokes on approach avoidance conflict which is stronger in open arm as compared to the enclosed arm. Rodents have an aversion for high and open space and prefer enclosed arm and therefore, spend a greater amount of time in the enclosed arm. When animals enter open arm, they freeze, become immobile, defecate and show fear-like movements. The plasma cortisol level is also reported to be increased, as a true reflection of anxiety. The elevated plus maze consisted of two opposite black open arms (50 cm × 10 cm), crossed with two closed walls of the same dimensions with 40 cm high walls. The arms were connected with a central square of dimensions 10 cm × 10 cm the entire maze was placed 50 cm high above the ground. Rats were placed individually at one end of the open arm facing away from the central square. The time taken by the animal to move from the open arm to the closed arm was recorded as transfer latency (TL) on 21st days (Puneet Kumar *et al.,* 2010).

➢ **Y-maze test**

The Y-maze test was a horizontal maze (40 cm long and 14 cm wide, with walls 22 cm high) made of polyvinyl chloride (PVC) material with three arms (labelled A, B, and C) disposed at 120 to each other. Each rat was placed at the centre of the apparatus and allowed to move freely through the maze for 5 min. The number of alternations (i.e., consecutive entry sequences of ABC, CAB or BCA, but not BAB) and the numbers of arm entries were recorded. Maze arms were thoroughly cleaned between tests with water spray to remove residual odours.

The percentage alternation was calculated according to the following equation: (Song X *et al.,* 2016).

Percentage alternation (%) = [(number of alternations)/ (total arm entries - 2)] × 100.

RESULTS

Effect of Naringenin on 3-Nitropropionic acid induced changes in the neurological score: The neurological score is based on movement analysis which is depicted in **Table 1.** 3-Nitropropionic acid treated groups resulted in motor abnormalities, showed normal behaviour or general slowness. General slowness of displacement resulting from mild hind limb impairment was observed in 1 animal, motor in-coordination and marked gait abnormalities were observed in 3 animals, hind limb paralysis was observed in 4 animals and 4 animals showed incapacity to move resulting from fore limb and hind limb impairment. Nar treated groups (25 mg/kg/day + 3 - NP and 75 mg/kg/day + 3 - NP) showed improvement in behavioural changes when compared with 3-NP treated group.

Table 1: Effect of Naringenin on 3-Nitropropionic acid induced changes in neurological score

Treatment groups	Normal behaviour/used (score – 0)	General slowness/used (score – 1)	In-coordination and marked gait abnormalities/used (score-2)	Hind limb paralysis/used (score – 3)	Incapacity to move/used (score-4)	Total mean
Normal	12/12	0/12	0/12	012	0/12	0.0
3-NP (15mg/Kg)	0/12	1/12	3/12	4/12	4/12	2.91
Nar (75mg/Kg)	10/12	2/12	0/12	0/12	0/12	0.16
3-NP (15 mg/kg) + Nar (25 mg/kg)	0/12	5/12	3/12	2/12	2/12	2.01
3-NP (15 mg/kg) + Nar (75 mg/kg)	0/12	8/12	3/12	1/12	0/12	1.41

Effect of Naringenin on 3-Nitropropionic acid induced changes in locomotor activity---- Table 2 and Fig. 1 show the locomotor activity which measures the locomotor counts per 5 min on the 7th day (before the day of administrating 3-NP), 14th day and 21st day. On the 7th day, there is no significant change within the groups. On the 14th day, locomotor counts showed a significant ($p<0.01$) decrease in 3-NP treated group when compared with control group. On the 21st day, locomotor counts showed a significant ($p<0.001$) decrease in 3-NP treated group when compared with control group. Only Nar treated group (75 mg/kg/day) has shown significant ($p<0.001$) increase in locomotor counts when compared with 3-NP and in Nar+

3-NP (25mg/kg/day & 75mg/kg/day) showed a significant ($p<0.001$) increase in locomotor counts when compared with 3-NP treated group.

