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Research Article

Hesperetin attenuates neuroprotective effect against 3-Nitropropionic acid induced Huntington's disease-like behavioral symptoms in rats

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Abstract

The disease Huntington's (HD) is an autosomal neurologic disorder characterized by inexorable loss of nerve cells in the brain accompanied with cognitive, motor and psychiatric disorders. In the present study, 3-Nitropropionic acid (3-NP), an inhibitor of mitochondrial citric acid cycle results in symptoms like HD. Hesperetin(HSP) is a flavanone rich in citrus species which possess neuroprotective effects. The aim of the present study was to evaluate the protective role of HSP against 3-NP induced symptoms. Pre-treatment of animals with HSP/normal saline for 7 days and from 8th day, 3-NP (10mg/kg) was co-administered with HSP. It is continued for 21 days of the treatment schedule. At the end day of the study, the results showed that HSP improved all the cognitive, motor and psychiatric symptoms induced by 3-NP significantly. Hence, these findings show the protective effect of HSP against 3-NP induced neurological disorder.

Keywords: Huntington's disease, Hesperetin, 3-Nitropropionic acid,

INTRODUCTION

Huntington's disease is a chronic neurological disorder, characterized by inexorable loss of neurons in a group of contiguous sub-cortical structures in the region of basal ganglia. It affects the motor control and reward system in the brain[1]. Striatum is the main region of basal ganglia facilitating the voluntary movements. This striatal cell loss leads to decline in cognitive, personality disorders and chorea. The pathogenesis of HD is mainly due to mitochondrial dysfunction[2]. Normal condition of mitochondria is altered by the interference of mutant Huntingtin protein (mHtt) causing inhibition of ATP production[3]. Early findings showed that mitochondrial complex II enzyme Succinate dehydrogenase (SDH) of respiratory chain functioning is also related to the excessive levels of oxidative stress in the brain[4]. Excess production of reactive oxygen species is responsible for oxidative stress and cell damage which ultimately causes atrophy. In the brain naturally occurring reactive oxygen species is controlled by the cellular antioxidant systems[5]. If the balance between them is impaired it leads to mitochondrial dysfunction, oxidative stress and neuronal cell death. Previous studies have been supported that HD may be triggered by the mitochondrial dysfunctions[6]. Similar symptoms like HD can be induced by the chemical compounds that inhibit proper functioning of mitochondrial complex. Evidence states that there is no particular treatment for HD[7]. The cognitive and motor disturbances along with biochemical estimations were used to find the proper medication to this disease[8]. Tetrabenazine was only approved drug by USFDA for the treatment of symptoms like chorea related to Huntington's disease, but found beneficial to some extent. However, different methods were used to treat HD but, unable to exhibit the particular reason to the cognitive impairment[9]. So, there is a need to focus on the alternative methods to decrease the symptoms of the Huntington's like disease.

Different models like injection of ibotenic acid, kainic acid, and quinolic acids in particular region of brain are exists. But past years the well popular model 3-Nitropropionic acid (3-NP) model is used. 3-NP is a mitochondrial toxin produced by the fungal species and also from plant species[7]. It has the ability to cross the

blood brain barrier; it acts as an irreversible mitochondrial SDH inhibitor. It shows mitochondrial dysfunction by decreasing ATP production which invariably leads to death of neurons[10]. Reports have been suggested that 3-NP acts primarily on striatal neurons causing atrophy. Moreover 3-NP also causes acute damage to the surrounding neuronal cells of the striatum such as cortex, hippocampus and hypothalamus[11].

Flavonoids are rich antioxidants which comprise a group of phytonutrients seen in fruits and vegetables[12]. They have great ability in inhibition of generative ROS and reduce the levels of formation of ROS [13]. Hesperetin (HSP; 3',5,7-Trihydroxy-4'-methoxy flavanone) a flavanone, from the group of flavonoids which is abundantly stored in the citrus species. It is aglycone of the flavonoid Hesperedin [14]. It was reported that Neuroprotective effects of the citrus flavanones against H_2O_2-induced cytotoxicity in PC12 cells[15]. Based on the above factors, the present study aimed to evaluate the potentials of Hesperetin may improve the neuroprotection against 3-NP induced Huntington's disease in rats.

