Novel Neuro protective mechanism of Flupirtine against 3-NPA induced memory impairment and motor incoordination in experimental model of Huntington's disease.

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ABSTRACT

Huntington's Disease (HD) is a progressive neurodegenerative disorder that gradually declines cognitive skills, impair memory and normal movements of affected individuals. Huntington's Disease (HD) is an autosomal dominant inherited disease caused by the repetition of CAG repeats on the short arm of chromosome 4p16.3 in huntingtin gene (Htt). On the Morris Water Maze test, 3-NPA treated group rats finds the platform in less time than control & Flupirtine group i.e. their spatial & learning memory was more than control group rats. The significant difference was found at Probe trial day between 3-NPA and Flupirtine group. Flupirtine treated rats at high dose spent more time in open arm than 3-NPA treated rats and less time in dark arm then 3-NPA treated rats. 3-NPA showed decreased retention time on the rotating rod than the control group & Flupirtine at high dose treated rats showed increased retention time on the rotating rod. Decreased GSH levels were observed in 3-NPA treated group than control group rats and Flupirtine at high dose increased the level of GSH in 3- NPA treated rats. High level of MDA was found in 3-NPA rats than control group rats and found to be low in the Flupirtine treated group. Flupirtine increase the level of SOD in 3-NPA treated rats as it was reduced due to the toxicity of 3- NPA. Low levels of CAT were also observed in 3- NPA treated rats compared to control group and Flupirtine increases the level of CAT in 3- NPA treated rats.

Keywords: *Huntington's Disease, Flupirtine, Morris Water Maze test, huntingtin gene, 3-Nitropropanoic acid (3-NPA), neurodegenerative disorder.*

INTRODUCTION

Huntington‘s disease (HD) is a devastating inherited monogenic autosomal dominant neuro-degenerative disorder caused by expansion of CAG in exon 1 of the gene coding for huntingtin, located in chromosome 4 short arm (1). It can be characterized by weight loss, impairment of motor function, cognitive dysfunction, neuropsychiatric disturbances and striatal damage (2). Huntington disease predominantly affects striatum, cerebral cortex and other areas of the brain controlling motor coordination and memory storage. Clear abnormalities were visible in the tissues of developing cortex, which includes defects in neuroprogenitor cell polarity and differentiation, mislocalization of mutant huntingtin and junctional complex proteins, abnormal ciliogenesis, and changes in mitosis and progression of cell cycle. (3,4) The most striking neuropathology in HD occurs within the striatum, in which gross atrophy of the caudate nucleus and putamen is accompanied by selective neuronal loss of the medium spiny GABAergic neurons and astrogliosis (5). Other regions, including the cerebral cortex, thalamus, globus pallidus, substantia nigra, subthalamic nucleus and cerebellum, show different levels of atrophy depending on the pathologic grade and also show cell-autonomous dysfunction caused by mutant huntingtin (mHTT) expression in particular type of glial cell is enough for inducing both pathology and HD related impairments both in cognitive and motor performance. (6, 7) Oxidative stress, mitochondrial dysfunction and neuro inflammation have been proposed as contributing factors for the appearance of motor alterations and cognitive decline. However, transcription factor PGC-1 alpha is relevant for the mitochondrial biogenesis in HD. (1,8). A consistent dysfunction in the mitochondrial respiratory chain complex II– III has been found in the caudate nucleus of patients with HD. It has been proposed that a disruption in redox balance could lead to increased oxidative stress, neuronal dysfunction and HD (9). Cortical atrophy and early degeneration of the hypothalamus are also important aspects of HD pathogenesis, and late stage HD patients show widespread brain degeneration (10). Detection of pre- symptomatic patients (i.e. carrying the mutation but asymptomatic) demonstrated significant atrophy of the caudate and putamen. However, one of the first observations made of pathological changes in this disease was cerebral cortex atrophy, but somewhat later, was neglected with the appreciation of the prominence of the striatum atrophy. (11). In the early stage of illness HD patients also suffer from impaired attention and visuo-spatial processing. As the disease progress inadequacy in executive tasks, planning, problem solving and cognitive flexibility is seen in HD patients. The complexity and unpredictability of this disease is a great problem in management and treatment for health and social care professionals. HD patients might also sometimes complain of other non-motor symptoms (NMS) different from cognitive and behavioural abnormalities. Though, the assessment of their clinical symptoms is limited often to individual symptoms rather than addressing the all patients. (12).

The incidence of Huntington disease is estimated to be 4.7–6.9 new cases per million per year in Western populations, but whether incidence is increasing in parallel with point prevalence, Which also represents increases over premolecular studies is unclear (13). Genetic confirmation of the CAG repetition is the hallmark of epidemiological measure of Huntington disease (14).

Flupirtine is a tri-amino pyridine derivative that functions as a centrally acting non-opioid (non-narcotic) analgesic (15). The unique pharmacological properties of FLU contributes to its therapeutic benefits, without undesirable adverse effects such as respiratory depression, tolerance and dependence that are typical of opioids, or the gastrointestinal and renal problems associated with non-steroidal anti-inflammatory drugs. A number of studies performed on Flupirtine report the important super additive (synergistic) effect between Flupirtine and opioid drugs (16).

Flupirtine (ethyl-N-[2-amino-6-(4-flurophenylmethyllamino) pyridine-3- yl] carbamate) has been in clinical use for many years as a centrally active analgesic with muscle relaxant properties. Due to its neuro-protective action, it has possible applications in multiple sclerosis, Batten disease, Alzeimer‘s disease and Creutzfeld- Jakob disease (16). It is very useful in reducing pain of fibromyalgia. It is also very effective in cancer and post-operative pain. It is used in Parkinson‘s disease as anti-cataleptic agent and also reverses muscle rigidity and akinesia. Flupirtine is a safe drug and has high tolerability. Drug tolerance does not develop in most cases (17).