S. No	Treatment Groups	Locomotor counts / 5 min (Mean ± SEM)		
		Before treatment	After treatment	
		7th day	14th day	21st day
1	Normal	196 ± 8.13	191 ± 8.18	187 ± 8.14
2	3-NP (15 mg/Kg)	210 ± 12.3	82 ± 546 ^{a***}	43.0 ± 5.98 ^{a***}
3	Nar (75 mg/Kg)	210 ± 12.9	195 ± 7.45 4 ^{b***}	175 ± 9.21 ^{b***}
4	3-NP (15 mg/kg) + Nar (25 mg/kg)	189 ± 8.07	137 ± 3.06 ^{b***}	101 ± 5.19 ^{b***}
5	3-NP (15 mg/kg) + Nar (75 mg/kg)	196 ± 6.36	164 ± 2.80 ^{b***}	120 ± 4.98 ^{b***}

Table 2 Effect of Naringenin on 3-Nitropropionic acid induced changes in locomotor activity. [Values are expressed as mean ± SEM (n=6). One way ANOVA followed by Tukey's post hoc test. ***p<0.001, ** p<0.01, *p<0.05 were considered as statistically significant. a) 3-NP compared with control group b) Treatment groups compared with 3-NP]

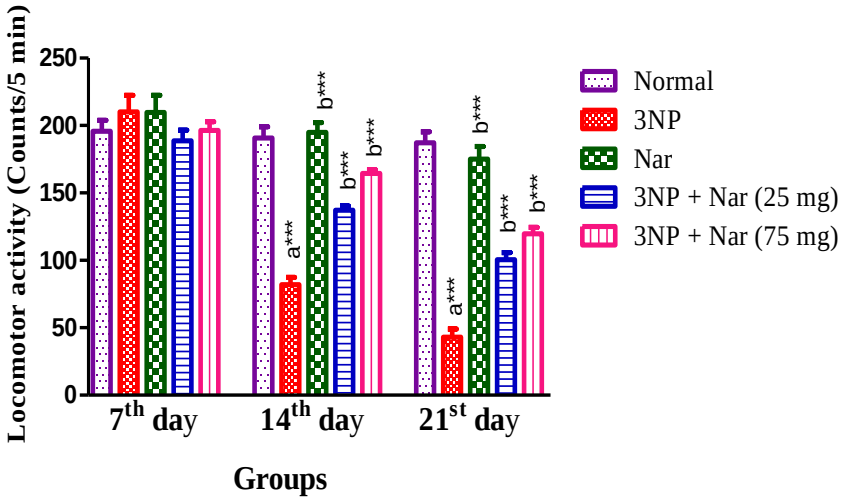

Fig. 1 Effect of Naringenin on 3-Nitropropionic acid induced changes in locomotor activity. [Values are expressed as mean ± SEM (n=6). One way ANOVA followed by Tukey's post hoc test. ***p<0.001, ** p<0.01, *p<0.05 were considered as statistically significant. a) 3-NP compared with control group b) Treatment groups compared with 3-NP]

Effect of Naringenin on 3-Nitropropionic acid induced changes in rotarod test---- During the treatment schedule, rotarod test measured the motor coordination by latency fall of time on the 7th day (before the day of administrating 3-NP), 14th day and 21stday represented in **Table 3 and Fig. 2**. On the 7th day, there is no significant change within the groups. On the 14th day, 3-NP treated group showed a significant decrease ($p<0.001$) in the motor coordination when compared with control group and only Nar treated group (75mg/kg/day) improved the motor coordination which showed the significance ($p<0.01$) when compared to the 3-NP treated group. On the 21st day, significant change in latency fall of time was reduced by showing a significant change ($p<0.001$) in 3NP treated group when compared with control group and only Nar treated group (75 mg/kg/day) has shown significant ($p<0.001$) change, when compared with 3-NP, treated group. Nar + 3-NP treated groups (25mg/kg/day and 75 mg/kg/day) improved the fall of time significantly ($p<0.001$) when compared to 3-NP treated groups.

S.No	Treatment Groups	Fall off time 180 sec (Mean ± SEM)		
		Before treatment	After treatment	
		7th day	14th day	21st day
1	Normal	157 ± 15.1	154 ± 9.82	156 ± 10.1
2	3-NP (15 mg/kg)	141 ± 11.8	61.6 ± 10.0^{a***}	30.0 ± 5.94 ^{a***}
3	Nar (75 mg/kg)	148 ± 13.1	141 ± 11.8 ^{b***}	149 ± 9.85 ^{b***}
4	3-NP (15 mg/kg) + Nar (25 mg/kg)	162 ± 3.81	91.2 ± 5.21^{b***}	86.2 ± 6.30 ^{b**}
5	3-NP (15 mg/kg) + Nar (75 mg/kg)	152 ± 16.9	126 ± 4.89 ^{b***}	96.2 ± 12.7 ^{b***}

Table 3 Effect of Naringenin on 3-Nitropropionic acid induced changes in rotarod test. [Values are expressed as mean ± SEM (n=6). One way ANOVA followed by Tukey's post hoc test. ***p<0.001, ** p<0.01, *p<0.05 were considered as statistically significant. a) 3-NP compared with control group b) Treatment groups compared with 3-NP]