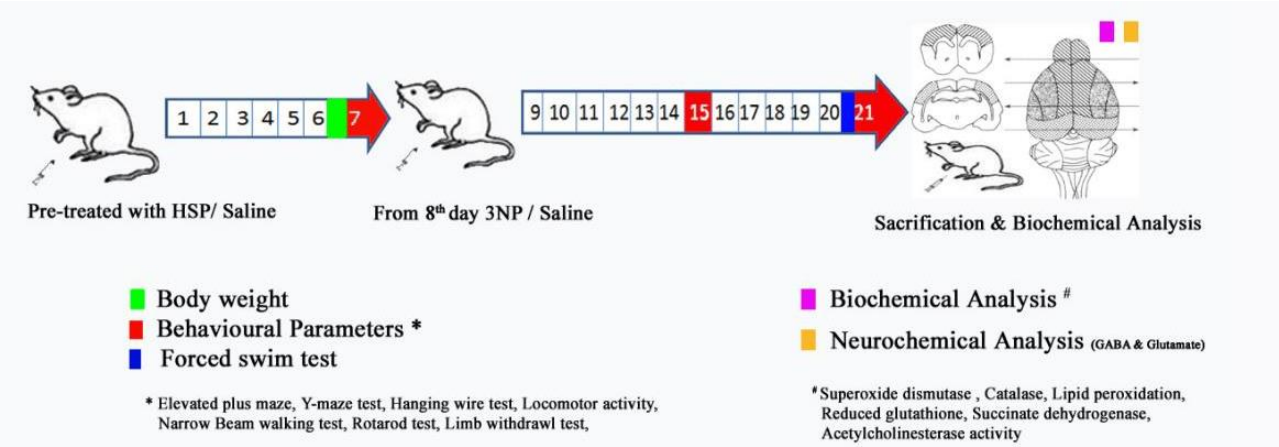


Figure 1: Experimental Schedule

2. MATERIALS AND METHODS

2.1. Chemicals

3-Nitropropionic acid (N5636-1 G, purity≥97%), Hesperetin purchased from Sigma (USA), TTC (Triphenyl Tetrazolium Chloride) and all required chemicals were of analytical grade purchased from Hi-Media Laboratories Pvt, Ltd., Mumbai

2.2. Experimental Animals

Adult Wistar rats of either sex (220-250 gm) of Forty Eight in number, were purchased for this study. They were obtained from Mahaveer Enterprises, Hyderabad and the animals were housed with maintaining under standard laboratory conditions of light-dark cycle. Food and water were available *ad libitum*. Approval of protocol (ANUCPS/IAEC/AH/P/9/2018) was done by the Institutional Animal Ethics Committee (IAEC) of University College of Pharmaceutical Sciences, Acharya Nagarjuna University, according to the guidelines of CPCSEA, Govt. of India.

2.3. Experimental design

Forty Eight male Albino Wistar rats were grouped into four containing 12 animals in each group. The entire study was designed for 21 days as following schedule

Group 1: Saline (i.p)
Group 2: 3NP (10mg/kg, i.p)
Group 3: HSP (25mg/kg, p.o) + 3NP (10mg/kg, i.p)
Group 4: HSP (50mg/kg, p.o) + 3NP (10mg/kg, i.p)

From the 8th day of the treatment schedule 3-NP was administered by dissolving in normal saline intraperitoneally (i.p) with a dose of 10mg/kg/day. HSP was suspended in 0.1% carboxymethyl cellulose (CMC) and administered at a dosage of 25mg/kg/day and 50mg/kg/day, through Per Oral route (p.o) from day 1 to day 21 according to the treatment schedule. From the day 8 to day 21, HSP was administered 1 hour prior to the 3-NP treatment. After 3-4 hrs of treatment of animals different physical and behavioral parameters were analyzed on 7th, 15th and 21st day.

2.4. Body weight assessment

Assessment of percentage change in body weights was done on the day 7 and the day 22 with the formula (Body weight on the day 22 – Body weight on the day 7 / Body weight on day 1 × 100)

2.5. Behavioral Assessments

Each group containing 12 animals was halved to reduce the stress of animals during behavioral assessment. Half of the group is used to assess the Neurological score, elevated plus maze test, Locomotor activity, Hanging wire test, Y- maze test. The remaining are for Narrow beam walking, Rotarod test, Limb withdrawal test, Forced swim test. All these assessments were carried out during a light between 9:00 am to 4:00 pm with the maximum of 20 min interval for each test.

2.5.1. Neurological score

Animals were scored based on disability in the normal behavioral functioning caused by the 3-NP. According to previous literature, scoring was on the scale 0-4 is as follows: 0- Normal behavior, 1-Slowness of general movements due to mild hind limb impairment, 2- loss of

co-ordination in movements and notable abnormality in gait, 3- paralysis of hind limbs, 4- both forelimbs and hind limb impairment which leads to inability in movement [11].

2.5.2. Elevated plus maze test

The long term spatial memory was assessed by using the elevated plus maze. The maze looks like '+' (plus) shape. The center of the maze is about 10cm×10cm, from here the arms (50cm, long×10cm, wide) are extended centrally towards opposite directions, two closed arms (40cm height wall) and two open arms. This is elevated at a height of 50cm above the ground. On 7th, 14th, 21st day the test was carried out, by mobilizing animals individually at one end of the open arm, and then the time taken by the animal to enter into the one of the closed arm is recorded as the Transfer Latency (TL)[16].

2.5.3. Y-maze test

The Y-maze apparatus consists of horizontally extended three arms (40cm long, 14cm wide, 20cm high) at an angle of 120̊ each. The rats were individually placed in the corner of an arm and allowed to move for 8min. Total number of arm entries were recorded and the number of alterations based on the triplets of the arm entries. The arms were cleaned thoroughly between the tests of each animal[17]. The percentage alternation was calculated as given formula: The percentage of spontaneous alternation (%SA) = [(no. of alternations)/ (total arm entries - 2)] × 100.

