Discussion

Naringenin is a selective neuroprotective flavonoid which is used to treat depression and many other neurodegenerative disorders (Peng HW, Cheng FC, Huang YT. et al., 1998). HD is an autosomal dominant inherited Neurodegenerative disease, characterised by motor co-ordination and behaviour assessments (Gilliam TC. 1987). In the present study, the protective effect of Naringenin was assessed against 3-NP induced memory dysfunction.

3-Nitroproponoic acid is a well known neurotoxin which produces HD like symptoms in animals and humans (Patocka J, 2000) established which is an irreversible inhibitor of the mitochondrial respiratory complex II and succinate dehydrogenase, recapitulates HD-like pathology and symptoms in primate and rodent models (Brouillet E. et al., 1999). In the present study, administration of 3-NP has shown significant decreased body weights on day 21 as compared to normal control group. Nar (25mg/kg/day and 75 mg/kg/day) when administered with 3-NP significantly decreased in body weight which is due to energy defects and decrease in ATP generation in the body because of mitochondrial complex II inhibition.

Striatal degeneration was induced by 3-NP as the striatum is the central core area in the basal ganglia that controls co-ordination of motor movement. Further, in narrow beam walking test 3-NP treated rats took longer time to cross the beam than the control group (Arpita.K. et al., 2017). On the 21st day, 3-NP treatment causes muscle weakness and rigidity. Subsequently animals took longer time to cross the beam and showed a significant change in 3-NP treated group when compared with control group and Nar + 3-NP treated groups (25mg/kg/day and 75mg/kg/day) showed significant increase in motor coordination when compared with 3-NP treated group. This observation is similar to the conclusion of dietary flavonoids which is significantly reversed 3-NP induced various behavioural, biochemical parameters and cellular changes. (Suganya SN, Sumathi T. et al., 2016)

There is strong impairment in motor functions that alter muscular movements and reduced locomotor activity. As per the reports intra-peritoneal administration of 3-NP cause muscular atrophy which leads to decrease in locomotion counts. Reduced number of locomotion also indicates central nervous
system (CNS) depression which is one of the symptoms of HD (Dilpesh Jain, Arti Gangshettiwar., 2014). In this study on 21st day, locomotor counts has shown a decrease in 3-NP treated group when compared with control group. Further treatment with Nar + 3-NP (25mg/kg/day & 75mg/kg/day) showed increased locomotor counts when compared with 3-NP treated group, which may be due to as they reduce oxidative stress and inflammation.

In the present study hanging wire latency fall of time showed reduction in 3-NP treated group when compared with control group (Dhadde SB. 2014). On treatment with Nar + 3-NP (25mg/Kg/day and 75 mg/Kg/day) the time spent holding the wire was significantly increased. These findings suggest improved motor coordination, decreased muscle rigidity and increased grip strength, indicating the protective effect of Naringenin against 3-NP-induced decrease in muscle strength.

In the present study, rotarod activity shown 3-NP (15 mg/Kg) treatment decrease muscle grip strength assessed by the rota rod test on the day 7th, 14th, 21st day compared to normal group (Manisha Sharma. 2012). Nar + 3-NP (25mg/kg/day and 75mg/Kg/day p.o) treatment significantly improved muscle grip strength of 3-NP (15mg/Kg i.p) treated rats.

Plus-maze was used to measure the loss of memory by transfer latency time. There was a decreased loss of memory in 3-NP treated and showed a significant change (p<0.001) when compared with control group and Nar + 3-NP treated group (25mg/kg/day and 75 mg/kg/day) showed its significant improvement in the loss of memory (p<0.001) when compared to the 3-NP treated group whereas in Nar alone treatment group (75 mg/kg/day) has shown significant decrease when compared with 3-NP treatment group.

Percentage spontaneous alterations 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 significant increased % spontaneous alteration 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 alteration when compared to the 3-NP treated group.
Depression in HD is associated with basal ganglia abnormality and neurodegeneration which can be measured with the help of forced swim test. Due to depression animal will not try to escape a stressful stimulus (water tank). Administration of 3-NP in rats produced neurodegeneration and exhibit depression. In the forced swim test the immobility time is directly proportional to the locomotor impairment. In this study, in 3-NP induced group, there was a significant (p<0.01) increase in immobility time as compared to normal control group. Further, Nar treatment (25mg/Kg and 75mg/Kg) significantly (p<0.05) attenuated the increase in immobility time.