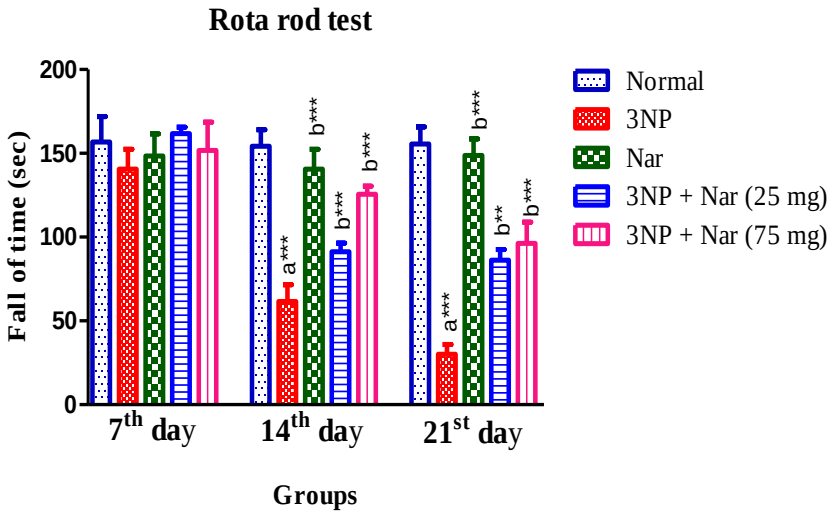

Fig. 2 Effect of Naringenin on 3-Nitropropionic acid induced changes in rotarod test. [Values are expressed as mean ± SEM (n=6). One way ANOVA followed by Tukey's post hoc test. ***p<0.001, ** p<0.01, *p<0.05 were considered as statistically significant. a) 3-NP compared with control group b) Treatment groups compared with 3-NP]

Effect of Naringenin on 3-Nitropropionic acid induced changes in elevated plus maze test ---- During the treatment schedule, Plus-maze was used to measure the memory by transfer latency time on the 7th day (before the day of administrating 3-NP),14th day and 21stday were represented in **Table 4 and Fig. 3**. On the 7th day, there was no significant change in the groups. On 14th day, 3-NP treated group showed decreased transfer latency time with the significance (p<0.01) when compared with control group and Nar + 3-NP treated group (25 mg/kg/day and 75 mg/kg/day) showed ameliorative effect with the significant (p<0.05) change by decreased transfer latency time when compared to the 3-NP treated group. On the 21st day, there was a decreased loss of memory in 3-NP treated groups when compared with control group and Nar + 3-NP treated group (25mg/kg/day and 75 mg/kg/day) showed significant improvement in the loss of memory (p<0.001) when compared to the 3-NP treated group.

S.No	Treatment Groups	Transfer Latency time (Mean ± SEM)		
		Before treatment	After treatment	
		7th day	14th day	21st day
1	Normal	10.8 ± 1.98	9.20 ± 2.58	19.2 ± 1.24
2	3-NP (15 mg/kg)	12.2 ± 1.80	37.6 ± 8.07 ^{a**}	78.8 ± 5.75 ^{a***}
3	Nar (75 mg/kg)	11.2 ± 2.08	9.60 ± 2.16 ^{b**}	16.4 ± 1.44 ^{b***}
4	3-NP (15 mg/kg) +Nar (25 mg/kg)	11.4 ± 2.42	17.0 ± 4.43 ^{b*}	43.4 ± 5.61 ^{b***}
5	3-NP (15 mg/kg) + Nar (75 mg/kg)	9.00 ± 1.52	14.6 ± 4.68 ^{b*}	33.2 ± 3.61 ^{b***}

Table 4 Effect of Naringenin on 3-Nitropropionic acid induced changes in elevated plus maze test. [Values are expressed as mean ± SEM (n=6). One way ANOVA followed by Tukey's post hoc test. ***p<0.001, ** p<0.01, *p<0.05 were considered as statistically significant. a) 3-NP compared with control group b) Treatment groups compared with 3-NP]