2.5.4. Hanging wire test

According to the treatment schedule, on 7th, 14th, 21st day the rats were placed to hang with forelimbs on a steel wire measuring a length of 80cm and the diameter of 2mm with their forelimbs to assess the grip strength of the animal. The wire is elevated at a height, 50cm from the ground and a foam cushion is padded to prevent the injury if it falls down. The cut-off time for the animal is 90sec, to calculate the latency of grip loss [18].

2.5.5. Locomotor activity

The monitoring of the locomotor activity was done by Actophotometer. Before the experiment animals were habituated for 2min. Later the rats were individually placed in the actophotometer about 5min in which the infrared sensitive photocell counts the motor activity of animals. The mobilization of animals was expressed as total counts in actophotometer per 5 min[19].

2.5.6. Narrow Beam walking test

Motor coordination mainly hind limb impairment was assessed by using Narrow beam. The narrow beam is a piece of wood with 2.3cm wide × 120cm long suspended 50cm from the ground by support on either side and the foam cushion padded on the ground to prevent the injury to the animal. On 7th, 14th, 21st day this test was carried out by placing the animals individually towards one end and allowed 2 min to traverse the beam. The test was considered as end when the animals were unable to mobilize on beam or slip from the beam. The latency to cross beam was recorded for successful traverse animals[20].

2.5.7. Rotarod test

Evaluation of gripping strength and motor coordination of animals can be done by rotarod apparatus. The animals were trained 3 trails per 3 days with an interval of 5min between each trail for the best performance in the main test. The rotarod is 7cm in diameter with a speed of 25 rpm and the cut-off time for each animal is measured up to 180sec. In the main test, the time of fall in the three trials was calculated as average[21].

2.5.8. Limb withdrawal test

Animals with functional disabilities in hind limbs due to striatal damage were assessed in this test. A platform of 20cm high, consisting of 4 holes of which two are having with a diameter of 4 cm for forelimbs and two are about 5cm for hind limbs were used for the test. By placing the limbs in respective holes, hind limb withdrawal was observed. The difference between the withdrawal of one hind limb and the successive limb is noted. An average of three trials was evaluated with an interval of 45min[22].

2.5.9. Forced swim test

Individual animals were placed in a Plexiglas cylinder (40cm high and 18cm diameter) filled with water up to 25cm at 23-25̊ on the day 21 of the scheduled treatment. Immobility of the animal was noted later it was taken out of the cylinder[23].

3. RESULTS

3.1. Body weight changes

Body weights of the rats were measured during the treatment as the percentage (%) weight change of the animals by comparison of the total body weight of 22nd day with total body weight of the 7th day. A significant decrease in the body weight of 3NP group ($p< 0.001$) with comparison of control group. HSP treated group's high dose and low dose (25mg/kg and 50mg/kg) prevented loss of weight of the body and showed significant change ($P< 0.05$ and $P< 0.01$).

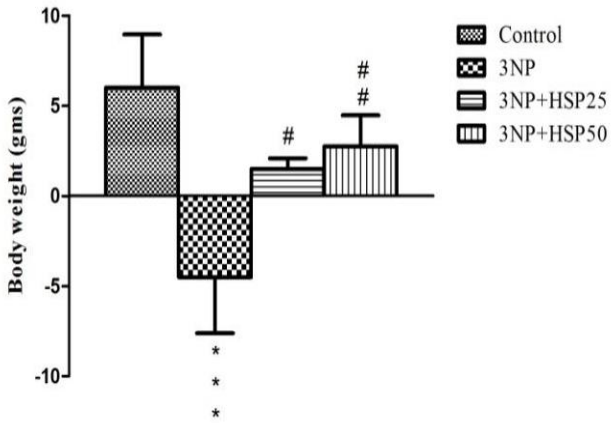


Figure 2: Effect of HSP on body weight changes in 3-NP induced animals. Results are expressed as mean ±SEM (n=6); *** indicate $P < 0.001$ Vs control; #, ## indicates $P < 0.05$, $P < 0.01$, respectively Vs 3-NP

3.2. Behavioral Assessments

3.2.1. Neurological score

Animals of 3-NP alone treated group showed abnormal functional disabilities like gait, paralysis of hind limbs and both fore limbs and hind limb paralysis ($P < 0.001$). The animals treated with low dose and high dose of Hesperetin (HSP 25mg and Hsp 50mg) showed improvement in behavioral functions, but 3-NP alone treated group was more significant than HSP treated groups.

3.2.2. Elevated plus maze test

Increase in Transfer latency was found to be significant ($P < 0.001$) in 3NP alone treated animals by retarding spatial memory, comparing to the normal group of animals. HSP treated animals at doses of 25mg and 50mg enhanced the impairment of spatial memory when compared to 3NP alone treated rats by significant ($P < 0.01$ and $P < 0.001$) reduction in the transfer latency.