MATERIALS AND METHODS

Chemicals and Drugs –

All the drugs (3-nitropropanoic acid (10mg/kg intra-peritoneally), Flupirtine (50 &100 mg/kg orally), imipramine (20 mg/kg orally) chemicals which were used are of analytical grade, and procured from Sigma chemicals Co., USA and Qualigens fine chemicals, Mumbai, India.

Animal Experiments:

Albino Swiss male rats in equal numbers per group (n=6) were taken. At the commencement of the study, the weight variations of animals used was kept minimal and not exceeded ± 20% of the mean weight of each animal. Normal weight of male rat was 200-250 gm. The temperature of the experimental animal room was maintained at 22ºC (±3ºC). Relative humidity was maintained between 50-60%. Lighting was artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets were used with drinking water supplied at libitum. Animals of the same group were caged in small numbers in a cage. Healthy male rats were assigned to the control, standard, and treatment groups. The animals were identified uniquely (i.e., via marking at the base of the tail) and acclimatized for not less than 5 days in their cages prior to the start of the study.

All experimental procedures involving animals were conducted in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The study protocols were approved by the Institutional Animal Ethics Committee (IAEC) of animals house of the Institute (Reg. no. Reg no - 711/Po/Re/S/02/CPCSEA).

Experimental Protocol:

Experimental protocol: Rats were allocated in to seven experimental groups and each group contained six rats.

Group 1: Control group: Rats were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.) 60 minutes before the acquisition trial for four consecutive days and 60 minutes before the retrieval trial on day 5.

Group 2: Negative control group (3- NPA Treated rats): Rats were administered with 3-NPA (10 mg/kg; i.p.) during acquisition trials conducted from day 1 to day 4 and then subjected to Morris water maze test. On day 5 animal were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.), and then subjected to retrieval trial after 30 minutes.

Group 3: Positive control group (Imipramine treated group): Rats were administered with Imipramine (20mg/kg, orally) during acquisition trials conducted from day 1 to day 4 and then subjected to Morris water maze test. On day 5 animal were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.), and then subjected to retrieval trial after 60 minutes.

Group 4: Prophylactic group (Flupirtine low dose + 3-NPA treated rats): Rats were administered with Flupirtine low dose (50mg/kg; orally)60 minutes before 3- NPA (10mg/kg, i.p.) before 30 minutes during acquisition trials conducted from day 1 to day 4 and then subjected to Morris water maze test. On day 5 animal were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.), and then subjected to retrieval trial after 60 minutes.

Group 5: Prophylactic group (Flupirtine high dose + 3-NPA treated rats): Rats were administered with Flupirtine high dose (100mg/kg; orally) 60 minutes before and 3-NPA (10mg/kg, i.p.) before 30 minutes during acquisition trials conducted from day 1 to day 4 and then subjected to Morris water maze test. On day 5 animal were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.), and then subjected to retrieval trial after 60 minutes.

Group 6: Positive control group (Flupirtine low dose in 3-NPA treated rats): Rats were administered Flupirtine (50 mg/kg, orally) during acquisition trials conducted from day 1 to day 4 and then subjected to Morris water maze test. On day 5 animal were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.), and then subjected to retrieval trial after 60 minutes.

Group 7: Positive control group (Flupirtine high dose in 3-NPA treated rats): Rats were administered Flupirtine (100 mg/kg, orally) during acquisition trials conducted from day 1 to day 4 and then subjected to Morris water maze test. On day 5 animal were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.) and then subjected to retrieval trial after 60 minutes.

Serum Collection: On day 6, blood was collected by retro-orbital plexus under mild ketamine anesthesia. The serum was separated by centrifugation at 3000rpm for 15 min, and subjected to estimation of various biochemical parameters including serum biomarkers and anti-oxidant parameters.

Assessments of oxidative stress markers: The oxidative stress markers are measured by various tests. Drug induced lipid peroxidation was measured by estimating the content of MDA, Glutathione (GSH), superoxide dismutase (SOD) & catalase (CAT) in blood sample. Determination will be done by precipitating the protein substance using tri chloro acetic acid (10% w/v). The protein free sample was used for estimation of various oxidative stress markers.

Assessments of Behavioural Studies: The effect of Flupirtine on spatial learning & memory using morris water maze, on anxiety using elevated plus maze, on locomotor activity using Actophotometer, on rota rod activity using Rota rod Apparatus in 3-NPA treated rats was studied in this activity assessment.

Statistical Analyses: The data were represented as mean ± standard error of mean (S.E.M.) for five rats. Student t-test was followed by individual comparison by Newman- Keuls test using GraphPrism Pad software (Version 6.05, GraphPad Software, Inc. USA) for the determination of level of significance. The value of probability less than 5% ($P<0.05$) was considered statically significant.

RESULTS:

Animal Experiment: Healthy animals was used for the study and they were divided into different groups and their weights were in the range of 150- 200 gm. Animals were monitored and weighed weekly to study the effects of the drug on body weight and that of the inducing agent. All the animals of each group weighed weekly. The control group rats were administered with normal saline.

Effect of Flupirtine on body weight: Flupirtine show significant effect on body weight of rats. A significant difference was observed between the 3-NPA treated group (143.11 ± 3.10, $P< 0.001$) and Flupirtine treated group (190.33± 2, $P< 0.001$) and Flupirtine + 3-NPA treated group (188.34 ± 1.34, $P < 0.001$). Less significant difference in the body weight was also observed between the 3- NPA treated group and the Flupirtine treated group with low dose (179.74 ± 1.81, $P< 0.01$).