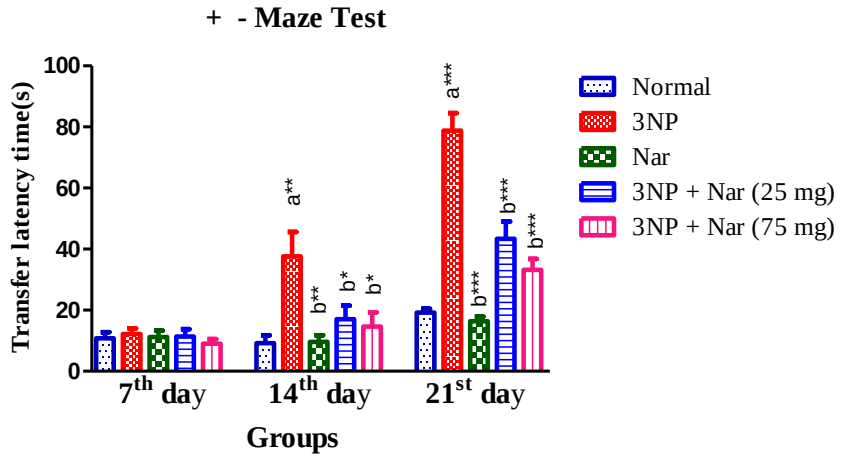

Fig. 3 Effect of Naringenin on 3-Nitropropionic acid induced changes in elevated plus maze test. [Values are expressed as mean ± SEM (n=6). One way ANOVA followed by Tukey's post hoc test. ***p<0.001, ** p<0.01, *p<0.05 were considered as statistically significant. a) 3-NP compared with control group b) Treatment groups compared with 3-NP]

Effect of Naringenin on 3-Nitropropionic acid induced changes in Y - maze test---- During the treatment schedule, Y-maze test is used for the measurement of % spontaneous alternations on the 7th day (before the day of administrating 3-NP), 14th day and 21st day. On the 7th day, there was no significant change within the groups. On 14th day, 3-NP treated group showed significance (p<0.001) attenuated response in % spontaneous alternations when compared with control group and Nar + 3-NP treated groups (25mg/kg/day and 75 mg/kg/day) showed a significant (p<0.05 and p<0.01) change in the % of spontaneous alternations when compared to the 3-NP treated group. On 21st day, % spontaneous alternations showed a significant decrease (p<0.001) in 3-NP treated group when compared with control group and Nar alone treatment group (75 mg/kg/day) has shown significantly increased % spontaneous alternation compared to 3-NP and also Nar + 3-NP treated groups (25mg/kg/day and 75mg/kg/day) showed its significant improvement (p<0.05 and p<0.001) in the spontaneous alternation when compared to the 3-NP treated group. **Table 5 and Fig. 4**

S.No	Treatment Groups	% Spontaneous alternations (Mean ± SEM)		
		Before treatment	After treatment	
		7th day	14th day	21st day
1	Normal	78.4 ± 5.25	77.2 ± 3.71	68.8 ± 5.18
2	3-NP (15 mg/kg)	75.0 ± 5.15	34.6 ± 5.62 ^{a***}	15.4 ± 2.16 ^{a***}
3	Nar (75 mg/kg)	77.0 ± 3.21	69.2 ± 2.13 ^{b***}	77.2 ± 3.71 ^{b***}
4	3-NP (15 mg/kg) + Nar (25 mg/kg)	75.2 ± 3.73	44.4 ± 1.78 ^{b*}	28.6 ± 2.77 ^{b*}
5	3-NP (15 mg/kg) + Nar (75 mg/kg)	65.2 ± 4.87	57.6 ± 3.57 ^{b**}	49.2 ± 2.85 ^{b***}

Table 5 Effect of Naringenin on 3-Nitropropionic acid induced changes in Y - maze test. [Values are expressed as mean ± SEM (n=6). One way ANOVA followed by Tukey's post hoc test. ***p<0.001, ** p<0.01, *p<0.05 were considered as statistically significant. a) 3-NP compared with control group b) Treatment groups compared with 3-NP].