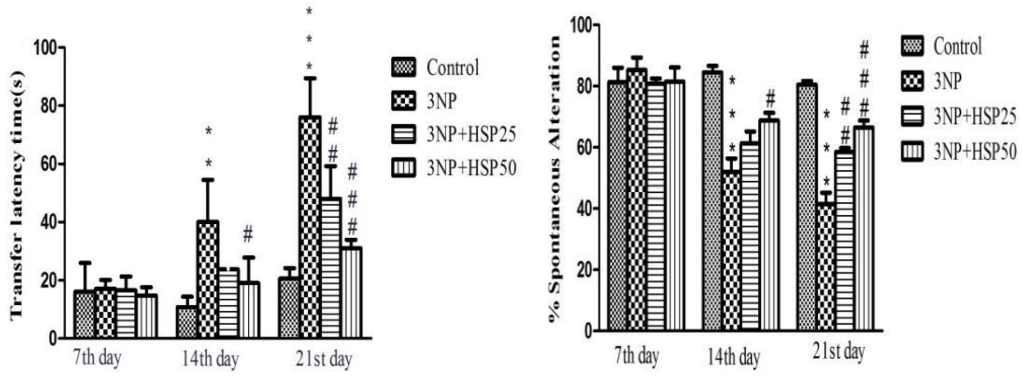
3.2.3. Y-maze test

Administered 3 NP alone group hindered short-term spatial memory significance ($P < 0.001$) by reduction in % SA, by comparison with normal group animals. The animals of HSP treated groups showed increased % SA, but only in high dose significantly ($P < 0.01$) when compared with 3NP alone group.

Table 1: Effect of HSP on neurological assessment in 3NP rats

Treatment groups	Animals Number					
	General behavior (score = 0)	Dull movement (score -1)	Incoordination and abnormalities in gait (score -2)	paralysis of hind limbs (score-3)	Incapable of movements (score-4)	Total mean score ± SEM
Normal	12/12	0/12	0/12	0/12	0/12	0.0
3-NP	0/12	2/12	3/12	3/12	4/12	2.65±0.421***
3-NP+HSP25	0/12	5/12	4/12	2/12	1/12	1.95±0.362
3-NP+HSP50	0/12	6/12	2/12	3/12	1/12	1.52±0.213

Values are mean ± SEM (n=12); *** indicates $P < 0.001$ Vs control. Kruskal Wall analysis of variance (non-parametric)



(a) (b)

Figure 3: Effect of HSP on (a) elevated plus maze and (b) Y-maze test in 3-NP induced animals. Results are expressed as mean ± SEM (n=6); **,*** indicate $P < 0.01$, $P < 0.001$, respectively, Vs control; #, # # and # # # indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, Vs 3-NP.

3.2.4. Hanging wire test

Decreased hanging latency of 25.5 ± 2.96 was observed in the 3 NP alone treated rats significantly ($P < 0.001$) when compared to the normal control animals with hanging latency of 78.3 ± 3.12. HSP treated animals of 25mg and 50mg showed 33.3 ± 2.69 ($P < 0.01$) and 40.5 ± 3.57 ($P < 0.001$) are significant when compared with 3NP alone treated animals.

3.2.5. Locomotor activity

Animals of 3NP alone administered group shows significant ($P < 0.001$) reduction in locomotor counts with comparison of normal control animals. Treatment with HSP of 25mg and 50mg has enhanced the reduction in locomotor counts significantly ($P < 0.05$ and $P < 0.01$) compared with 3NP alone treated group.

3.2.6. Narrow Beam walking test

Significant decrease in the motor co-ordination observed in the 3NP alone treated animals compared to normal control animals with a significantly ($P < 0.001$) increase in transfer latency while passing through beam. The low dose and high dose of HSP treated animals reduce the transfer latency time significantly ($P < 0.01$ and $P < 0.001$) compared with 3-NP alone treated group by surpass motor coordination.

3.2.7. Rotarod test

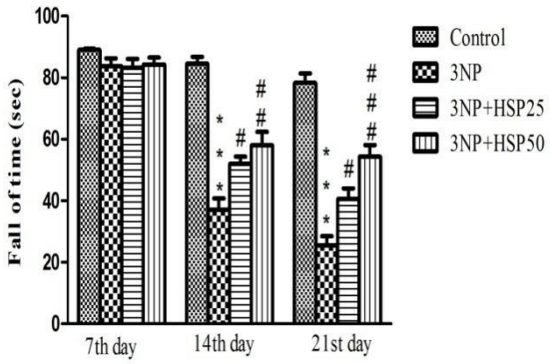
3NP administration significantly (P < 0.001) decreased the falling time of the animal when comparing to normal control animals. The HSP treated groups of low and high doses significantly (P < 0.05 and P < 0.01) ameliorate the motor co-ordination and enhanced the falling time of animal when analyzed with 3-NP alone treated group.