TAB. 1 Effect of Flupirtine on body weight

S.No	Groups	Treatment	Body Weight (in grams)			
			Week 1	Week 2	Week 3	Week 4
1	Group1	Normal Saline	180.26 ± 1.22	179.83 ± 1.08	176.04 ± 1.46	182.59 ± 3.07
2	Group2	3-NPA (10 mg/kg)	183.74 ± 2.0	175.67 ± 1.72	158.92 ± 2.10	143.11 ± 3.11
3	Group3	Imipramine (20 mg/kg)	174.64 ± 1.28	182.41 ± 0.99	179.28 ± 1.09	194.33 ± 2.06
4	Group4	Flupirtine (50 mg/kg)	154 ± 1.13*	163.82 ± 0.92	171.24 ± 0.98**	179.74 ± 1.81**

5	Group5	Flupirtine (100 mg/kg)	172.3 ± 0.95	180.17 ± 0.86**	187.83 ± 1.01***	190.33 ± 2.14***
6	Group6	Flupirtine (50mg/kg + 3- NPA)	162.17 ± 1.70*	173.5 ± 0.40	181.22 ± 0.44*	167.83 ± 1.52**
7	Group7	Flupirtine (100 mg/kg + 3-NPA)	152.01 ± 1.12	158.33 ± 0.73**	161.17 ± 0.26*	188.34 ± 1.63***

Values are mean ± SEM, n = 6.3-NPA = 3- Niropropanoic acid. Analysis were done by ANOVA followed by Dunnet test. All groups were compared with positive control. Where *P<0.05, **p<0.01 and ***p<0.001, When 3-NPA treated group is compared with other treated groups.

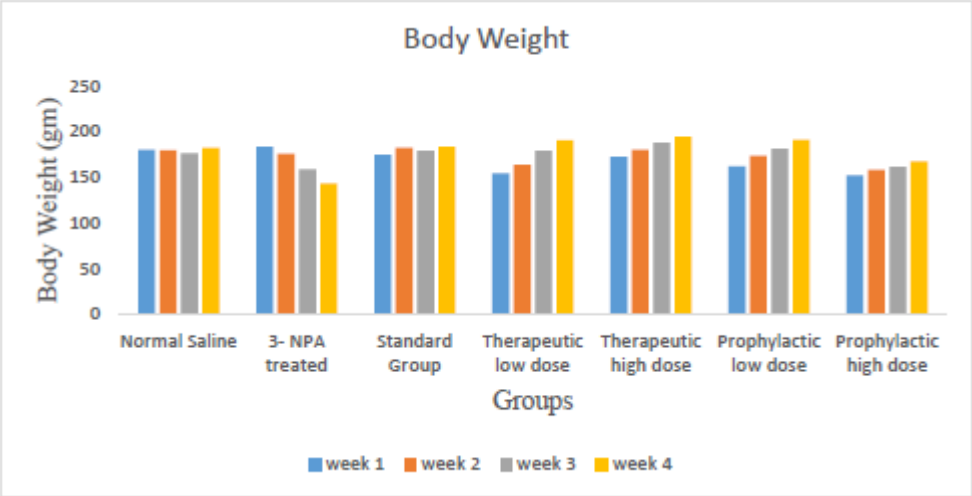

Fig. 1 Effect of Flupirtine on body weight. Values are mean ± SEM, n = 6. 3- NPA = 3-Nitropropanoic Acid. Analysis were done by ANOVA followed by Dunnet test. All groups were compared with positive control. Where *P<0.05, **p<0.01 and ***p<0.001, When 3-NPA treated group is compared with other treated groups.

Effect of Flupirtine on spatial learning & memory using morris water maze: Significant difference was found between 3-NPA treated, Flupirtine (100 mg/kg) and control group as shown in Fig. 3.2. Rats were administered with Flupirtine (50mg/kg, p.o and 100mg/kg, p.o) and 3-NPA (10 mg/kg, i.p.) 60 min. and 30 min. respectively prior to acquisition trials conducted on day 1 to day 4 and normal saline (10 ml/kg, i.p.) administered 60 min & 30 min prior to retrieval trial conducted on day 5 markedly attenuated increase in ELT noted during acquisition trials and increased in time spent in target quadrant in search of platform during retrieval trial.

TAB. 2 Effect of Flupirtineon ELT during Acquisition Trials in 3-NPA induced memory impairement in rat.

Groups	Treatments	Dose	ELT(sec) on Acquisition days			
			Day 1	Day 2	Day 3	Day 4
1	Normal saline	10 ml/kg	117 + 0.9	110.25 + 4.46	94 + 4.42	74.50 + 7.67
2	3- NPA	10 mg/kg	119.25 + 0.9	115.35 + 3.12	108 + 5.33	97 + 4.96
3	Imipramine	20 mg/kg	115.5 + 1.2	106.5 + 2.23	88.25 + 5.75	68 + 4.26
4	Flupirtine	50 mg/kg	112.65 + 5.20	98.75 ± 6.21**	89.35 + 5.32*	70.26 + 3.97**
5	Flupirtine	100 mg/kg	101.35 + 4.80*	96.5 + 3.20	71 + 5.20**	62.5 + 4.32***
6	Flupirtine + 3- NPA	50 mg/kg + 10 mg/kg	71 + 4.8	66.5 + 1.12	51.75 + 1.24**	45.6 + 1.32*
7	Flupirtine + 3-NPA	100 mg/kg + 10 mg /kg	74.65 + 3.23	62.5 + 4.21*	56.75 + 2.13*	53.25 + 7.12**

during Acquisition Trials in 3-NPA induced memory impairement in rat.