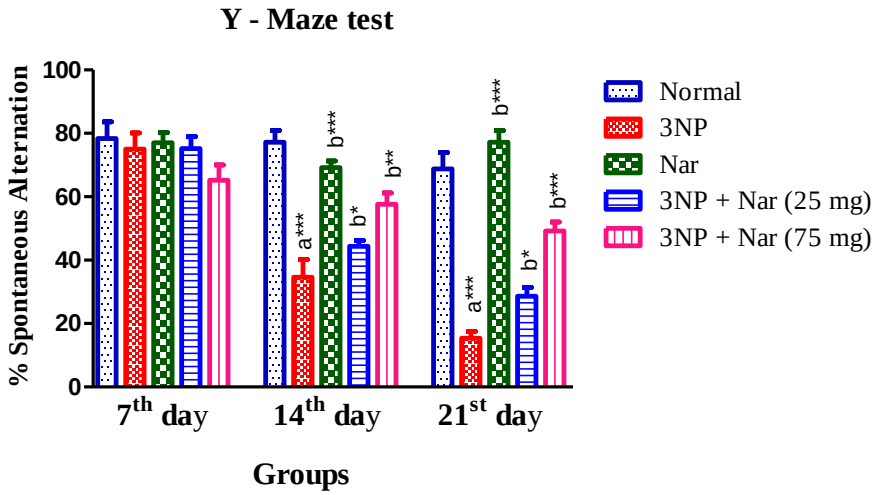

Fig. 4 Effect of Naringenin on 3-Nitropropionic acid induced changes in Y - maze test. [Values are expressed as mean ± SEM (n=6). One way ANOVA followed by Tukey's post hoc test. ***p<0.001, ** p<0.01, *p<0.05 were considered as statistically significant. a) 3-NP compared with control group b) Treatment groups compared with 3-NP].

DISCUSSION

3-Nitropropionic acid (3-NP) is a natural environmental toxin obtained from various plants and fungi. Oxidative stress is a major cause of cellular injuries in a variety of human diseases including neurodegenerative disorders (Keating, 2008). HD is an autosomal dominant inherited disorder characterized by progressive decline in motor and cognitive functions (Nakamura et al., 2001). HD is characterized by destruction of cholinergic receptors leading to a decreased level of acetylcholine (Kumar et al., 2009). The exact pathogenesis of the disease is not yet known, but evidence suggests that impairment in energy metabolism and oxidative stress play a major role in the pathogenesis of the Huntington's disease (Browne et al., 1999). Recently, researchers have made considerable efforts on searching natural antioxidants, in particular, plant derived polyphenolic compounds with neuroprotective potential for the treatment of neurodegenerative diseases (Kelsey et al., 2010; Craggs and Kalaria, 2010). 3-NP is a neurotoxin that irreversibly inhibits succinate dehydrogenase (SDH), a relevant enzyme constituting the complex II of the respiratory chain during mitochondrial electron transport (Kumar et al., 2006). 3-NP is known to produce oxidative/nitrosative stress and evokes an experimental model of Huntington's disease (Tunez et al., 2007).

In the present study, we report the neuroprotective activity of Naringenin against 3- nitropropionic acid induced Huntington's disease like symptoms produced motor and behavioral abnormalities, weakness and rigidity of muscles. Administration of 3-NP (15 mg/kg, i.p. for 21 days) produced significant increased brain oxidative stress and induced motor abnormalities in animals, these observations are in agreement with the above findings.

Both the doses of Naringenin with 3-NP-injected rats restored the changes towards the normal indicating protective potential of Naringenin against 3-NP-induced mitochondrial toxicity. Administration of 3-NP also associated with movement disorders and hypoactivity in the animals (Ahuja et al., 2008). Treatment with Naringenin (25 and 75 mg/kg) with 3-NP-challenged rats significantly reversed the movement disorders and increased locomotor counts. Naringenin treatment of 3-NP-administered animals showed 70–80% reduction in neurological scoring and increased locomotor counts when compared with 3-NP alone, indicating its potential protective effect against 3-NP-induced toxicity.

Intraperitoneal administration of 3-NP in rats produces striatal lesions with neurobehavioral changes like impairment in locomotor activity, rotarod performance, elevated plus maze and Y-maze. Naringenin caused significant improvement in motor activity in 3-NP treated animals. Further, the 3-NP treatment caused loss of grip strength on rotarod performance suggesting motor coordination impairment. Naringenin treatment significantly improved grip strength indicating an improvement in motor performance, alterations in % spontaneous alternations. A Recent study also reported that Naringenin improved the motor symptoms in Neurodegeneration in Alzheimer's disease (Kwatra et al., 2016). Naringenin treatment (25 and 75 mg/kg) to 3-NP-administered rats significantly restored the antioxidant status and decreased oxidative stress level, supporting the antioxidant potential of Naringenin (Katzung, 2001). Hence, the observed beneficial effect of Naringenin against 3-NP toxicity may be due to its antioxidant properties.

In summary, the present study suggests the antioxidant potential of Naringenin flavonoid against 3-Nitropionic acid induced neurotoxicity. However, more investigations are required to elucidate the cellular mechanisms of Naringenin against 3-Nitropropionic acid induced Huntington's disease like symptoms.

CONCLUSION

The data combined together suggests that Naringenin treatment protects against behavioural deficits caused as a result of 3-NP induced oxidative stress in transfer latency, motor coordination, muscle weakness, and rigidity. Our data show that both the doses (25 mg/kg and 75 mg/kg) significantly ameliorated the altered behavioural parameters, but Naringenin (75 mg/kg) showed better results in most of the behavioural parameters. This shows that Naringenin scavenges free radicals and possess antioxidant properties.

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