3.2.8. Limb withdrawal test

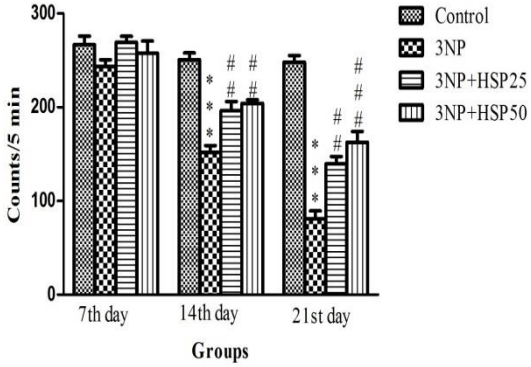
The normal retraction time significantly (P < 0.001) increase by 3-NP administration when compared with normal control animals. Both the doses (25mg/kg and 50mg/kg) HSP treated animals reduced the retraction time significantly (P < 0.01 and P < 0.001) while comparison with 3-NP alone treated group.

3.2.9. Forced swim test

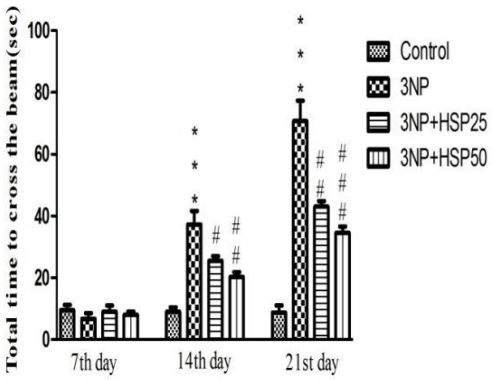
Mobility of the hind limbs were significantly (P < 0.001) inhibited by 3-NP administration when compared to normal control animals. Hence the HSP treatment groups, both the doses (25mg/kg and 50mg/kg) reduce the condition of immobility significantly (P < 0.05 and P < 0.001) by improving the hind limbs when compared to 3-NP alone treated group.



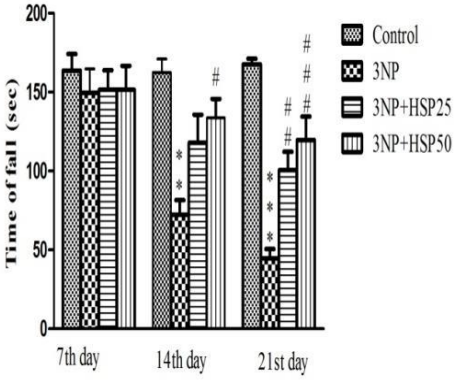
(a) Hanging wire



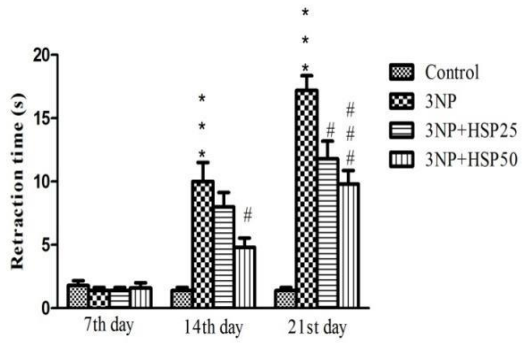
(b) Locomotor activity



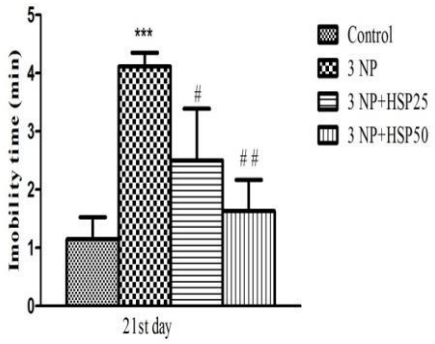
(c) Narrow beam walking test



(d) Rotarod activity



(e) Limb withdrawal test



(f) Forced swim test

Figure 4: Effect of HSP on (a) Hanging wire test (b) Locomotor activity (c) Narrow beam walking (d) Rotarod test (e) Limb withdrawal test and (f) Forced swim test in 3-NP induced animals. Results are expressed as mean ± SEM (n=6); **,*** indicate P < 0.01, P < 0.001, respectively, Vs control; #,## and ### indicate P < 0.05, P < 0.01, and P < 0.001, respectively, Vs 3-NP.

4. DISCUSSION

Neurodegenerative disorder is assessed by progressive neuronal loss in specific areas of nervous system. Huntington's disease (HD) is a rare neuronal degenerative disorder which shows symptoms like, chorea, cognitive dysfunction, oxidative stress and mitochondrial damage in rat brain[33].The present study was planned to assess the protective neuronal effect of Hesperetin (HSP) by using 3-NP, a toxic induction model of HD. Induction of 3NP, a mitochondrial toxin will produce oxidative stress and mitochondrial dysfunction thereby similar symptoms are seen in HD[3]. Recent studies had reported that antioxidants present in root extract of *Withania somnifera*[34], flavonoid kameferol[35], naringenin[36], sartraline[37], L-carnitine[38], taurine[39] and resveratrol[40] play an important role in the management of HD like symptoms. Based on these results, the present study was to assess the neuroprotective effect of Hesperetin in animal model of HD.