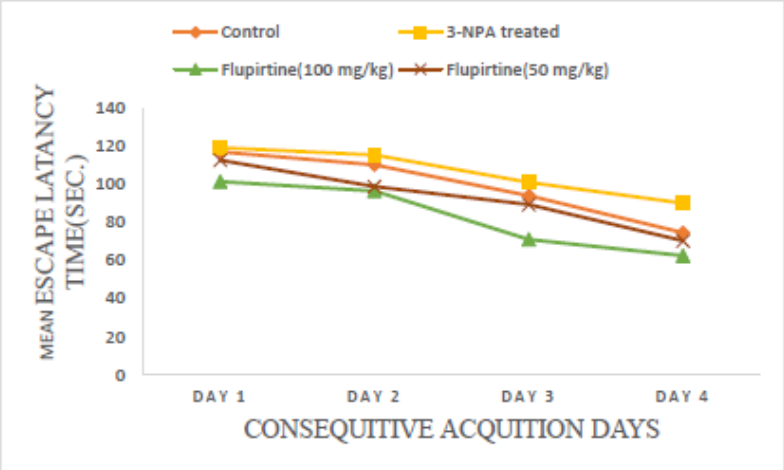

Fig. 2(a) Effect of Flupirtineon ELT

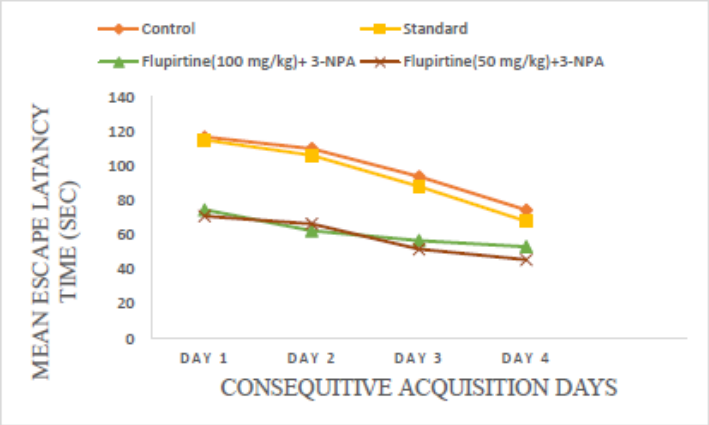

Fig. 2 (b) Effect of Flupirtineon ELT during Acquisition Trials in 3-NPA induced memory impairement in rat.

TAB. 3 Effect of Flupirtine in 3-NPA Induced Changes in Time Spent In Target Quadrant (TSTQ) during Retrieval Trial

Groups	Treatments	Time spent in target Quadrant (TSTQ) in sec
1	Normal saline	36.92 ± 2.61
2	3- NPA	20.90 ± 1.34
3	Imipramine	38.27 ± 1.23
4	Flupirtine	33.42 ± 1.02**
5	Flupirtine	37.44 ± 1.10***
6	Flupirtine + 3-NPA	29.50 ± 4.13*
7	Flupirtine + 3-NPA	30.32 ± 3.10*

Values are mean ± SEM, n = 6. 3- NPA = 3-Nitropropanoic Acid. Analysis were done by ANOVA followed by Dunnet test. All groups were compared with positive control. Where *P<0.05, **p<0.01 and ***p<0.001, When 3-NPA treated group is compared with other treated groups.

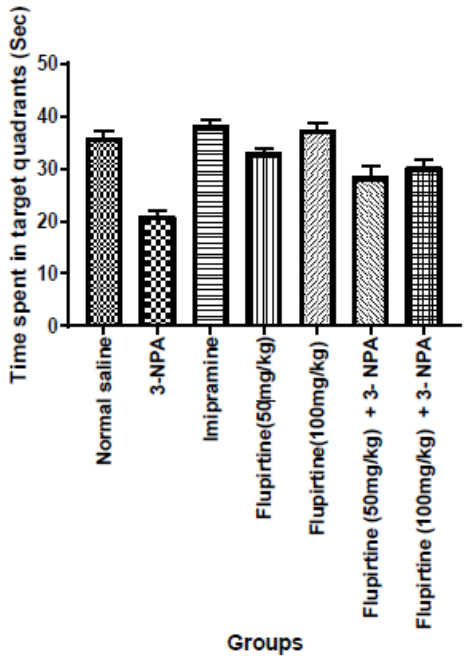

Fig.3 Effect of Flupirtine in Time Spent in Target Quadrant (TSTQ) during Retrieval Trial. 3-NPA treated rats showed decreased time spent in target quadrant as compared to target quadrant (Q4) of control group. But Flupirtine showed increased time spent in target quadrant and showed protection against 3-NPA induced memory loss. Results were expressed as mean ± SEM. n = 6 in each group.

Effect of Flupirtine on Anxiety using elevated plus maze in 3-NPA treated rats: Number of entries was lower in 3-NPA treated group (33.41±2.12, P< 0.07) than Flupirtine (100 mg/kg) treated group (77.27±0.04, P< 0.001). A significant difference was found between 3-NPA treated group and standard group in both open arms and dark arms of elevated plus maze. Lower time had been spent by Flupirtine (100 mg/kg) + 3-NPA treated group (132.13±0.18, P<0.003) than 3-NPA treated group (173.28±0.98, P< 0.001) in dark arm and was being significantly different. There was a significant difference between 3-NPA treated group and control group.

TAB. 4 Effect of Flupirtine on Anxiety in 3-NPA treated rats

S. No.	Groups	Treatment	No. of Entries		Time spent (Sec.)	
			Open arm	Dark arm	Open arm	Dark arm
1	Group 1	Control	7.30 ± 2.18	7.09 ± 2.13	71.34 ± 2.02	135.65 ± 0.42
2	Group 2	3-NPA (10 mg/kg)	3.95 ± 1.03	10.35±0.99	33.41 ± 2.12	173.28 ± 0.98
3	Group 3	Imipramine (20 mg/kg)	8.13 ± 1.28	6.02±0.05	95 ± 0.13	120.19 ± 1.96
4	Group 4	Flupirtine (50 mg/kg)	5.59 ± 1.32***	6.02 ± 2.15*	77.27 ± 0.04***	125.77 ± 2.10***

5	Group 5	Flupirtine (100 mg/kg)	6.72 ± 0.02***	6.18 ± 0.56*	87.85 ± 0.90***	119.56 ± 0.92**
6	Group 6	Flupirtine (50 mg/kg) + 3-NPA	5.76 ± 1.01***	5.65 ± 0.87**	70.78 ± 0.94**	129.63 ± 0.18***
7	Group 7	Flupirtine (100 mg/kg) + 3-NPA	6.59 ± 0.02**	6.57 ± 0.04*	85.08 ± 0.88***	128.43 ± 0.10***

Values are mean ± SEM, n = 6. 3-NPA = 3- Niropropanoic acid. Analysis were done by ANOVA followed by Dunnet test. All groups were compared with positive control. Where *P<0.05, **p<0.01 and ***p<0.001, When 3-NPA treated group is compared with other treated groups.