Cognitive dysfunction associated with hippocampal CA1 pyramidal neuronal damage of the brain caused by 3-NP treated group. Administration of Nar with 3-NP (25mg/Kg and 75mg/Kg) treated groups showed significant improvement in cognitive performance and memory retention in animals reflecting its protective ability on hippocampus region against 3-NP. In the Morris water maze test, the mean escape latency of trained rats gradually increased during training session in 3-NP treated rats. However escape latency was significantly decreased as compared with the normal group. Administration of Nar (75mg/Kg, p.o) treatment showed a significant improvement in memory performance as compared with 3-NP treated groups (p<0.05).

AchE is found in cholinergic synapses. There it hydrolyses the neurotransmitter acetylcholine to choline and acetate, thereby, playing an important role in cholinergic neurotransmission. Decreasing levels of acetylcholine has been implicated in many neurodegenerative disorders. 3-NP being a potent neurotoxin causes a decrease in levels of AchE. Results of the present study support the previous studies suggesting 3-NP causes memory dysfunction. Previous reports also suggested that 3-NP produced hippocampal CA1 pyramidal neurons lesions, the areas of the brain that are associated with cognitive performance (Arpita K. et al., 2017). Further, 3-NP treatment significantly increase Acetylcholinesterase enzyme activity in hippocampus as compared to other areas (cortex and striatum), suggesting the involvement of hippocampus in cognitive dysfunction and Nar + 3-NP treatment decrease acetylcholinesterase enzyme levels in all regions of brain with associated improved memory performance in 3-NP treated rats.
In the other conditions, the activity of LDH increases in the cell. In this study, 3-NP treatment significantly increased the level of LDH in the striatum, cortex and hippocampal regions of the brain. Nar + 3-NP treatment significantly attenuated the increase in LDH level, suggesting its potential role against 3-NP induced neurotoxicity.

The most significant alteration in antioxidant defence is a decrease in GSH concentration. GSH is one of the essential compounds responsible for maintaining cell integrity because of its reducing properties and participation in the cell metabolism. Glutathione is synthesized and degraded in most cell types by a series of well characterized enzymatic reactions. It exists in both reduced (GSH) and oxidized (GSSG) form, the former by far the largest fraction. GSH and GSSG are interconvertible by the action of two enzymes, GSH–Peroxidase and glutathione reductase. Reduced GSH strongly modulates redox state ratio of oxidizing to reducing equivalents of the cell, a role which is critical for cell survival. GSSG is formed in antioxidant reactions that involve GSH, and can accumulate with increased oxidative processing in the cell. The ratio of GSSG and GSH serves as a sensitive index of oxidative stress. GSH synthesis has been shown to be decreased in cells exposed to oxidative stress as an adaptive process.

In the present study, 3-NP significantly decrease reduced glutathione levels in the striatum, cortex and hippocampal regions of the brain indicating weak anti-oxidant defence. Nar treatment with administration of 3-NP significantly increases the glutathione levels in the same areas of brain.

Oxidative stress has been linked to neurodegeneration. Oxidative stress results in production of free radicals which damage tissues and DNA, causes inflammation and subsequently cellular apoptosis. Free radicals are molecules containing an unpaired electron. Free radicals are constantly produced in human body as by-products of aerobic metabolism. Brain is highly metabolically active with high oxygen demand and high levels of polyunsaturated fatty acids (PUFA), which makes brain highly vulnerable to oxidative stress. 3-NP is a neurotoxin inhibiting succinate dehydrogenase, targeting striatum, resulting in mitochondrial dysfunction. SDH is Complex II of mitochondrial electron transport chain and also a membrane bound enzyme playing a role in Kreb’s cycle by oxidising fumarate to succinate. In our
study, there is significant decreased levels of SDH in 3-NP induced rats in the targeting region striatum and the other areas of brain. Whereas there is a significant increased levels of SDH in Nar treatment in the striatum, cortex and hippocampal region.

3-NP induction results in oxidative stress due to production of hydrogen peroxide, superoxide, and hydroxide radicals. These free radicals are normally scavenged by catalase (CAT) and superoxide dismutase (SOD) the main anti-oxidant enzymes. SOD is known to convert two superoxide anions into one molecule each of hydrogen peroxide and oxygen. This hydrogen peroxide produced is further converted to water by CAT. 3-NP induction causes a decrease in levels of these enzymes. In this study, these enzyme levels were significantly increase on treatment with Nar (25mg/Kg and 75mg/Kg) which may be due to superoxide scavenging activity of Naringenin.

The data combined together suggests that Naringenin treatment protects against biochemical and behavioural deficits caused as a results of 3-NP induced oxidative stress in striatum, cortex and hippocampus. Both the doses of Naringenin with 3-NP (25mg/Kg and 75mg/Kg) significantly ameliorated the altered biochemical and behavioural parameters, but Naringenin dose of 75mg/Kg showed better results in most of the behavioural and biochemical parameters. This shows that Naringenin scavenges free radicals and possess antioxidant properties.
References