In the present study, the body weights of the animals with the induction of 3-NP produce significant weight loss. It might be due to the metabolic impairment and also due to the neuronal degeneration in hypothalamus[32, 41]. Other side striatal lesions and bradykinesia caused by 3NP may be possible reason for the weight loss of the animals[42]. The treatment with HSP significantly enhanced the body weight of the animals. HD is assessed by the major symptoms like chorea and cognitive impairment. According to previous literature chorea is treated as a movement disorder due to the specific striatal lesions in the brain. Administration of 3-NP produce significant motor impairment in animals. Neurobehavioral parameters such as beam walking, hanging wire, locomotor activity, rotarod, limb withdrawal is used to identify chorea[43, 44]. In this study, HSP treated animals significantly attenuated the motor impairment caused by 3NP induced striatal lesions.

CONCLUSION

In the present study, HSP could protect from 3-NP induced huntington's disease like behavioral changes due to motor dysfunction. These findings may be contributed to the antioxidant mechanism of mitochondrial oxidative injury. Hence HSP will be treated as a therapeutic agent in the HD like symptoms but further studies needed to evaluate the molecular mechanisms involved in neuroprotection of HSP against 3-NP induced neuronal toxicity.

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Conflicts of interest: The Authors declare that no conflicts of interest

Source of Support: Nil

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Ethics approval: Approval of protocol (ANUCPS/IAEC/AH/P/9/2018) was done by the Institutional Animal Ethics Committee (IAEC) of University College of Pharmaceutical Sciences, Acharya Nagarjuna University, according to the guidelines of CPCSEA, Govt. of India

REFERENCES

1. Leegwater-Kim J, Cha JJ, The paradigm of Huntington's disease: therapeutic opportunities in neurodegeneration, NeuroRx, 2004:128-138. https://doi.org/10.1602/neurorx.1.1.128 PMid:15717013 PMCid:PMC534918

2. Wang LH, Qin ZH, Animal models of Huntington's disease: implications in uncovering pathogenic mechanisms and developing therapies. Acta Pharmacol Sin 2006; 27:1287-1302. https://doi.org/10.1111/j.1745-7254.2006.00410.x PMid:17007735

3. Garcia M, Vanhoutte P, Pages C, et al, The mitochondrial toxin 3-nitropropionic acid induces striatal neurodegeneration via a c-Jun N-terminal kinase/c-Jun module, J Neurosci, 2002; 22: 2174-2184. https://doi.org/10.1523/JNEUROSCI.22-06-02174.2002 PMid:11896157 PMCid:PMC6758250

4. Tunez I, Inmaculada T, Perez-De LCV, Santamaria A, 3-Nitropropionic acid as a tool to study the mechanisms involved in Huntington's disease: past, present and future, Molecules, 2008;15: 878-916. https://doi.org/10.3390/molecules15020878 PMid:20335954 PMCid:PMC6263191

5. Wang M, Tsao R, Zhang S, et al, Antioxidant activity, mutagenicity/ anti-mutagenicity, and clastogenicity/anti-clastogenicity of lutein from marigold flowers, Food Chem Toxicol, 2006; 54: 7998-8005. https://doi.org/10.1016/j.fct.2006.04.005 PMid:16757077

6. Schulz JB, Matthews RT, Henshaw DR, Beal MF, Neuroprotective strategies for treatment of lesions produced by mitochondrial toxins: implications for neurodegenerative diseases, Neuroscience, 1996; 71: 1043-1048 https://doi.org/10.1016/0306-4522(95)00527-7 PMid:8684608

7. Tariq M, Khan HA, Elfaki I, Deeb SA, Moutaery KA, Neuroprotective effect of nicotine against 3-nitropropionic acid induced experimental Huntington's disease in rats, Brain. Res. Bull, 2005; 67: 161-168. https://doi.org/10.1016/j.brainresbull.2005.06.024 PMid:16140176

8. Saydoff JA, Liu LS, Garcia RA, Hu Z, Li D, Borstel RW, Oral uridine pro-drug PN401 decreases neurodegeneration, behavioral impairment, weight loss and mortality in the 3-nitropropionic acid mitochondrial toxin model of Huntington's disease. Brain Res, 2003; 994:44-54. https://doi.org/10.1016/j.brainres.2003.09.049 PMid:14642447

9. Setter S, Neumiller J, Dobbins E, Wood L, Clark J, DuVall C, Santiago A, Treatment of chorea associated with Huntington's disease: focus on tetrabenazine, Consul. Pharm, 2009; 24: 524-537. https://doi.org/10.4140/TCP.n.2009.524 PMid:19689181

10. Ahuja M, Bishnoi M, Chopra K, Protective effect of minocycline, a semi-synthetic second-generation tetracycline against 3-nitropropionic acid (3-NP)-induced neurotoxicity, Toxicology, 2008; 244: 111-122. https://doi.org/10.1016/j.tox.2007.11.003 PMid:18164115