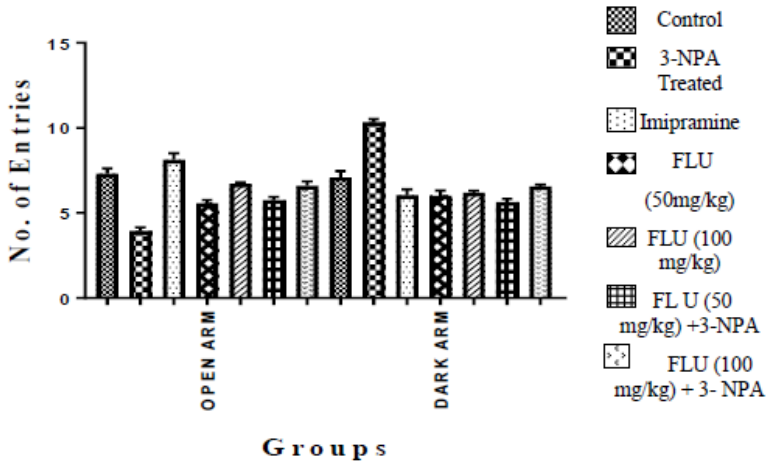

Fig.4 (a) Effect of Flupirtine on anxiety in 3- NPA treated rats, Values are mean ± SEM, n = 6. 3- NPA = 3- Nitropropanoic acid. Analysis were done by ANOVA followed by Dunnet test. All groups were compared with positive control. Where *P<0.05, **p<0.01 and ***p<0.001, When 3-NPA treated group is compared with other treated groups.

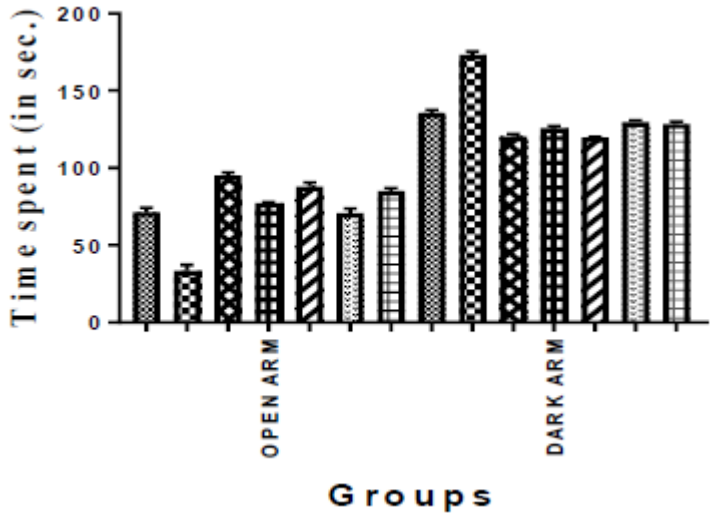

Fig.4 (b)Effect of Flupirtine on anxiety in 3- NPA treated rats, Values are mean ± SEM, n = 6. 3- NPA = 3- Nitropropanoic acid. Analysis were done by ANOVA followed by Dunnet test. All groups were compared with positive control. Where *P<0.05, **p<0.01 and ***p<0.001, When 3-NPA treated group is compared with other treated groups.

Effect of Flupirtine on locomotor activity using Actophotometer in 3- NPA treated rats: Locomotor activity was performed by using Actophotometer. The results were shown in Tab. 3.4. A significant difference was found between the 3-NPA treated group (122.81± 2.86, P < 0.001) and the Flupirtine (100mg/kg) treated group (189.02 ± 3.62, P < 0.001). Flupirtine has shown less significant effect in the prophylactic treatment and therapeutic treatment at low dose (147 ± 3.51, P < 0.01). Significant difference was found between the control group and the 3-NPA treated group.

TAB. 5 Effect of Flupirtine on locomotor activity in 3- NPA treated rats

S.No.	Groups	Treatment	Counts in 10 min.			
			Week 1	Week 2	Week 3	Week 4
1	Group1	control	188.34 ± 3.50	186.96 ± 2.85	188.52 ± 1.98	189.37 ± 2.72
2	Group2	3-NPA (10 mg/kg)	183.46 ± 3.42	172.75 ± 3.10	151±2.88	122.81 ± 2.86
3	Group3	Imipramine (20 mg/kg)	175 ± 3.50	184.83 ± 3.18	188.83 ± 2.66	191.64 ± 3.08
4	Group4	Flupirtine (50 mg/kg)	98.17 ± 3.76	108.33 ± 3.12**	126.19 ± 2.92*	147 ± 3.51**
5	Group5	Flupirtine (100	137.21 ± 4.01*	158.67 ± 3.70*	173.33 ± 2.36**	189.02 ± 3.62***

		mg/kg)				
5	Group5	Flupirtine (100 mg/kg)	137.21 ± 4.01*	158.67 ± 3.70*	173.33 ± 2.36**	189.02 ± 3.62***
7	Group7	Flupirtine (100 mg/kg + 3-NPA)	176.83 ± 3.36*	180.33 ± 3.10**	181.61 ± 2.68**	184.67 ± 2.42***

Values are mean ± SEM, n = 6. 3-NPA = 3- Nitropropanoic acid. 3- NPA = 3-Nitropropanoic acid. Analysis were done by ANOVA followed by Dunnet test. All groups were compared with positive control. Where *P<0.05, **p<0.01 and ***p<0.001, When 3-NPA treated group is compared with other treated groups.

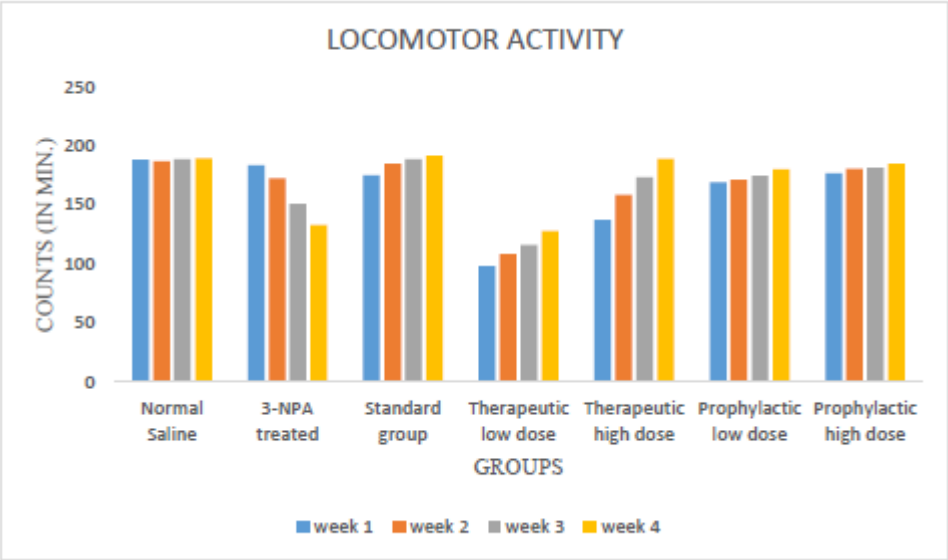

Fig.5 Effect of Flupirtine on Locomotor activity in 3-NPA treated rats, values are mean ± SEM, n = 6. 3-NPA = 3-Nitropropanoic acid. Analysis were done by ANOVA followed by Dunnet test. All groups were compared with positive control. Where *P<0.05, **p<0.01 and ***p<0.001, When 3-NPA treated group is compared with other treated groups.

Effect of Flupirtine on rota rod activity using Rota rod Apparatus in 3- NPA treated rats:

Rota rod activity was performed by using the rota rod apparatus. From the fig.3.6, it was found that Flupirtine treated group and standard group rats spent more time on rota rod comparable to 3-NPA treated group. At the week 4, a significant difference was found between the 3-NPA treated group (6.17 ± 5.01, P < 0.001) and the Flupirtine (100 mg/kg) treated group (35.33 ± 7.01, P < 0.001) and the standard group.

TAB 6 Effect of Flupirtine on rota rod activity in 3- NPA treated rats

S.No.	Groups	Treatment	Counts in 10 min.			
			Week 1	Week 2	Week 3	Week 4
1	Group1	control	24.5 ± 4.12	22.5 ± 2.32	23.17 ± 3.06	21.17 ± 4.84
2	Group2	3-NPA (10 mg/kg)	30.5 ± 3.91	19.33 ± 2.26	11.83 ± 2.63	6.17 ± 5.01
3	Group3	Imipramine (20 mg/kg)	32.5 ± 2.99	28.83 ± 2.38	30.59 ± 2.29	37.83 ± 5.11
4	Group4	Flupirtine (50 mg/kg)	9.67 ± 3.56	16.5 ± 2.12*	26.71 ± 2.98**	29.17 ± 4.05**
5	Group5	Flupirtine (100 mg/kg)	12 ± 4.52*	16.67 ± 3.18*	28.48 ± 3.92**	35.33 ± 7.01***
5	Group5	Flupirtine (100 mg/kg)	14.17 ± 5.10*	11.67 ± 2.26	17.17 ± 3.47**	18.5 ± 6.03**
7	Group7	Flupirtine (100 mg/kg + 3-NPA)	11.17 ± 4.90*	16.5 ± 2.51	19 ± 3.12**	21.62 ± 6.11**

Values are mean ± SEM, n = 6. 3-NPA = 3-Nitropropanoic acid. Analysis were done by ANOVA followed by Dunnet test. All groups were compared with positive control. Where *P<0.05, **p<0.01 and ***p<0.001, When 3-NPA treated group is compared with other treated groups.

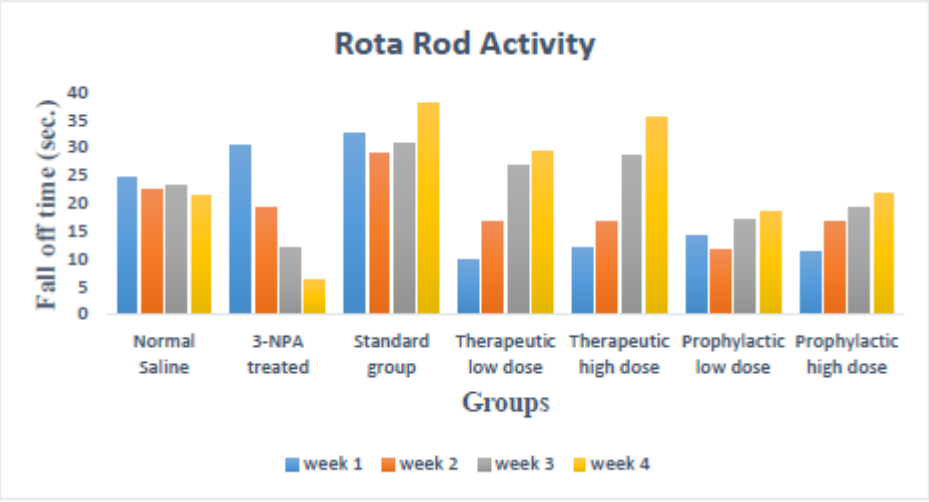

Fig.6 Effect of Flupirtine on Rota rod activity in 3-NPA treated rats, values are mean ± SEM, n = 6. 3-NPA = 3-Nitropropanoic acid. Analysis were done by ANOVA followed by Dunnet test. All groups were compared with positive control. Where *P<0.05, **p<0.01 and ***p<0.001, When 3-NPA treated group is compared with other treated groups.