11. Ludolph AC, He F, Spencer PS, Hammerstad J, Sabri M, 3-Nitropropionic acid-exogenous animal neurotoxin and possible human striatal toxin, Can. J. Neurol. Sci, 1991; 18: 492-498. https://doi.org/10.1017/S0317167100032212 PMid:1782616

12. Heim KE, Tagliaferro AR, Bobilya DJ, Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem, 2002; 14(10): 572-584.13.

https://doi.org/10.1016/S0955-2863(02)00208-5 PMid:12550068

13. Agati G, Azzarello E, Pollastri S, Tattini M, Flavonoids as antioxidants in plants: Location and functional significance, Plant Science, 2012; 196: 67-76. https://doi.org/10.1016/j.plantsci.2012.07.014 PMid:23017900

14. Garg A, Garg S, Zaneveld LJD, Singla AK, Chemistry and pharmacology of the citrus bioflavonoid hesperidin, Phytother Res, 2001;15:655-69. https://doi.org/10.1002/ptr.1074 PMid:11746857

15. Hwang SL, Yen GC, Neuroprotective effects of the citrus flavanones against H2O2-induced cytotoxicity in PC12 cells, J Agric Food Chem, 2008;56:859-64. https://doi.org/10.1021/jf072826r PMid:18189359

16. Shepherd, J.K., Grewal, S.S., Fletcher, A., Bill, D.J., Dourish, C.T., 1994. Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety, Psychopharmacol Berlin, 116: 56-64. https://doi.org/10.1007/BF02244871 PMid:7862931

17. X. Song, B. Zhou, P. Zhang, D. Lei, Y. Wang, G. Yao, T. Hayashi, M. Xia, S.I. Tashiro, S. Onodera, T. Ikejima, Protective effect of silibinin on learning and memory impairment in LPS-treated rats via ROS-BDNF-TrkB pathway, Neurochem. Res, 2016;41:1662-1672. https://doi.org/10.1007/s11064-016-1881-5 PMid:26961891

18. Hunter AJ, Hatcher J, Virley D, Nelson P, Irving E, Handubgham SJ, Functional assessments in mice and rats after focal stroke, Neuropharmacology, 2000; 39:806-816. https://doi.org/10.1016/S0028-3908(99)00262-2 PMid:10699446

19. Kulkarni SK, Hand Book of Experimental Pharmacology, Vallabh Prakashan, New Delhi, 1999.

20. Yan X, Wang S, Hou H, Ji R, Zhou J, Lithium improves the behavioral disorder in rats subjected to transient global cerebral ischemia, Behav. Brain Res, 2007; 177: 282-289. https://doi.org/10.1016/j.bbr.2006.11.021 PMid:17210190

21. Song YN, Li HZ, Zhu JN, Guo CL, Wang JJ, Histamine improves rat rota-rod and balance beam performances through H2 receptors in the cerebellar interpositus nucleus, Neuroscience, 2006; 140:33-43. https://doi.org/10.1016/j.neuroscience.2006.01.045 PMid:16533576

22. Vis JC, Verbeek MM, Waal RM, Donkelaar HJ, Kremer HP, 3-nitropropionic acid induces a spectrum of Huntington's disease-like neuropathology in rat striatum, Neuropath. Appl. Neuro, 1999; 25: 513-521. https://doi.org/10.1046/j.1365-2990.1999.00212.x PMid:10632901

23. Porsolt R.D., Bertin, A., Jalfre, M., Behavioral despair in mice: a primary screening test for antidepressants, Arch. Int. Pharmaco¬dyn. Ther, 1977; 229: 327-336.

24. Sandhir R, Mehrotra A, Kamboj SS, Lycopene prevents 3-nitropropionic acidinduced mitochondrial oxidative stress and dysfunctions in nervous system, Neurochem.Int,2010;57:579-587. https://doi.org/10.1016/j.neuint.2010.07.005 PMid:20643176

25. Marklund S, Marklund G, Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, Eur. J. Biochem, 1974;47: 469-474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x PMid:4215654

26. Claiborne A, Catalase activities, in: R.A. Greenwald (Ed.), CRC Handbook of Methods in Oxygen Radical Research, CRC Press, Boca Raton, 1985.