Effect of Flupirtine on Biochemical Parameters in 3- NPA treated rats:

Measuement of oxidative stress: Drug induced oxidative stress was measured by estimation the content of GSH, MDA, SOD, CAT & ACh in blood sample. Determination will done by precipitating the protein substance using trichloroacetic acid (10% w/w). The protein free sample was used for the estimation of oxidative stress parameters. The following parameters are to be estimated as follows:

Glutathione (GSH): There is no significant difference in the GSH content between control and Flupirtine group. A significant difference was observed between 3- NPA treated group (7.67± 1.78, P< 0.001) and FLU + 3- NPA treated group (15.30 ± 1.02, P<0.001), 3-NPA treated also significantly different from control and prophylactic groups.

Malonaldehyde (MDA): There is no significant difference in the MDA content between control and Flupirtine (100mg/kg) group. A significant difference was observed between 3-NPA treated group (17.77 ± 1.10, P<0.001) and Flupirtine (100 mg/kg) (7.26 ± 0.98, P<0.001). The equal amount of MDA content was observed in control and standard.

Superoxide Dimutase (SOD): Identical amount of SOD content was observed in control group and Flupirtine (100 mg/kg) group but a significant difference was observed in 3-NPA (58.99 ± 0.03, P<0.001) and 3-NPA + Flupirtine (100 mg/kg) treated group. The amount of SOD content was more in control group than 3-NPA treated group.

Catalase (CAT): There was no significant difference in the amount of CAT content between control and Flupirtine (100 mg/kg). A significant difference was observed between 3-NPA (43.65 ± 1.88, P< 0.001) and Flupirtine (50 mg/kg + 3-NPA) (70.95 ± 1.71, P< 0.01) and Flupirtine (100 mg/kg + 3-NPA) (67.34 ± 3.06, P< 0.001) and Flupirtine (100 mg/kg) (70.95 ± 1.71, P<0.001).

TAB. 7 Effect of Flupirtine on Biochemical Parameters in 3-NPA treated rats

S.No.	Groups	Treatment	Biochemical parameters			
			GSH (µm/mg)	MDA (nM/mg)	SOD (Units/mg)	CAT (µg/min./mg)
1	Group1	control	17.85± 2.51	10.06 ± 1.63	77.33± 0 .05	80.52 ± 1.43
2	Group2	3-NPA (10 mg/kg)	7.67 ± 1.78	17.77±1.10	58.99 ± 0.03	43.65 ± 1.88
3	Group3	Imipramine (20 mg/kg)	15.81 ± 2.35	8.39 ± 1.09	54.91 ± 0.42	73.87 ± 1.68
4	Group4	Flupirtine (50 mg/kg)	14.64 ± 1.15**	6.52 ± 1.06**	48.69± 0. 59*	50 ± 1.92**
5	Group5	Flupirtine (100 mg/kg)	16.08 ± 2.96***	5.91 ± 1.02***	33.74 ± 0.98***	70.95 ± 1.71***
5	Group5	Flupirtine (100 mg/kg)	13.02 ± 2.30**	8.27 ± 1.85*	42.13 ± 0.92**	58.5 ± 1.20***
7	Group7	Flupirtine	15.30±	7.26 ±	31.27 ±	67.34 ±

		(100 mg/kg + 3-NPA)	1.02***	0.98*	0.04***	3.06***

Values are mean ± SEM, n = 6. 3-NPA = 3- Niropropanoic acid. Analysis were done by ANOVA followed by Dunnet test. All groups were compared with positive control. Where *P<0.05, **p<0.01 and ***p<0.001

Effect of Flupirtine on AChE level: Bar graph representing effect of Flupirtine on AChE level: As compared to control group, 3-NPA treated group show significant increase in AChE level was noted. Imipramine and Flupirtine (50 mg/kg) treated group decreased AChE level. Significant difference was observed between 3-NPA (16.67 ± 3.89, P <0.001) Flupirtine (100 mg/kg) treated group (9.68 ± 2.94, P < 0.001) and Flupirtine (100 mg/kg + 3-NPA, P<0.05). Difference was also observed between 3-NPA and Flupirtine (50 mg/kg) (10.23 ± 2.42, P < 0.01).

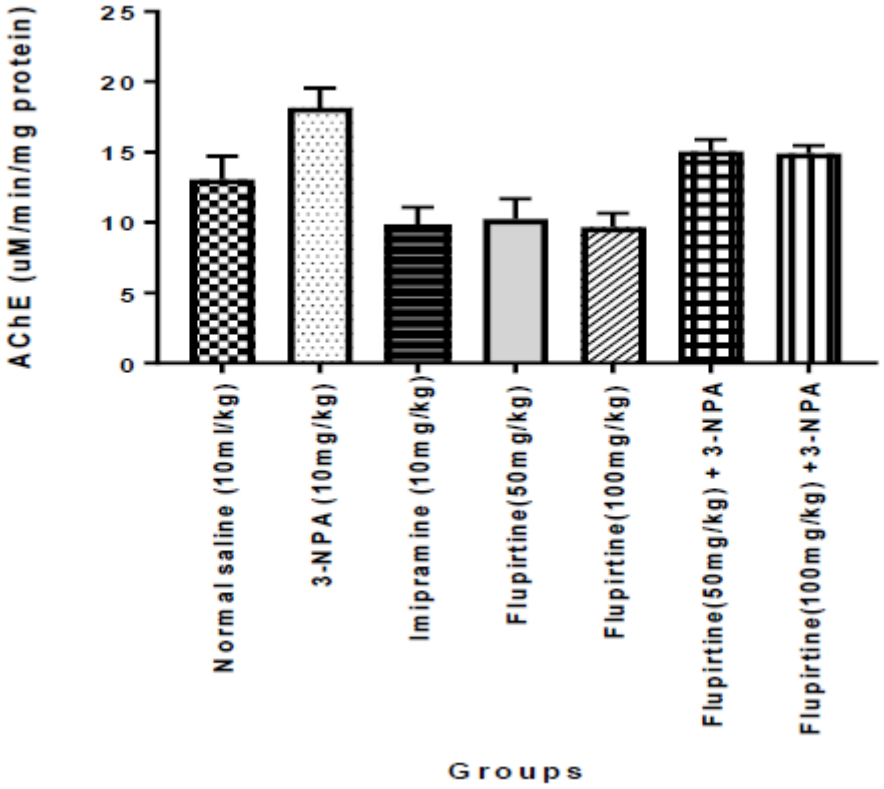

Fig.8 Effect of Flupirtine on AChE in 3-NPA treated rats, Values are mean ± SEM, n = 6. 3-NPA = 3- Niropropanoic acid. Analysis were done by ANOVA followed by Dunnet test. All groups were compared with positive control. Where *P<0.05, **p<0.01 and ***p<0.001, When 3-NPA treated group is compared with other treated groups.

DISCUSSION:

The present study aimed at exploring the impact of Flupirtine on rats exposed to 3-NPA. 3-NPA induces behavioural behavioural alterations and mitochondrial dysfunction in the striatum of rat brain on exposure to that seen in huntington‘s disease patients (Sarumani & Thangarajan, 2014). The animal model of huntington‘s disease induced by 3-NPA, has a significant number of characteristics observed in humans with Huntington‘s disease. Among those are repetitive and stereotypic behaviour, weight loss, impaired locomotion, motor coordination and learning memory and biochemical impairement (2).

Behavioural changes in rats showed the following results: Flupirtine at high dose show significant increase in body weight. 3-NPA treated rats shows a significant decrease in the body weight over the time. Flupirtine treatment with 3-NPA do not show significant alterations in body weight over the time.

On the Morris Water Maze test, 3-NPA treated group rats finds the platform in less time than control & Flupirtine group i.e. their spatial & learning memory was more than control group rats. Flupirtine treated rats find platform in less time than 3-NPA group rats. The significant difference was found at Probe trial day between 3-NPA and Flupirtine group, at the 5th day probe trial was conducted in which platform was removed and Flupirtine treated group spent more time in the quadrant in which platform was placed in previous 4 days.

Treatment with 3-NPA is believed to evoke anxiety and exaggerated fear identical to that seen in huntington‘s disease subjects. Flupirtine treated rats at high dose spent more time in open arm than 3-NPA treated rats and less time in dark arm then 3-NPA treated rats i.e. Flupirtine reduced the anxiety in huntington model rats. 3-NPA treated rats showed decreased no. of entries than control group rats in both arms and there was no difference found out between 3-NPA group and 3-NPA + Flupirtine group in the no of entries in both arms.

Locomotor activity test showed that 3- NPA treated rats had impaired locomotion due to the striatal damage in rat brain. Flupirtine helps in improving the locomotion in huntington‘s disease animal models.

Motor clumsiness is an evident symptom of Huntington‘s disease and can be easily tested on the rota rod. The cerebellum plays a pivotal role in modulating muscular coordination. At the 4th week 3-NPA showed decreased retention time on the rotating rod than the control group & Flupirtine at high dose treated rats showed increased retention time on the rotating rod.

Rats treated with 3-NPA showed enhanced formation of oxidative markers like thiobarbituric acid reactive substance which can interrupt neuronal integrity. The cytotoxic activity of 3-NPA is due to the generation of hydrogen peroxide, hydroxyl as well as due to reduced levels of enzymatic and non- enzymatic anti-oxidants. Diminished glutathione peroxidase, superoxide dismutase and catalase enzyme activities have been associated with huntington‘ disease. Level of SOD, and GSH-Px were significantly lower in huntington‘s model compared with their controls.

Low level of GSH were observed in 3-NPA treated group than control group rats and Flupirtine at high dose increased the level of GSH in 3- NPA treated rats. High level of MDA was found in 3-NPA rats than control group rats and found to be low in the Flupirtine treated group. It was found that Flupirtine increase the level of SOD in 3-NPA treated rats as it was reduced due to the toxicity of 3- NPA. Low levels of CAT were also observed in 3- NPA

treated rats compared to control group and Flupirtine increases the level of CAT in 3- NPA treated rats.

CONCLUSION:

The study of Flupirtine on Huntington‘s disease model showed that Flupirtine may act by preventing and relieving the symptoms of Huntington‘s disease like memory impairement and motor incoordination etc. by decreasing the level of AChE enzyme in 3-NPA treated animals. The Neuro-protective effect may be by reducing the oxidative stress markers like GSH, MDA, SOD and CAT in Huntington‘s disease model.

Flupirtine may have potential in the prevention and therapeutic effectiveness of Huntington‘s disease. Other experimental studies are necessary to evaluate the unambiguous effect of Flupirtine in Huntington‘s disease, so that it can be used as a rational therapy in HD.

Flupirtine has Neuro protective property and NMDA receptor antagonist property which will reduce the incidence of nerve cell damage.

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