27. Gelvan D, Saltman P, Different cellular targets of Cu- and Fe-catalyzed oxidation observed using a Cu-compatible thiobarbiturate acid assay, Biochim. Biophys. Acta, 1990; 1035: 353-360. https://doi.org/10.1016/0304-4165(90)90100-B

28. Kumar P, Kumar A, Effect of lycopene and epigallocatechin-3-gallate against 3-nitropropionic acid induced cognitive dysfunction and glutathione depletion in rat: A novel nitric oxide mechanism, Food Chem Toxicol, 2009; 47:2522-2530. https://doi.org/10.1016/j.fct.2009.07.011 PMid:19616597

29. King TE, Preparation of succinate dehydrogenase and reconstitution of succinate oxidase Method, Enzymol, 1967;10: 322-331. https://doi.org/10.1016/0076-6879(67)10061-X

30. Ellman GL, Courtney KD, Andres V, Featherstone RM, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol, 1961; 7: 88-95. https://doi.org/10.1016/0006-2952(61)90145-9 PMid:13726518

31. Kola PK, Akula A, Nissankara Rao LS, Danduga RC, Protective effect of naringin on pentylenetetrazole (PTZ)-induced kindling; possible mechanisms of antikindling, memory improvement, and neuroprotection, Epilepsy Behavi, 2017;72: 114-126. https://doi.org/10.1016/j.yebeh.2017.07.011 PMid:28846920

32. Bederson JB, Pitts LH, Germano SM, Nishimura MC, Davis RL, Bartkowski HM, Evaluation of 2, 3, 5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats, Stroke 1986;17: 1304-1308. https://doi.org/10.1161/01.STR.17.6.1304 PMid:2433817

33. Ramaswamy S, McBride JL, Kordower JH, et al, Animal models of Huntington's disease, ILAR J, 2007;48:356-373. https://doi.org/10.1093/ilar.48.4.356 PMid:17712222

34. Kumar P, Kumar A, Possible neuroprotective effect of Withania somnifera root extract against 3-nitropropionic acid-induced behavioral, biochemical, and mitochondrial dysfunction in an animal model of Huntington's disease, J Med Food, 2009;12: 591-600. https://doi.org/10.1089/jmf.2008.0028 PMid:19627208

35. Lagoa R, Lopez-Sanchez C, Samhan-Arias AK, Ganan CM, Garcia-Martinez V, Gutierrez-Merino C, Kaempferol protects against rat striatal degeneration induced by 3-nitropropionic acid, J Neurochem, 2009; 111:473-487. https://doi.org/10.1111/j.1471-4159.2009.06331.x PMid:19682208

36. Kumar P, Kumar A, Protective effect of hesperidin and naringin against 3-nitropropionic acid induced Huntington's like symptoms in rats: possible role of nitric oxide, Behav Brain Res, 2010; 206:38-46. https://doi.org/10.1016/j.bbr.2009.08.028 PMid:19716383

37. Kumar P, Kumar A, Possible role of sertraline against 3-nitropropionic acid induced behavioral, oxidative stress and mitochondrial dysfunctions in rat brain, Prog Neuropsychopharmacol Biol Psychiatry, 2009;33:100-108. https://doi.org/10.1016/j.pnpbp.2008.10.013 PMid:19022325

38. Binienda ZK, Ali SA, Neuroprotective role of L-carnitine in the 3-nitropropionic acid induced neurotoxicity, Toxicol Lett, 2001; 125:67-73. https://doi.org/10.1016/S0378-4274(01)00415-5 PMid:11701224

39. Tadros MG, Khalifa AE, Abdel-Naim AB, Arafa HM, Neuroprotective effect of taurine in 3-nitropropionic acid-induced experimental animal model of Huntington's disease phenotype, Pharmacol Biochem Behav, 2005; 82:574-582. https://doi.org/10.1016/j.pbb.2005.10.018 PMid:16337998

40. Kumar P, Padi SS, Naidu PS, Kumar A, Effect of resveratrol on 3-nitropropionic acid-induced biochemical and behavioral changes: possible neuroprotective mechanisms, Behav Pharmacol, 2006;17:485-492. https://doi.org/10.1097/00008877-200609000-00014 PMid:16940769

41. S.H. Li, Z.X. Yu, C.L. Li, H.P. Nguyen, Y.X. Zhou, C. Deng, X.J. Li, Lack of huntingtin- associated protein-1 causes neuronal death resembling hypothalamic degeneration in Huntington's disease, J. Neurosci, 2003; 23: 6956-6964. https://doi.org/10.1523/JNEUROSCI.23-17-06956.2003 PMid:12890790 PMCid:PMC6740731

42. Guyot MC, Palfi S, Stutzmann JM, Maziere M, Hantraye P, Brouillet E, Riluzole protects from motor deficits and striatal degeneration produced by systemic 3-nitropropionic acid intoxication in rats, Neuroscience, 1997; 81: 141-149. https://doi.org/10.1016/S0306-4522(97)00192-9 PMid:9300407

43. Silva RH, Ablio VC, Kameda SR, et al, Effects of 3-nitropropionic acid administration on memory and hippocampal lipid peroxidation in sleep-deprived mice, Prog Neuropsychopharmacol

Biol Psychiatry, 2007;31:65-70. https://doi.org/10.1016/j.pnpbp.2006.06.019 PMid:16876303

44. Lee WT, Chang C, Magnetic resonance imaging and spectroscopy in assessing 3-nitropropionic acid-induced brain lesions: an animal model of Huntington's disease, Prog Neurobiol, 2004; 72: 87-110. https://doi.org/10.1016/j.pneurobio.2004.02.002 PMid:15063527